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Improving the safety and quality of eggs and egg products

Volume 1: Egg chemistry, production and consumption

Edited by Yves Nys, Maureen Bain and Filip Van Immerseel

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Improving the safety and quality of eggs and egg products

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Contents

Part III Egg production and quality

Contributor contact details

(* = main contact)

Editors

Yves Nys
Institut National de la Recherche Agronomique
INRA
UR83 Recherches Avicoles
F-37380 Nouzilly
France

E-mail: Yves.Nys@tours.inra.fr

M. Bain
Institute of Biodiversity, Animal Health and Comparative Medicine
College of Medical, Veterinary and Life Sciences
University of Glasgow
Garscube Estate
Bearsden Road
Glasgow G61 1QH
UK

E-mail: Maureen.Bain@glasgow.ac.uk

F. Van Immerseel
Department of Pathology, Bacteriology and Avian Diseases
Faculty of Veterinary Medicine
Ghent University
Salisburylaan 133
B-9820 Merelbeke
Belgium

E-mail: Filip.VanImmerseel@UGent.be

Chapter 1
P. Magdelaine
ITAVI
28 rue de Rocher
75008 Paris
France

E-mail: magdelaine@itavi.asso.fr

Chapter 2
N. Yang
College of Animal Science and Technology
China Agricultural University
Beijing 100193
China

E-mail: nyang@cau.edu.cn

Chapter 3
H. M. Tukur
Department of Animal Science
Faculty of Agriculture
Usmanu Danfodiyo University
P.M.B. 2346
Sokoto
Nigeria

E-mail: hussaini_tukur@yahoo.com

Chapter 4
F. Hansstein
Department of Statistics
University of Bologna
Via Zamboni 18
40122 Bologna
Italy

E-mail: francesca.hansstein@unibo.it

Chapter 5
F. C. Schwägele
Max Rubner-Institut
Federal Research Institute of Nutrition and Food
Analysis Division
E.-C.-Baumann-Straße 20
95326 Kulmbach
Germany

E-mail: fredi.schwaegele@mri.bund.de

Chapter 6
Yves Nys* and N. Guyot
Institut National de la Recherche Agronomique
INRA
UR83 Recherches Avicoles
F-37380 Nouzilly
France

E-mail: Yves.Nys@tours.inra.fr
Nicolas.Guyot@tours.inra.fr

Chapter 7
J. Gautron*, S. Réhault-Godbert and Y. Nys
Institut National de la Recherche Agronomique
INRA
UR83 Recherches Avicoles
F-37380 Nouzilly
France

E-mail: Joel.Gautron@tours.inra.fr

K. Mann
Max-Planck-Institut für Biochemie
Germany

P. G. Righetti
Politecnico di Milano
Italy

Chapter 8
M. Hincke*
Cellular and Molecular Medicine
University of Ottawa
451 Smyth Road
Ottawa
K1H 8M5
Canada

E-mail: mhincke@uottawa.ca

J. Gautron and Y. Nys
Institut National de la Recherche Agronomique
INRA
UR83 Recherches Avicoles
F-37380 Nouzilly
France

E-mail: Joel.Gautron@tours.inra.fr
Yves.Nys@tours.inra.fr

A. B. Rodriguez-Navarro
Minerology and Petrology
University of Granada
18002 Granada
Spain

E-mail: anava@ugr.es

M. D. McKee
Faculty of Dentistry
McGill University
Montreal, Quebec
H3A 2B2
Canada

E-mail: marc.mckee@mcgill.ca

Chapter 9

S. Réhault-Godbert*
Institut National de la Recherche Agronomique
UR83 Recherches Avicoles
F-37380 Nouzilly
France

E-mail: Sophie.Rehault@tours.inra.fr

V. Hervé-Grépinet
INSERM-U618 Protéases et Vectorisation Pulmonaires
10 boulevard Tonnellé BP 3223
F-37032 Tours
Cedex 1
France

E-mail: virginie.herve-grepinet@univ-tours.fr

J. Gautron
Institut National de la Recherche Agronomique
UR83 Recherches Avicoles
F-37380 Nouzilly
France

E-mail: Joel.Gautron@tours.inra.fr

C. Cabau
Institut National de la Recherche Agronomique
UR83 Recherches Avicoles
F-37380 Nouzilly
France

E-mail: Cedric.Cabau@tours.inra.fr

M. Hincke
Division of Clinical and Functional Anatomy
Cellular and Molecular Medicine
University of Ottawa
451 Smyth Road
Ottawa
K1H 8M5
Canada

E-mail: mhincke@uottawa.ca

Y. Nys
Institut National de la Recherche Agronomique
UR83 Recherches Avicoles
F-37380 Nouzilly
France

E-mail: Yves.Nys@tours.inra.fr

Chapter 10
K. Mertens
Department of Biosystems
Division of Mechatronics, Biostatistics and Sensors (MeBioS)
Katholieke Universiteit Leuven
Kasteelpark Arenberg 30 – bus 2456
B-3001 Heverlee
Belgium

E-mail: kristof.mertens@biw.kuleuven.be

Chapter 11
I. Dunn
The Roslin Institute and Royal (Dick) School of Veterinary Studies
University of Edinburgh
Easter Bush
Midlothian EH25 9RG
UK

E-mail: ian.dunn@roslin.ed.ac.uk

Chapter 12
I. Bouvarel*
ITAVI
Centre INRA de Tours
F-37380 Nouzilly
France

E-mail: Bouvarel.itavi@tours.inra.fr

Y. Nys and P. Lescoat
Institut National de la Recherche Agronomique
UR83 Recherches Avicoles
F-37380 Nouzilly
France

Chapter 13
A. Travel*
ITAVI
Recherches Avicoles
Centre INRA de Tours
F-37380 Nouzilly
France

E-mail: travel.itavi@tours.inra.fr

Y. Nys
Institut National de la Recherche Agronomique
INRA
UR83 Recherches Avicoles
F-37380 Nouzilly
France

E-mail: Yves.Nys@tours.inra.fr

M. Bain
Institute of Biodiversity, Animal Health and Comparative Medicine
College of Medical, Veterinary and Life Sciences
University of Glasgow
Garscube Estate
Bearsden Road
Glasgow G61 1QH
UK

E-mail: Maureen.Bain@glasgow.ac.uk

Chapter 14
F. Baron* and S. Jan
Equipe de microbiologie de l'œuf et des ovoproduits
UMR1253 STLO
Agrocampus Ouest
INRA, F-35042
65 rue de Saint Brieuc
CS 84215
F-35042 Rennes
France

E-mail: florence.baron@agrocampus-ouest.fr
sophie.jan@agrocampus-ouest.fr

Chapter 15
M. Rossi*
Università degli Studi di Milano
Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche
via Celoria 2
20133 Milano
Italy

E-mail: margherita.rossi@unimi.it

K. De Reu
Institute for Agricultural and Fisheries Research (ILVO)
Technology and Food Science Unit
Brusselsesteenweg 370
9090 Melle
Belgium

E-mail: Koen.Dereu@ilvo.vlaanderen.be

Chapter 16
J. R. Roberts*
University of New England
Armidale
NSW 2351
Australia

E-mail: jrobert2@une.edu.au

R. Souillard
Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (Anses)
BP 53
22440 Ploufragan
France

E-mail: r.souillard@anses.fr

J. Bertin
Coopérative Le Gouessant
22402 Lamballe

E-mail: joel.bertin@legouessant.fr

Chapter 17
O. A. E. Sparagano*
School of Life Sciences
Northumbria University
Ellison Building
Newcastle upon Tyne NE1 8ST
UK

E-mail: Olivier.sparagano@northumbria.ac.uk

A. Giangaspero
Facoltà di Agraria
University of Foggia
Via Napoli 25
71100 Foggia
Italy

E-mail: a.giangaspero@unifg.it

Chapter 18
S. Le Bouquin* and A. Huneau-Salaün
Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (Anses)
BP 53
F-22440 Ploufragan
France

E-mail: Sophie.Lebouquin-Leneveu@anses.fr

Chapter 19
M. G. MacLeod
Roslin Institute and Royal (Dick) School of Veterinary Studies
University of Edinburgh
Roslin
Midlothian EH25 9PS
UK

E-mail: murdo.macleod@roslin.ed.ac.uk

Chapter 20
M. Hammershøj
Department of Food Science
Faculty of Science and Technology
Aarhus University
Blichers Alle 20
8830 Tjele
Denmark

E-mail: mah@agrsci.dk

Chapter 21
J. F. Huang*
Ilan Branch, Livestock Research Institute
Council of Agriculture, Executive Yuan
28-1 Jeshui Road
Jehsin Village
Wuchieh, Ilan 268
Taiwan

E-mail: huangajf@mail.tlri.gov.tw

C. C. Lin
Department of Animal Science/ Graduate Institute of Biotechnology
National Ilan University 1
Sec. 1
Shen-Lung Road
Ilan 260
Taiwan

E-mail: lincc@niu.edu.tw

Chapter 22
A. Tserveni-Goussi* and P. Fortomaris
Faculty of Veterinary Medicine
Aristotle University of Thessaloniki
541 24 Thessaloniki
Greece

E-mail: goussi@vet.auth.gr
fortomap@vet.auth.gr

Chapter 23
V. Lechevalier*, T. Croguennec and F. Nau
UMR1253 Science et technologie du lait et de l'œuf
Agrocampus Ouest
INRA
F-35000 Rennes
France

E-mail: francoise.nau@agrocampus-ouest.fr

M. Anton
INRA Nantes UR 1268
Biopolymères Interactions Assemblages
F-44000 Nantes
France

E-mail: marc.anton@nantes.inra.fr

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Preface

Over the last 50 years or so there have been dramatic changes in the way we keep laying hens as well as in hen performance both in terms of the numbers and quality of eggs laid. In the 1920s and 1930s, farmers traditionally kept a few hens in backyard systems to supply their own families with eggs. However, as egg production became more profitable some of these farmers started to build up the size of their flocks so that they could supply eggs to local shops and markets. By the early 1960s it was recognised that production could be dramatically improved if hens were kept indoors especially in cage-based systems. This together with the development of genetic, nutrition, rearing system, sophisticated mechanical equipment and hen management resulted in a shift to the larger intensive commercial operations we typically associate with egg production today.

Eggs have a high nutritive value (OMS reference 100 for biological value of protein until the recent replacement virtual protein) and they also provide consumers with a cheaper animal protein (~0.01USD/g) than other foodstuffs such as meat and milk. This might explain why world egg production has dramatically increased over the last 20 years (+78%) to reach 1140 billion eggs (61 million tons; FAO (2010) *Statistical Year Book*, Food and Agriculture Organisation of the United Nations, Rome, Italy. www.fao.org.). Most of this growth (70%) has taken place in Asian countries (China and India), which now represents 59% of world egg production. In contrast, in Africa, which is one of the world's second largest populated continents, egg production has increased by just 29% (2.2% per annum) and egg consumption is still much lower in these countries than the world average (36 eggs per capita vs. 145 eggs per capita in 2007). In the EU, egg production has been stable for the last 10 years possibly as a consequence of an increased awareness in consumer expectations in terms of animal welfare and the pending implementation of the EU directive banning conventional cages in favour of more welfare friendly systems. This ban will mark the beginning of a new era in the history of egg production which will require the application of new and existing technological advances to ensure that

egg quality is optimised and consumer safety remains assured. In the light of these changes the publication of this book is particularly timely.

In the first part of this volume (chapters 1–5), the socio-economical changes in egg production are discussed in the context of the current systems of production found throughout the world with a special focus on Europe, China and Africa. A description of the consumers' perception of egg quality and a review of changes in egg production systems which will arise from the implementation of the EU directive banning conventional cages are also provided.

In the second part (chapters 6–10), classical information on egg formation and composition is provided along with innovation in this area with a focus on novel measurements of egg quality. The advances in our knowledge which have resulted from the application of new molecular techniques including proteomic and transcriptomic approaches are subsequently presented. It is because of these molecular techniques that the number of identified proteins in eggs for example has increased by 10-fold!! The accurate evaluation of egg quality is crucial in the identification of risky eggs. The final chapter in this section looks at the techniques which are now available to evaluate both the internal quality and eggshell quality including fast physical non-invasive methods suited to online application.

Part III (chapters 11–18) analyses the origins of changes in egg quality and describes a number of ways in which persistency in lay and optimal egg quality characteristics can be maintained. The role played by genetic selection, nutrition, hen physiology, the laying environment and layer management techniques are each explored in terms of their effects for example on egg weight, albumen/yolk proportion, egg composition or eggshell integrity. Some of the information provided in this section is admittedly not new but where ever possible we have tried to show that the concepts remain valid in our modern laying strains by including more recent data. Information relating to the level of egg contamination in each of the main systems of management is then discussed since the hygienic quality of eggs is crucial in terms of consumer safety. A more detailed account of the microbiology of eggs, however, is provided in Volume 2. This section concludes with a review of the effects of hen pathology and parasitism on egg quality and a rather innovative review on the health risks for people working in egg production units and methods of control.

The final part (chapters 19–23) attempts to address consumer and public concerns about sustainability and bird welfare by first introducing the concept of life-cycle analysis and then focusing on organic and free-range system, the popularity of which is growing in highly developed countries. The peculiarities associated with duck egg production which is important in Asia along with some of the less common types of egg production from species such as quail, pheasant, geese or turkey are then presented. Egg processing is a major activity in developed countries. No book on the subject of eggs would be complete without a detailed description of the main processes for

producing and stabilising liquid and dried egg products, their functionality and their uses. These topics are therefore covered in the last chapter of this volume.

This book is intended to provide a comprehensive description of the myriad of information that exists in the scientific literature on egg and egg quality. To achieve this, the editors have brought together many renowned international experts who have willingly contributed to this collective work which is dedicated to the egg community.

The editors would like to express their sincere thanks to each and every one of these experts who contributed to chapters in this book as without their input the compilation of this book would not have been possible. The editors would also like to thank the large number of individuals from within the scientific egg community who willingly gave up their time to review and improve the quality of the chapters presented in each volume. Finally Woodhead Publishing is also gratefully acknowledged for their direction and help in the completion of this book.

Yves Nys
Maureen Bain
Filip Van Immerseel

1

Egg and egg product production and consumption in Europe and the rest of the world

P. Magdelaine, ITAVI, France

Abstract: World egg production and consumption have been increasing for the last ten years whereas egg production in the EU has remained stable in volume. EU production systems, however, have evolved dramatically in relation to consumer expectations regarding animal welfare and the variety of products and also due to the implementation of the EU welfare regulation. The situation remains contrasting within the EU. European egg consumption amounts to 240 eggs per person per year, with strong variations among countries, and a rise in the share of egg products in global consumption. Various steering factors will determine the future of the European egg industry, such as European regulation (animal welfare, environment, sanitary requirements), feedstuff price volatility, changes in consumers' preferences and finally the conclusions of World Trade Organization (WTO) negotiations, which could lead to an opening of the European market to products (egg powder for example) from emerging countries.

Key words: egg production, market, competitiveness, consumption.

1.1 Introduction

World egg production has shown strong development for the last 20 years and has, along with poultry meat production, registered the most important growth in the coverage needs of protein for the world population. This chapter will begin by discussing world egg production before presenting its dynamics for past 20 years, with some particular analysis of the main producing countries (the US and China). Trade changes such as trends in global consumption

for table eggs and egg products will be analysed. The chapter will then go on to present an overview of the European egg industry, focusing on the main producing countries and on the European regulation concerning animal welfare and its impact on production and market segmentations within the European Union. The diversity of the national situations will be analysed in relation to specific national rules and contexts.

The chapter will conclude by presenting the international production costs of selected countries. It will also present their future prospects in the global and the European markets in relation to different scenarios concerning international and European policies and regulations, global and European growth and demand in egg and egg products.

1.2 Worldwide overview

1.2.1 World production

According to the FAO, world egg production reached 60.7 Mt in 2008 (i.e. 1140 billion eggs; Table 1.1). China is by far the largest producer with 22.7 Mt, i.e. 37% of the world production, followed by the EU-27 and the US. World egg production rose on average by 2.4% per year during the ten last years, compared with a 3.5% growth during the previous decade, 1987–1997. Most of the world's growth originates from traditional Asian producing countries, which represent 59% of world production and are responsible for 70% of the world growth during the last ten years.

China is the main egg-producing country in the world and has shown strong development over the past 20 years, although production has slowed somewhat in the last few years. Ten Chinese provinces produced 80% of the national egg production in 2006 (vs. 70% 20 years ago). The Shandong

Table 1.1 World egg production (tonnes) (source: FAO March 2010)

	1988	1998	2008	Average annual change 1988–1998	Average annual change 1998–2008
China	5 738 105	17 531 550	22 749 200	11.8%	2.6%
EU-27	6 718 737	6 716 421	6 539 986	0.0%	–0.3%
US	4 069 000	4 731 000	5 338 700	1.5%	1.2%
India	1 044 000	1 621 000	2 740 000	4.5%	5.4%
Japan	2 400 061	2 536 035	2 554 000	0.6%	0.1%
Mexico	1 090 164	1 461 153	2 337 215	3.0%	4.8%
Russia	–	1 827 930	2 118 500	–	1.5%
Brazil	1 178 359	1 389 539	1 825 000	1.7%	2.8%
Other countries	11 731 051	10 274 865	14 475 790	–	–
Total	**33 969 477**	**48 089 493**	**60 678 391**	**3.5%**	**2.4%**

and Hebei provinces represent a third of the Chinese production. Apart from a few areas which have large industrialized farms, Chinese farms are often little family production units, with the majority of their output going to family or local markets. A billion farms keep less than 200 hens each and produce only 10% of the Chinese production, whereas a few big farms keep (or integrate) more than a million billion hens each. Less than 5% of Chinese egg production was processed towards egg products in 2002. After a strong rise, the avian flu epidemic has slowed down the development of egg consumption since 2005–2006. Chinese egg consumption reached 333 eggs per capita in 2008 (source IEC, 2010).

The **United States of America** ranks in third place behind China and the EU-27. For the last ten years, US production has been more dynamic than its European counterpart (+1.2% a year, vs. stability in the EU). American farm sizes are also quite different from those in Europe, and very concentrated: in 2008, 255 farms kept 95% of the total 284 millions hens. Six major companies kept one-third of total livestock. According to Hans Windhorst, (2010a) the composition of the ten leading states in egg production has not changed very much for the last ten years, but the spatial pattern has changed a great deal. In 2008, Iowa ranked first with 16% of the US production, followed by Ohio (8%), Indiana (7.2%) and Pennsylvania (6.9%). Egg consumption was very high in the 1950s, around 380–400 eggs per capita but there followed a long decline until consumption reached 235 eggs per capita in 1995. For the last ten years, however, consumption has again increased and reached 248 eggs in 2008 (IEC, 2010).

1.2.2 World trade

According to FAO data, international trade reached 1.4 Mt for shell eggs, 55 000 t for egg powder and 218 000 t for liquid egg products in 2007, i.e. approximately 1.94 Mt of shell egg equivalent, intra-EU trade included. This accounts for only 3% of the global production. Nevertheless, international trade has increased during the last ten years by about 5% per year (6% per year for egg powder). Most of the trade for shell eggs and liquid egg products is within the EU. Three European countries rank in the top five exporting countries. The main shell egg exporting countries were, in 2008, the Netherlands (24% of the global trade), China (8%) and Spain (7%), followed by the US and Germany. The main importing countries were Germany (21%), followed by the Netherlands and France (12% each). The main exporting countries for liquid eggs trade were the Netherlands (40%) and France (13%).

The egg powder trade, after a strong development, has been stable for the past three years. The EU (12 400 t exported in 2007) and the US (11 300 t) are the leading exporters in this market, followed by India (7 700 t).

1.2.3 Consumption

The average consumption level in the world was assessed by the FAO to be 9.1 kg per person in 2005, which is to say about 145 eggs (Fig. 1.1). Strong variations exist according to different countries, however. For example, more than 300 eggs per capita were consumed in Japan and Mexico, 230–240 eggs in the EU and the US, and fewer than 100 eggs in most African countries and South East Asia. In developing countries, demand and supply should continue to grow more rapidly than in developed countries, due to a strong demographic growth in the former and to the already high consumption levels in most developed countries (Windhorst, 2008).

According to the FAO, eggs (together with poultry meat) have registered the most important growth in the coverage of protein needs for the world population in the past 20 years (Table 1.2). The high level of egg consumption in Asia can be partly explained by a low level of milk consumption. Indeed, although, on average, egg protein supply/milk protein supply ratios are around 0.6 for the world, this figure varies greatly from more than 2 in most Asian countries (5 in Korea, 3 in Thailand, 2.7 in China, 1.7 in Japan) to less than

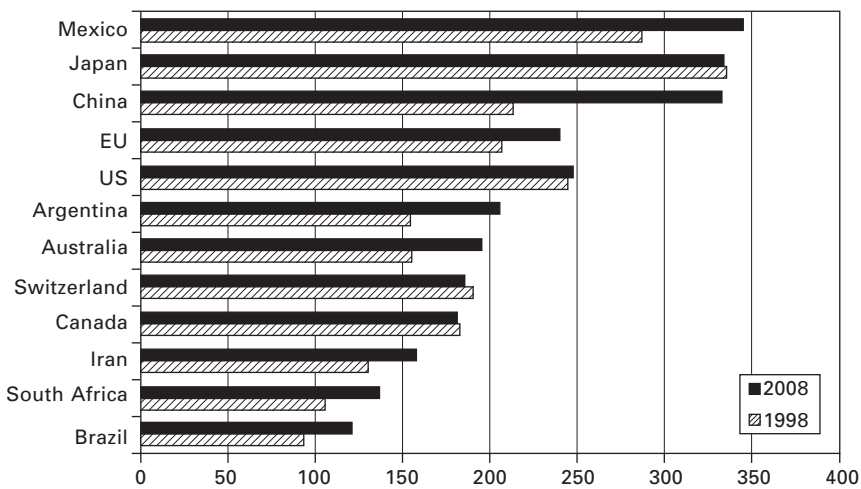

Fig. 1.1 Egg consumption levels in selected countries (egg/head 1998–2008) (source: IEC).

Table 1.2 Protein supply quantity (g/capita/day) (g) (source: FAO March 2010)

	1985	1995	2005
Bovine Meat	3.98	3.74	3.59
Eggs	**1.84**	**2.23**	**2.53**
Milk, whole	4.13	4.16	4.36
Pig meat	3.41	4.05	4.4
Poultry meat	2.28	3.3	4.33

1 in Europe and America (1.1 in Canada, 0.9 in France, 0.5 in Germany and 0.4 in the US).

1.3 European overview

1.3.1 Production

EU-27 egg production has been quite stable for the last ten years: it was up to 6.44 Mt in 2008 and was approximately 6.25 Mt in 2009 (this is the equivalent of a little more than 102 billion eggs), with a decrease of 3% compared with 2008 (Table 1.3). The EU produces about 1.5 Mt of egg products in equivalent liquid, i.e. a little less than a quarter of European egg production. The European self-sufficiency level reached 102.3% in 2008. Egg and egg product exports reached 191 000 t shell egg equivalent in 2008, of which one-third were egg products. Import levels remain very low (24 400 t shell egg equivalent in 2008).

1.3.2 European production systems diversification

For over ten years, production systems within the EU have shown strong diversification, with a switch from cage to alternative housing systems, due firstly to new consumer expectations concerning animal welfare, and secondly to the European welfare regulation implementation (EC/99/74 Directive), which, from 1st January 2012, will no longer allow the use of traditional cages.

Table 1.3 Major European egg producers (source: ITAVI from SSP and European Commission)

	Production 2009 (billion eggs)*	Average annual change 2000–2009 (%)	Change estimation 2009/2008 (%)
France	13.8	– 0.7	– 2.3
Italy	11.6	– 1.5	– 0.8
Spain	11.4	– 0.9	– 2.0
Germany	9.9	– 3.8	– 17.1
United Kingdom	9.5	+ 0.2	+ 0.2
The Netherlands	9.5	– 0.4	+ 0.2
Poland	8.8	+ 2.6	stable
EU-15	77.3	– 1.3	– 3.5
10 NMS^{+}	18.4	+ 2.6	– 1.9
EU-25	95.7	– 0.7	– 3.2
Romania	5.3	n.a.	stable
Bulgaria	1.4	n.a.	stable
EU-27	102.5	n.a.	– 3.0

*16.4 eggs per kilo
$^{+}$NMS is new member states 2004

1.3.3 Some housing systems definitions according to the EU regulation

- *Enriched cages*: Cage area per hen should be at least 750 cm^2. Cages should be enriched with a nest and litter so that pecking and scratching are possible, as well as appropriate perches.
- *Barn production*: Hens can be kept on the floor or in multi-tier systems. The stocking density must not exceed nine laying hens per m^2 of usable area. If systems of housing hens are used where the laying hens can move freely between different levels, there should not be more than four levels.
- *Free-range production*: The requirements are the same in the building as for barn production but hens also have continuous daytime access to an open-air area with a maximum stocking density of one hen per 4 m^2.
- *Organic production*: Hens are kept in a free-range area and specific rules should be followed regarding using organic feed and limiting the use of veterinary treatments.

Marketing standards have been defined for the European market from 2001, and since 2004, egg farming methods have to be mentioned on the eggs with a code shown clearly on the boxes. In 2008, 125 million laying hens were kept in alternative systems within the EU-27, which is approximately 32% of all European laying hens (source European Commission n.d; Table 1.4).

Within the EU, regulatory backgrounds are quite different which explains the diversity of situation concerning the different housing methods. Currently, three countries impose further requirements on the housing of laying hens than the standard EU requirements. In Sweden, a ban was introduced 20 years ago against keeping hens in cages. The law was later amended to allow furnished cages. In Germany and the Netherlands, regulations ban cage systems (even furnished cages), and allow so-called colony systems which are more demanding in terms of available space per hen: keeping hens in small groups in higher (60 cms) and larger cages in which space per hen is between 800 and 900 cm^2. Austria also has additional regulations above

Table 1.4 Share of alternative production systems within the EU in 2008 (ITAVI from national sources and European Commission)

	Cage (%)	Free range (%)	Barn (%)	Organic (%)	Alternative systems (%)
1996 (EU-15)	92	4	4	ND	8
2000 (EU-15)	89	6	5	ND	11
2008 (EU-27)	68	17	13	2	32
France	81	12	3	4	19
Spain	97	1	2	–	3
Germany	60	13	21	5	40
The Netherlands	45	12	41	2	55
United Kingdom	48	36	8	5	52
Italy	80	1	16	2	20

the EU requirements. The diversity of national demands also explains the diversity of national supplies and housing systems.

1.3.4 Focus on the main European producing countries

France remains the main egg producer within the EU with 13.6 billion eggs produced in 2009 (–2.3% compared to 2008), and around 43 million laying hens in production in 2008. After a growth in the 1990s, French production has been slightly decreasing since 2000, with some exceptions as in 2004–2005, when an overproduction induced a crisis and very low producer prices. For the last two years, demand has been going up and a new rise in production can be observed (Fig. 1.2).

In France, Brittany produces about 42% of the national egg production, but is responsible for only 30% of the packing station activity and less than 20% of the processing industry. Rhone Alpes and the Pays de la Loire rank second and third, with around 9.5% and 9% of the national production in 2008. A national survey, carried out by the French Ministry of Agriculture in 2009, showed that 1750 farms kept around 43 millions laying hens in 2008. Two types of farms can be identified: cage egg production with an average capacity of 54 000 hens per farm, and alternative systems with an average capacity between 7000 and 8000 hens. In 2008, approximately 80% of laying hens were still kept in cages and approximately 40% of these were kept in furnished cages.

Germany was the second highest European producing country in 2008, but German production dropped by 15–17% in 2009, in relation with a new regulation which banned both conventional cages and enriched cages from January 2010. Germany's self-sufficiency level was only 67% in 2008

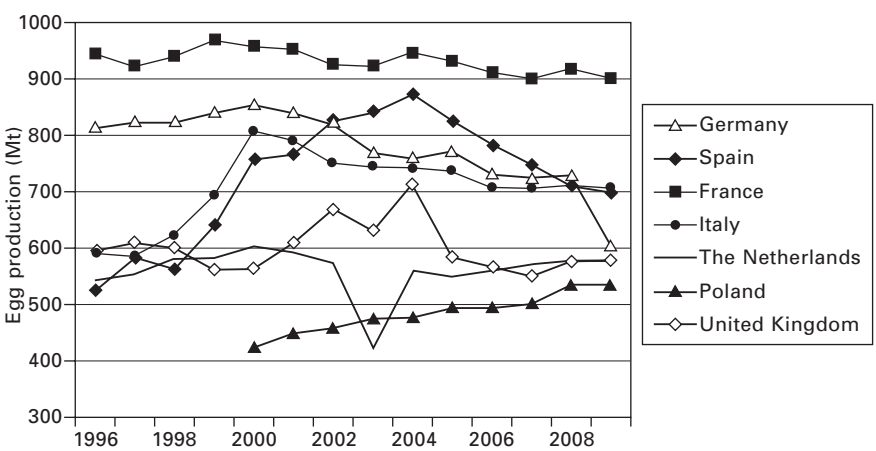

Fig. 1.2 Development of egg production in European leading egg producing countries (source: European Commission).

and approximately only 57% in 2009, in relation with the drop in local production (Table 1.5). In December 2008, 60.4% of hens were still kept in cages (Windhorst, 2010b) of which about 4% were colony systems (German Kleingruppenhaltungen, which are a form of enriched cage holding up to 60 hens per compartment), 12.8% were kept in free range systems, 21.4% in barn systems and 5.4% were kept in organic production systems. During 2010, most of the laying hens kept in cages were transferred to barn systems (Windhorst, 2010b).

The **Spanish** egg industry showed significant development from the end of the 1990s until 2004, but more recently has shown a drop in egg production. Until 2004 the sharp increase was initiated by a fast growing demand and high exports (Windhorst, 2010b); from 2004 on, production collapsed because of a drastic decrease in the per capita consumption of shell eggs and falling exports.

In 2000, Spain ranked as number 5 among the leading egg producing countries in the EU; in 2008, it shared the number three ranking with Italy, and again in 2009 when both ranked in second place, ahead of Germany. Spain is one of the member countries with the highest share of layers still kept in conventional cages. At the present time, this situation may be an economic advantage because of lower production costs, but in the long run, very high investment will be necessary to fulfil EU directive 99/74/EC. The concentration of the Spanish egg industry is high, with a million farms producing cage eggs (the average size is approximately 47 000 laying hens) and approximately 500 small production units in alternative systems producing less than 3% of the national egg production. The autonomous regions Castilla-La Mancha and Castilla-Leon account for approximately 50% of Spanish laying hens, followed by the regions of Valencia and Catalunia (Windhorst, 2010b).

Italy was the third largest European egg-producing country along with Spain in 2008. Its main production regions are in the north of the country, near Venice and Milan. Production is very integrated by leading groups, from upstream activities to the distribution. The share in alternative systems has

Table 1.5 Self-sufficiency level within the EU (ITAVI from national sources, ZMP and MEG)

	1998	2008
Germany	73	67
France	101	95
Italy	103	105
The Netherlands	226	230
United Kingdom	98	82
Spain	103	110
EU-15	103	
EU-27		101

grown rapidly over the last two years and now accounts for approximately 20% of global production.

1.3.5 Consumption

Within the EU-27, egg and egg product average consumption reached 6.4 Mt in 2008, i.e. around 14.7 kg per capita or 240 eggs per capita and per year, with strong variations between countries. Some countries, such as Belgium, Spain, the UK and the Netherlands have a consumption level of approximately 180 eggs per capita, whereas Hungary and Denmark's levels reached almost 300 eggs per capita (Table 1.6).

Two main trends are common to all countries. On the one hand, there is an increasing share of egg products in the total egg and egg product consumption (from 20% in Sweden, the UK and the Netherlands, to 40 or 50% in Denmark and Belgium), and on the other hand, there is an increasing share of alternative eggs in table egg consumption. This trend is more marked in home consumption than in restaurant or catering. Thus, segmentation of the table egg market and the share of different types of eggs vary greatly according to the national markets (Fig. 1.3).

1.4 Conclusion and future trends

Development perspectives remain favourable in the world egg market, driven by a strong demand from emerging countries such as China. New producers, such as Argentina, India and Brazil, have begun to appear on the international scene.

The European situation is more stable, with production even declining slightly during the last few years. However, the European market is heading towards further segmentation, both in favour of egg products (25% of the European production) and of alternative systems, which represent nearly 30%

Table 1.6 Egg consumption in different EU Member States (ITAVI from IEC and national statistics)

	Consumption 2008 (eggs and egg products) (eggs/capita)	Egg product share in total consumption (%)
France	248	31
Italy	224	33
Belgium	184	50
Germany	212	ND
Spain	189	ND
The Netherlands	182	22
United Kingdom	183	20
Denmark	300	42
Sweden	197	19

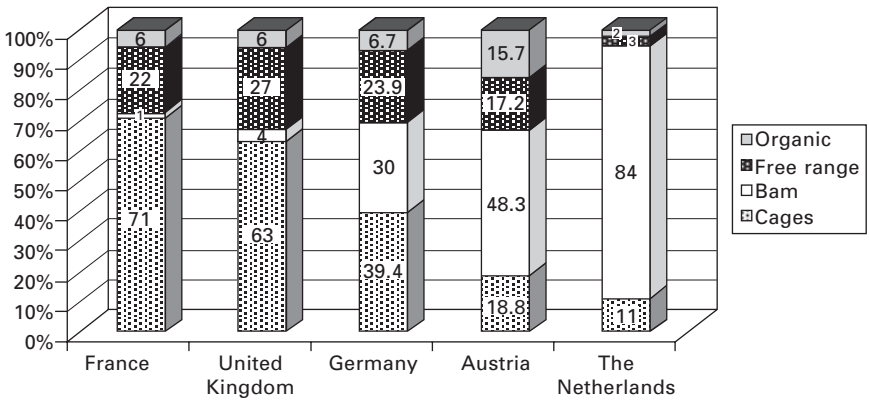

Fig. 1.3 Egg shell markets segmentation within the EU in 2007 (ITAVI from Nielsen, BEIC, Gfk).

of the European table egg demand. These changes remain much contrasted within the EU member countries.

The European egg industry is at a very important stage of its history. In the future, European welfare regulations could have a negative impact on the European egg industry's competitiveness. Currently, there is little trading with countries outside the EU in shell eggs and liquid egg products. But the situation is quite different for powdered eggs because of the long lifespan qualities of this product and the relatively low transport costs. The extra costs due only to national and European regulations have been assessed by Peter van Horne (LEI, 2007): egg production costs (at the farm level) were, in 2006, 32–33% lower in the US or in Brazil than in the EU. US and Brazil competitiveness can be explained by a low cost of inputs (feed and labour), but also by lower welfare, environmental and food safety regulatory requirements. These regulations could explain 20–25% of the gap between EU and US or Brazil egg production costs. Figure 1.4 shows the impact of welfare criteria on production costs.

The European market is at present protected by import duties which, together with transport costs, compensate for the difference in production cost (Van Horne, 2007). The European purchase price of eggs has increased due to costs such as animal welfare measures but, at the same time, the EU intends to reduce the import duties in the context of the WTO or bilateral negotiations. In this situation it is economically more attractive for the food industry to replace European liquid egg products with powdered egg from countries outside the EU. One consequence of this would be that egg products will be purchased from third world countries where the animal welfare standard is markedly lower than in the EU. A recent study showed that in Argentina laying hens are kept in cages measuring 371 cm^2 on average (van Horne, 2010).

The regulatory, economic and food safety context is changing significantly,

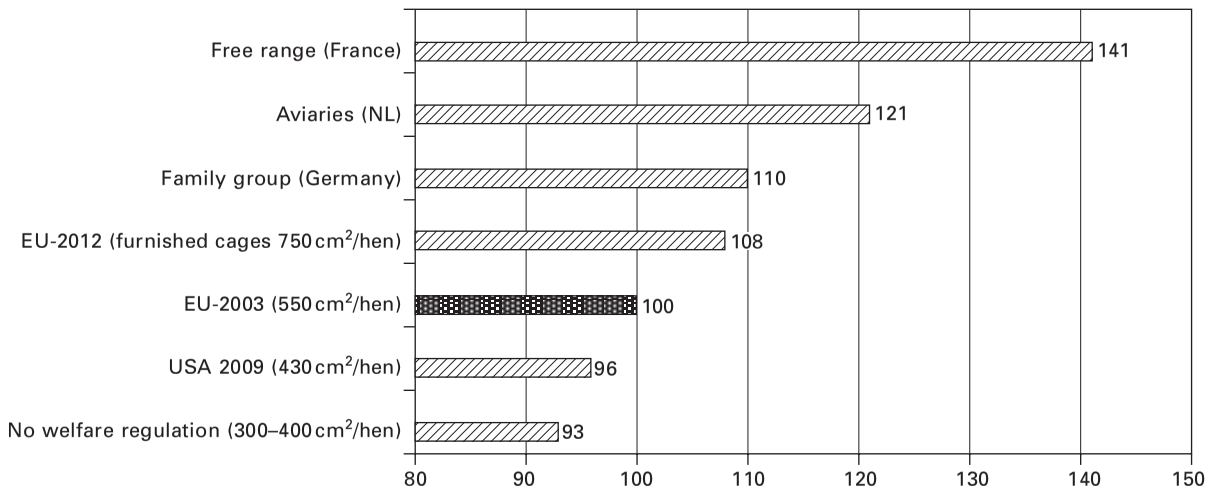

Fig. 1.4 Cost of housing systems related to animal welfare constraints in 2007 (source: LEI and ITAVI for free range).

within the EU and at the international level. The causes of these changes, which will determine the possible scenarios for the future, are the following:

- The European regulation about animal welfare. The European Commission has recently confirmed that the ban on traditional cage housing systems will be effective in 2012. This should contribute to emphasize the diversification of production systems and to the development of alternative systems.
- Increasing requirements regarding environmental conditions, For example, the Dutch regulations regarding a reduction of ammonia emissions, and of food safety, e.g. Salmonella prevention, which could further increase production costs. Again, great differences can be observed between member countries on this.
- High volatility in the price of raw materials (for animal feed) and incidences of changes to production costs.
- Trends in purchase behaviours and consumption habits, which remain uncertain. On the one hand, the economic crisis reinforces the consumers' price sensitivity, which is in favour of egg consumption: first, because egg protein remains the cheaper option, and second because in a difficult economy, consumers return to cooking, using basic products like eggs. On the other hand, more and more consumers are aware of animal welfare issues, housing methods, and the way animals are fed, as shown by the increase in the demand for organic products. Consumers' expectations are taken up (and even emphasized) by supermarkets, catering companies and other major food industries.
- A sanitary background demonstrated by the endemic avian flu situation in some parts of the world.
- The WTO (World Trade Organization) negotiations which could, if an agreement were concluded, lead to a reduction of import duties and to an opening of the European market to more competitive third world countries' products, particularly egg products such as egg powder.

The European egg industry may therefore have to go to great lengths to adapt production tools in light of the 2012 changes to egg production. Egg producers have to make investment decisions regarding the type of hen farming system they will use (furnished cages, colony systems or aviaries), which they may then be involved with for the next 20 years, yet technical models have yet to be tested or improved, and the future prospects of the market are still uncertain. These choices will have to take into account during the development of non-cage egg production, which should keep pace with changes in demand in order to avoid over supply in alternative eggs. In conclusion, future production systems should both comply with animal welfare regulations, respect and provide sanitary guarantees and be competitively priced for supplying the catering and food industries, which currently represent 40–50% of total egg consumption, and could reach more than 50% by 2020. This competitiveness requirement will be increased by

globalization and by the opening of the European market to supply from third world countries which will not support equivalent constraints. Indeed, if world trade liberalization is expected for the next decade, the integration of animal welfare standards in international negotiations may be far more difficult.

To help French stakeholders design strategies for the future, a technical institute (ITAVI) and a research institute (INRA) conducted a scenario-building exercise based on interviews with stakeholders and an 18-month-long series of discussions by a panel of poultry experts. Four future scenarios were developed for the whole poultry meat and egg sector, taking into account uncertainties such as potential shifts in European policies and regulations, consumer attitudes and stakeholder strategies. The outlook was favourable for the French egg sector, and stated that the forecasted decline in meat consumption and egg assets in terms of nutrition, low environmental impact and low price will have a positive effect on egg consumption. All the scenarios emphasize the importance of multidisciplinary research to characterize the quality and sustainability of poultry production, which in turn can improve its competitiveness.

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2

Social economic aspects of egg production in China

N. Yang, China Agricultural University, China

Abstract: China is the world's leading country in egg production. This chapter describes the historical development of the Chinese egg production, and introduces the current status of the Chinese egg industry with regard to geological distribution, structure of farms, breeding, economic parameters, feeds, market, waterfowl eggs, egg processing and egg trade. Total egg production was 27.41 million tons in 2009, with the average egg consumption close to 20 kg per capita. Sufficient and constant supply of feed grains will be the greatest constraint for the future development of Chinese egg production. In addition, pollution and biosecurity will also be major limiting factors.

Key words: China, egg production, historical development, production patterns, economic parameters.

2.1 Introduction

China has a long history of over 5000 years of poultry production, but before the 1980s, the poultry production in China was traditional, where eggs were produced from small household backyard farming. Since 1979 China has undergone a thorough social and economic reform and adopted the open-up policy. This country, with its enormous population, vast lands and rich resources, has witnessed its golden age in the egg industry, and has become the world's largest egg producer and consumer, with more than 40% of the world market share. Table eggs, traditionally regarded as one of the highest nutritive foods, are the cheapest source of animal proteins. It

is estimated in 2010 that each gram of protein from eggs costs only 0.066 CNY (~0.01USD), which is much lower than any other animal products including meats and milk. Table egg plays an important role in the daily diet of Chinese people.

Total egg production was 27.41 million tons in 2009, with average egg consumption per capita close to 20 kg. Chickens produce more than 80% of the total eggs, while ducks and quails are also important providers of table eggs. This has been a special feature of Chinese egg industry. Within hen eggs, brown-shell eggs are the majority with around 70% of total production and consumption, while white-shell eggs are not popular in China. Light brown eggs, sometimes called tinted eggs, produced mainly by hybrids of a brown-egg layer with a white-egg layer, have gained popularity of Chinese customers due to their similarity in eggshell colour to eggs from indigenous chicken breeds. Customers can also find blue-shell eggs in the markets.

2.2 Historical development of egg production in China

There is a long tradition of raising chickens and other poultry to produce eggs in China, but generally as by-products of rural life. Like many other countries of the world, backyard farming has existed for thousands of years. Urbanization during the past decades has created an increasing need for table eggs, which is a major stimulus for the egg industry. Driven by a huge market need and economic growth, China has experienced four stages of development in egg production (Fig. 2.1).

2.2.1 The natural growth stage before 1978

In this period China was in its traditional economic state, and egg production was only a small and integrated part of the agriculture. Eggs were produced

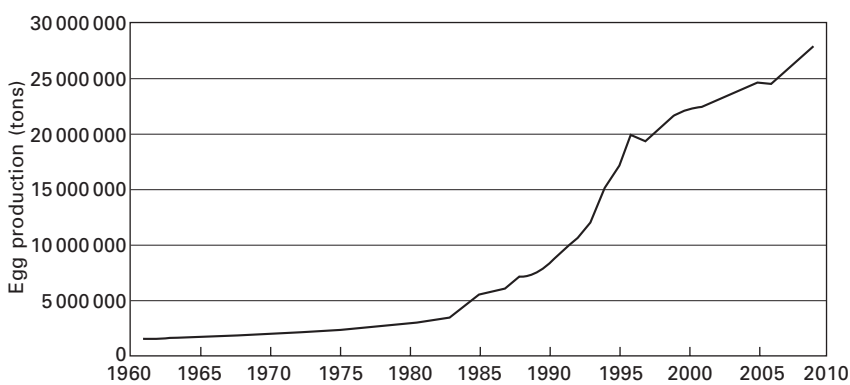

Fig. 2.1 Egg production over the past 50 years in China (data from www.fao.org).

in the backyards of small household farmers. The chicks were reproduced from indigenous breeds with poor performance. A family normally had a small flock of hens. No specialized feeds were formulated and provided to the poultry. For a long period of time, total egg production in the country was less than 3 million tons whereas egg consumption per capita was less than 3 kg per year.

2.2.2 The initial period of fast development from 1979 to 1990

In 1979, China started a thorough social and economic reform and adopted the opening-up policy. Enjoying the benefits from the reform, people's income increased significantly and higher demand arose for eggs and other animal products. New layer farms with better facilities were constructed and elite chicken breeds were imported from Europe and the USA. The annual growth rate of egg production in China was 7.64% in this stage.

The most significant change in this period was the high input of the Chinese Government in investment, education, technology transfer and economic policies related to poultry production. Egg production was an important part of the famous 'shopping basket' projects, which aimed to provide urban people with affordable vegetables, meat and eggs. A large number of layer and breeder farms with modern facilities were built in the suburbs of large cities such as Beijing and Shanghai. A new generation of college graduates majoring in animal science and veterinary medicine joined these farms to improve the production management. Hybrid chickens, advanced feed technologies and vaccines were introduced into China. Grains with government subsidies were provided to these state-run farms so that they could produce table eggs at a lower cost, which were then sold to urban citizens at a reasonable price. While poultry production in the vast rural lands remained in a traditional pattern, the interest brought by the modern layer farms provided a great boost for the formation of the egg industry in China. During this stage, the egg industry began to transform from small, traditional backyard farming to a market-oriented production system. In the late 1980s, China overtook the USA to become the top egg producer in the world.

2.2.3 The explosive development period from 1991 to 2000

This decade was the most dynamic period of the egg production in China. Egg production increased from 9.46 million tons in 1991 to 22.21 million tons in 2000 with an annual growth rate of 8.02%. The most significant change in this period was the widespread involvement of small farmers in commercial egg production. Profits from egg production attracted farmers to get into the egg business. Efficient technology transfer involving government extension agencies and private companies in feeds, medicine, vaccines and breeder production helped small household farmers to learn the know-how

of modern egg production. Farmers took advantage of the lower cost of feed grains, labour and land to develop their own business in egg production. Millions of farmers across the country participated in the commercial egg production. A few concentrated areas of egg production were formed in northern China, located in the corn-producing regions, and were close to the large cities and the main transportation lines. At the same time, many state-run chicken farms lost subsidies for grain feeds and met difficulties in market competition with rural producers. Urbanization of China has also driven most farms in the city suburbs out of the egg business due to concerns of environment protection and the high production costs. Some farms have transferred into private companies and upgraded their business into breeding or high-end brand products, and have even become among the most powerful companies in Chinese egg industry.

2.2.4 A steady stage since 2001

Strong economic growth, increased supply and favourable prices have led to an increase of egg consumption in China. In the new century, the supply and demand of eggs have reached a state of balance. Eggs are turned from expensive food to ordinary food that people can afford, and that are a major component of the average Chinese citizen's diet. People across all ranges and regions of the country either consume them as table eggs or use them as ingredients and food additives. As egg production increases, the market competition has become more severe. Outbreaks of infectious diseases, including Avian influenza, caused serious damage to the egg industry. More importantly, customers are more concerned on the quality and safety of the egg products. Therefore, the development of Chinese egg industry is changing from quantity to quality of eggs.

2.3 Current status of the Chinese egg industry

2.3.1 Geological distribution

Poultry egg producers in China are mainly located in the north coastal region of the country, along with the grain-concentrated areas. The six largest egg-producing provinces are Hebei, Shandong, Henan, Liaoning, Jiangsu and Sichuan, accounting for nearly 65% of the total production of the country (see Fig. 2.2). During the 1990s egg production concentrated in grain feed production areas of northern China, and the products have been transported across the country. In recent years, however, there has been a tendency for egg production to spread nationwide owing to the need to minimize disease risk, reduce transportation cost and market fresh eggs. The egg industry in many provinces in southern China, with its large population and strong food industry, has undergone rapid development.

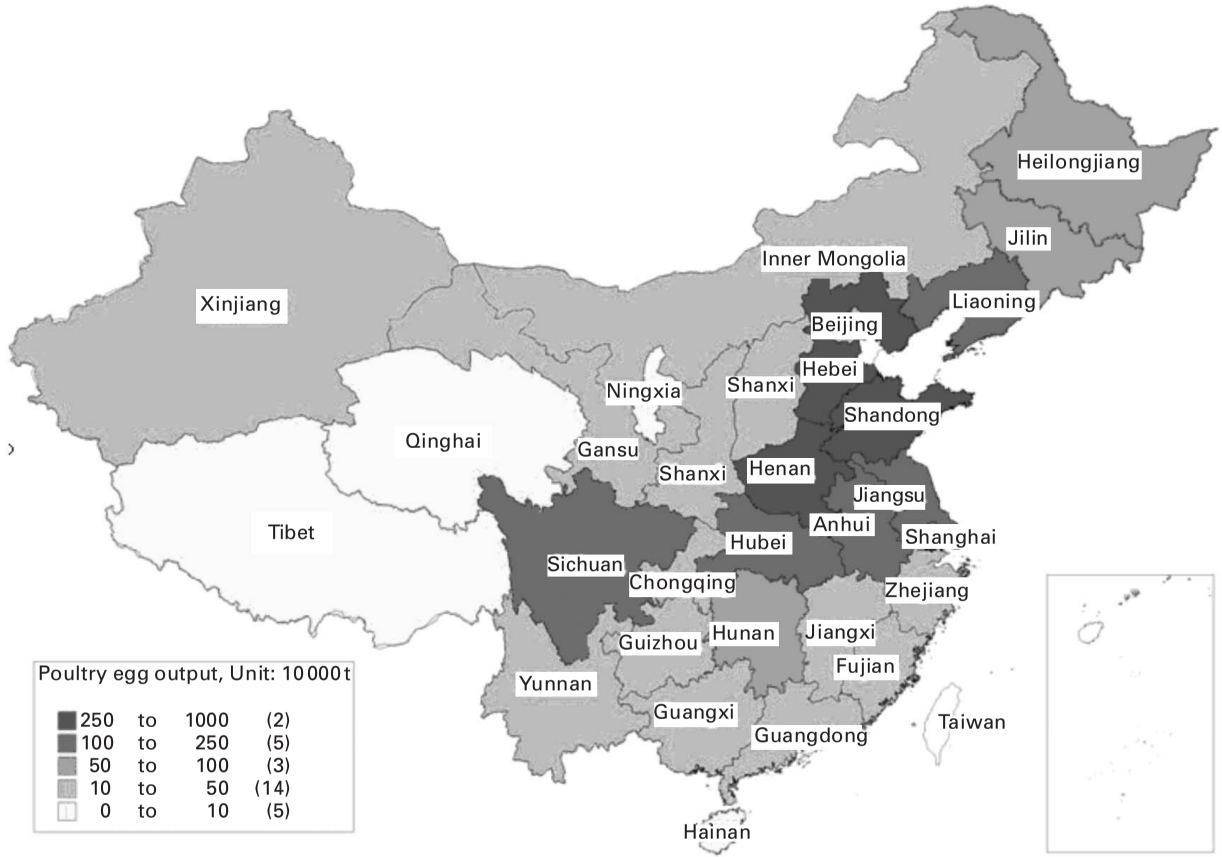

Fig. 2.2 Poultry egg output by provinces in 2008. (Source: Ministry of Agriculture (2009) *Yearbook 2008 for China Animal Husbandry Statistics*.) *Note*: Data for Taiwan, Hongkong and Macao are not shown.

2.3.2 Structure of farms

China has been developing very fast and has diversified development of the egg industry. Many different methods are used for keeping poultry. These production systems range from small, village-level poultry flocks to modern integrated intensive operations in which large companies control all aspects of the production and marketing chain. In between is a range of production systems, from individual farms practising industrial-type production to hundreds of caged laying hens in simple shelters in the same yard where farmers live. Table 2.1 shows the most recent data on the structure of chicken egg production in China. A total of 22.97 million tonnes of table eggs were produced from chickens in 2008. Among them over 40% were produced in small farms or households with flock sizes of fewer than 2000 hens. Although this percentage is decreasing, small farms still represent the majority of Chinese egg industry. In this category, many peasants keep indigenous chickens for egg production. Their laying performance is poorer than the highly selected commercial layers, but customers prefer to pay higher price for these products that are presumed to have a better taste. On the other hand, large companies are developing very fast with better priced and branded egg products. There are at least five layer farms with a capacity of over a million hens in China. DQY, GeGeDa and Shendan are the most famous brands for egg products in China.

As the farm scale goes up, the productivity is getting better (Table 2.1). For the smallest group, the poor performance may be due to less productive breeds and poor management. There is a trend for scaling-up in the villages, but the small flocks of production will remain for a long time before they are replaced. A large operation with better facilities and production management may be more efficient in egg production. More and more large layer farms are being built.

Table 2.1 Chicken farm scale and egg production in China (2008)

Farm size (chickens housed)	No. of farms	Chicken layers		Egg production		Average egg production (kg/hen)
		Total no. (million)	%	Total (in 1000 tons)	%	
<2000	n.a.	637.35	43.0	9402	40.9	14.8
2000–9999	236 993	511.51	34.6	8125	35.4	15.9
10 000–49 999	25 516	252.38	17.0	4144	18.1	16.4
50 000–99 999	1138	43.06	2.9	718	3.1	16.7
100 000–490 000	312	29.34	2.0	466	2.0	15.9
500 000 +	13	6.64	0.5	114	0.5	17.2
Total/average		1480.28	100	22 969	100	15.5

Source: Ministry of Agriculture (2009) *Yearbook 2008 for China Animal Husbandry Statistics*

2.3.3 Breeding

Modern breeding technologies based on genetics have developed many commercial lines with outstanding performance. Commercial hybrid laying hens can produce as many as 325 eggs per year. This is the result of a long structured and intensive selection for egg production by a handful breeding companies, which reach the world market through a multiplication and distribution network. China now imports hundreds of thousands of grandparent (GP) chicks every year for the multiplication pipeline, whereas native chicken breeding companies are distributing their own breeding products too. Consequently, the diversified need of commercial egg producers are met by these breeds suitable for specialized production conditions and markets in China. Dwarf layers CAU 3, developed by China Agricultural University, are welcomed by Chinese egg producers due to their excellent feed conversion rate as well as the suitable size and colour of the eggs laid by dwarf hens. It is estimated that 25 million parent stock chicks and 1.5 billion commercial chicks are produced each year in China. Among them, both imported and native breeds play important roles. Beijing Huadu Yukou Poultry Company produced over 100 million of commercial pullets in 2009, and has become the largest egg-type chick supplier in Asia.

2.3.4 Economic parameters

Qin *et al*. (2010) calculated the economic parameters of egg production in selected Chinese chicken farms from 2005 to 2008 (Table 2.2). The results indicated a steady improvement of annual egg production from 15.44 kg/hen in 2005 to 16.70 kg/hen in 2008, which has been mainly due to scaling-up of the chicken farms, application of new technology including better feed and breeds, and improved management. Their samples were from larger farms, which had better production performance than the national average shown in Table 2.1. The production cost increased over the period, mainly due to the higher cost of main feed ingredients: corn and soybean. This is a serious situation for Chinese egg industry, because China has to import a large amount of feed grain for the needs of the animal industry.

Table 2.2 Farm-level cost, income and profit for egg production in China (2005–2008)

Year	Egg production (kg/hen)	Production cost (CNY/hen)	Total income (CNY/hen)	Net profit (CNY/hen)
2005	15.44	82.20	89.58	7.37
2006	15.61	79.36	90.00	10.64
2007	16.51	100.51	115.53	15.02
2008	16.70	109.28	118.17	8.89

Note: The exchange rate between the Chinese currency CNY to Euro ranged from 11 in early 2005 to 9.5 by the end of 2008. The exchange rate was 9.2202 RMB to 1 Euro on 20 October 2010. Adapted from Qin *et al*. (2010).

The total income of egg production also increased over the period, reflecting the improvement of productivity and stable egg supply. Marginal profits were recorded in the period, demonstrating a relatively stable economic status for the egg industry. Net profit per hen varied from 7.37 CNY in 2005 to 15.02 CNY in 2007, which is not significant compared with those of feed mill and breeder producers. However, in China the total income of egg production includes sales of table eggs, spent hens and manure. Spent hens are referred to those having completed egg production, and used as food for human, especially for making chicken soups.

2.3.5 Feeds

Along with the development of intensive animal production, feed industry has become a powerful agro-business in China. Industrialized feed production increased from 1.1 million tons in 1980 to 137 million tons in 2008 at an average annual rate of 18.8%. Total consumption of feed grains reached 205 million tons in 2008 for the whole animal industry, which accounted for 40.82% of total grain production.

Compound feeds produced by specialized feed mills are commonly used in large layer farms. Small farmers prefer to mix their own layer feeds with premixes from feed companies plus locally produced corn. Feeds for pullets and hens are generally provided in the form of mash. Corn and soybean are two most important sources of feed ingredients for chickens,. Cottonseed and rapeseed cakes are also used as protein sources. Fishmeal is less used for commercial layers due to the concern for odours in eggs.

Corn production in China could meet the demand of animal industry for a long time, and corn used to be an export item of Chinese agro-business. In recent years, however, the exportation of corn has been reduced dramatically while import increased. A shortage of corn supply is predicted in the near future as the animal industry develops. China has suffered a shortage of protein feedstuffs for a long time. More than 60% of the soybeans, fishmeal and synthetic amino acids are dependent on imports from South America and the USA.

2.3.6 Market

Traditionally, table eggs are sold in the open markets throughout the country. Eggs are transported and marketed in crates or large boxes without labelling on the eggshell. It is therefore almost impossible to trace the source of these products. Customers in the cities generally purchase branded eggs in the supermarkets or convenience stores. Table eggs labelled with producer's brand and production date that are carefully washed, oiled, sorted and packaged in modern processing factories are generally welcomed by customers. It is estimated that at the moment less than 10% of table eggs are marketed in this way, but the proportion is increasing. This has created a substantial need

for egg processing facilities. A big problem for the Chinese egg industry is the lack of a cold chain system for egg storage and transportation, which may cause problems in maintaining the freshness of eggs and increase the danger of contamination.

In recent years, food production has changed from being producer-driven to consumer-driven. Consumers are increasingly concerned by health, environment, ethical issues, and demands for certified products such as a free-range or organic eggs have emerged. The need has been gradually realized by the producers, and the Green-Food eggs certificated by a national agency are popular and welcomed by the customers. Free-range eggs are popular too all over the country, and organic eggs can also be found in the supermarkets of large cities.

2.3.7 Waterfowl eggs

China has long been acknowledged as the kingdom of waterfowls with rich duck and geese resources. A few duck breeds, such as Shaoxin, Gaoyou and Jinding ducks, are famous for their high performance in egg production. Ducks produced 3.5 million tons of eggs in 2008, which was the highest in the world. Salted and other preserved duck eggs are common products in China. Duck eggs are also widely used for food industry, such as for making Moon Cake. Since the traditional processing methods are simple and the flavour of duck egg products is special, the processed duck eggs are now desirable foods for people in China and many other countries. This, in turn, has encouraged the development of Chinese duck production.

2.3.8 Egg processing

Although China is the largest egg production country, the egg processing industry lags far behind. Less than 10% of table eggs are washed and packaged, and only 0.26% of hen eggs are further processed as liquid or dry products. There are less than 500 major egg processing plants in China, and these companies have limited scale and products. However, processing of waterfowl eggs, mainly duck eggs, in traditional ways is very popular, and it is estimated that 45% of duck eggs are sold as processed products, such as salty eggs and the ‘thousand-year’ eggs.

With the development of new technology and changes in customer preferences, egg production and processing present new challenges and opportunities. Under this new situation, different kinds of eggs along with various forms of egg products should be explored to meet the market requirements. Examples are blue-shelled eggs produced by Chinese native chickens, and double-yolk duck eggs. Additionally, healthy eggs or medicinal eggs with a higher content of iodine, zinc, iron, selenium and certain vitamins have been developed to suit public tastes. Moreover, traditional products, such as preserved eggs, quail eggs, eggs pickled with grains or

in wine and dried minced egg, can be produced in a more efficient and safe way.

2.3.9 The egg trade: imports and exports

Although China is the top egg producer in the world, it has never been a major player in the world trade of eggs. Import volume has always been negligible. In 2001 egg exports of China were estimated at only 933 million eggs, which were roughly 56 000 tons, accounting for about 0.25% of the total output. Of this total a large volume was exported as processed products, of which the major portion was salted eggs for Hong Kong, the USA and Canada. Shell eggs, used by the food processing industry, were shipped to Japan and Hong Kong. Preserved eggs were mainly exported to Hong Kong and Singapore and also to the USA, Japan and Malaysia. In 2007, egg export amounts in total increased to 89 000 tons (including shell eggs), accounting for only 0.4% of the total egg outputs in China. The major markets for Chinese egg exports are Oman, Korea, Japan and its two special administration regions: Hong Kong and Macao.

2.4 Future trends

Egg production in China will remain as a huge and strong industry for the future development, and egg products will still be a popular and affordable food for Chinese people, but growth will be at a slower pace. It is estimated that the annual egg consumption in 2020 and 2030 will be 25.03 and 30.51 million tons, respectively. Sufficient and constant supply of feed grains will be the greatest constraint for the future development of Chinese egg production. In addition, pollution and biosecurity will also be major limiting factors. The future may witness a deceasing number of small household farmers, and more modern layer farms of moderate size. Customers will find more processed egg products in better quality. Technology development and better management will facilitate sustainable development of the Chinese egg industry.

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3

Egg production in Africa

H. M. Tukur, Usmanu Danfodiyo University, Nigeria

Abstract: Egg production in Africa is estimated at 2 367 000 tonnes per annum (representing only 3.7% of the global egg output). There is considerable variation among countries – from as high as 533 000 tonnes for Nigeria to as low as 1000 tonnes for countries such as Central African Republic, Comoros, Congo, Gambia, Guinea and Swaziland. This low level of productivity translates into low per capita egg consumption of 36 eggs/person/year, much lower than the world average of 145 eggs. The low performance of the poultry industry in Africa could be attributed to the inefficient scavenging management system that is predominant in most rural communities. Improvement of the performance of the industry could be achieved by adapting more efficient production systems.

Key words: egg production and consumption in Africa, egg production systems in Africa.

3.1 Introduction

Africa is the second largest continent in the world (after Asia), with a land area of 30.2 million km^2 (equivalent to 6 and 20.4% of the total surface and land area of the Earth respectively). With a population of about 1 billion people, it is also the second most populous continent. Poultry farming is an important aspect of the continent's agricultural activity, and plays a role in sustaining the livelihoods of a number of rural communities. Like in other continents, the domestic chicken (*Gallus domesticus*) is the most widely reared poultry specie in Africa, consisting of the specialized egg and meat

types as well as the dual purpose local species predominant in virtually all African villages.

3.2 Egg production

As at 2007, total egg production in Africa was estimated at 2 367 000 tonnes (representing 3.7% of the global egg output), with considerable variation among countries (FAO 2009). African countries can therefore be categorized into three groups on the basis of egg production (Fig. 3.1):

- *High egg producing countries.* The highest egg producing country in Africa is Nigeria, with a total output of 553 000 tonnes per annum; followed by South Africa (435 000), Egypt (240 000), Morocco (200 000), Algeria (185 000), Tunisia (85 000), Kenya (69 000) and Libya (60 000).

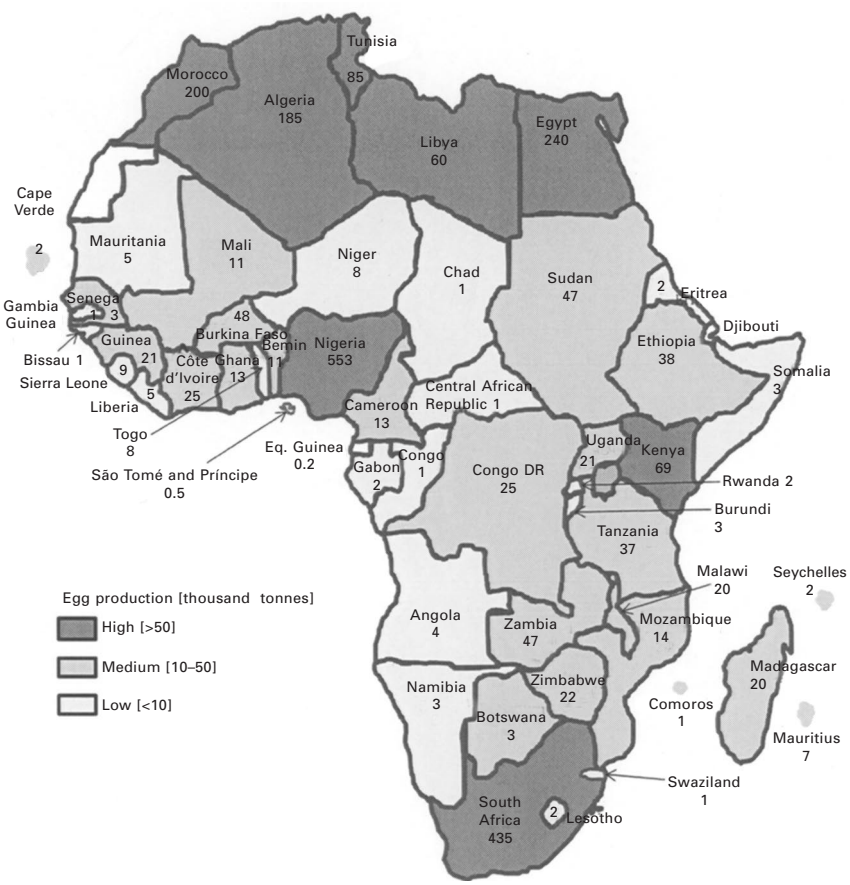

Fig. 3.1 Map of Africa showing egg production values.

These eight countries (15% of the number of countries in Africa), with a combined population of 396 million (representing 41% of the population of the continent), produce 1 827 000 tonnes, equivalent to 77% of the continental egg output.

- *Medium egg producing countries*: 17 countries (33% of the number of countries in the continent) belong to this category; with a total population of 389 million, also representing 41% of the population of the continent. These countries produce 453 000 tonnes per year, representing 19% of the egg production for the continent. Production levels vary from 11 000 tonnes for countries like Benin and Mali to 47 000 tonnes for Sudan and Zambia, with an average of 26 647 tonnes per country.
- *Low egg producing countries*: 25 countries (48% of the countries in Africa) are in this category; with a total population of 176 million, equivalent to 18% of the population of the continent. Total egg production for these countries is 87,000 tonnes per year, equivalent to only 4% of the total continental output. Here also, production varies from as low as 1000 tonnes for countries like Central African Republic, Comoros, Congo, Gambia, Guinea and Swaziland to 9000 tonnes for Sierra Leone, with an average of 3480 tonnes per country.

Egg production in Africa rose by 29% (from 1.7 to 2.4 million tonnes) from 1994 to 2007, representing an increase of 2.2% per annum (Fig. 3.2). This rate of growth is close to the world average of 2.4% during the last ten years. The rate of increase in egg production varies greatly among countries. Those with the highest increase are Swaziland (68%), Senegal (64%), Guinea (54%), Guinea Bissau (51%), Ghana (49%), São Tomé and Príncipe (47%), South Africa (41%), Benin (41%) and Algeria (40%). Some countries recorded decrease in egg production during the same period. They are: Eritrea (–130%), Congo DR (–19%), Burundi (–17%), and Mali (–13.1%). Countries in which the industry recorded low level of growth (i.e. less than 10% increase) are:

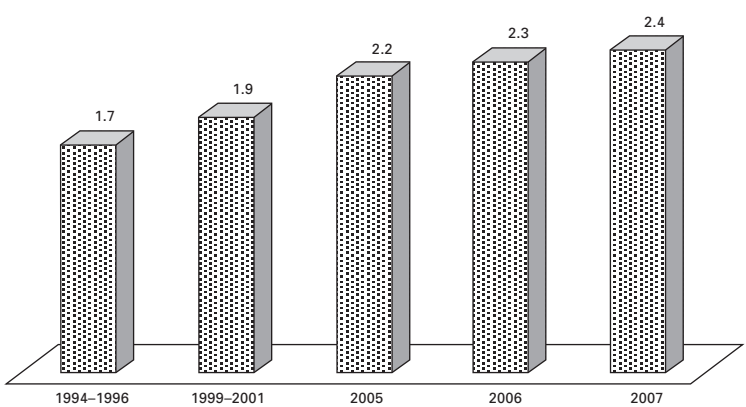

Fig. 3.2 Egg production in Africa between 1994 and 2007 (million tonnes) (source: FAO 2009).

Angola, Cameroon, Cape Verde, Central African Republic, Chad, Comoros, Congo, Equatorial Guinea, Malawi, Morocco, Niger, Somalia and Tanzania. The remaining countries witnessed moderate increase of 10 to 39% in egg production during the same period.

3.3 Egg consumption, marketing and trade

Most of the eggs produced in Africa are consumed locally – especially in urban centres. There is, however, some level of international egg trade. Even though the value of agricultural imports to Africa declined from 32.1% in 1994 to 18.5% in 2007, the value of food in agricultural imports remained relatively constant (average of 82%). However, share of eggs in food imports increased from 0.2% to 2.78% during the same period (Fig. 3.3).

In monetary terms, the value of egg imports into Africa rose from 34 million USD in 1994 to 97 million in 2007 (country average of 0.7 to 2.4 million USD) (Fig. 3.4), with considerable variation among countries. The highest egg importing countries are Angola, Botswana, Congo, Equatorial Guinea, Liberia, Libya, Mauritania, Sierra Leone, Sudan, Swaziland and Tunisia. In 2007, these countries imported eggs worth 83 million USD, equivalent to 86% for the whole continent. Export values fluctuated between 1994 and 2007, with an average value of 10.2 million USD (Fig. 3.4).

Generally, import and export values are related to egg production levels. Thus average values of imports for the low, medium and high egg producing countries were 2.4, 2.7 and 1.1 million USD, respectively in 2007. Inversely, export values were higher for the high egg producing countries (0.8 million USD) compared with the low and medium egg producing countries (0.1 million USD) (Fig. 3.5).

In terms of quantity, data from 16 African countries show that total imports averaged 7.5% of total production, while exports (data from eight

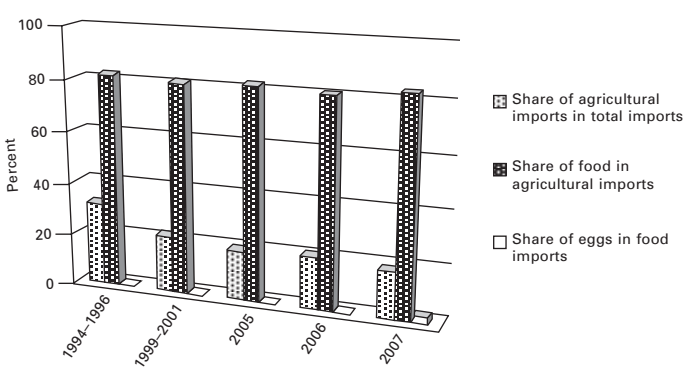

Fig. 3.3 Importation of agricultural products, food and eggs into Africa between 1994 and 2007 (source: FAO 2009).

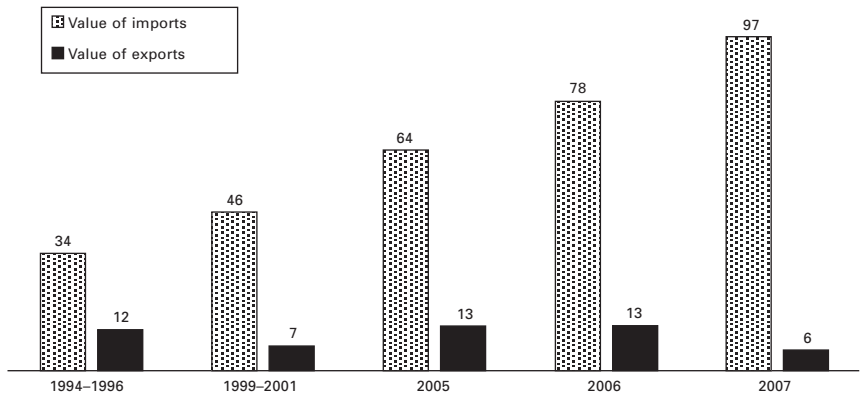

Fig. 3.4 Value of imports and exports of eggs (million USD) for Africa from 1994 to 2007 (source: FAO 2009).

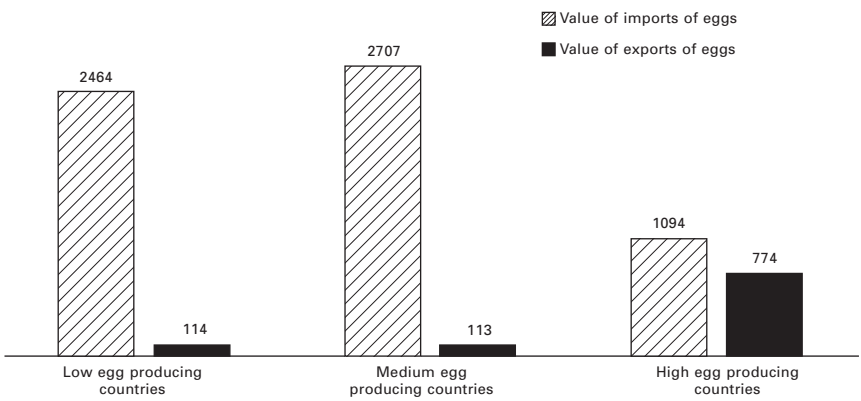

Fig. 3.5 Import and export values of eggs (thousand USD) for low, medium and high egg producing African countries (source: FAO 2009).

countries) represent 5.7% of production. In addition, international egg trade in Africa is mostly between countries within the continent. Thus, assuming that all the eggs produced in the continent are consumed locally, per capita egg consumption (calculated from population figures and egg production values for 2007; FAO 2009) averages 2 kg/year, equivalent to 36 eggs/person/year; which is much lower than the world average of 145 eggs. As noted for egg production, there is considerable variation in egg consumption among African countries (Fig. 3.6):

- *Countries with high per capita egg consumption*: The only African countries whose egg consumption level is above 100/person/year are Libya (174), South Africa (158), Tunisia (151) and Morocco (114). The total population of these five countries is 97 million, representing only 10% of the population of the continent.

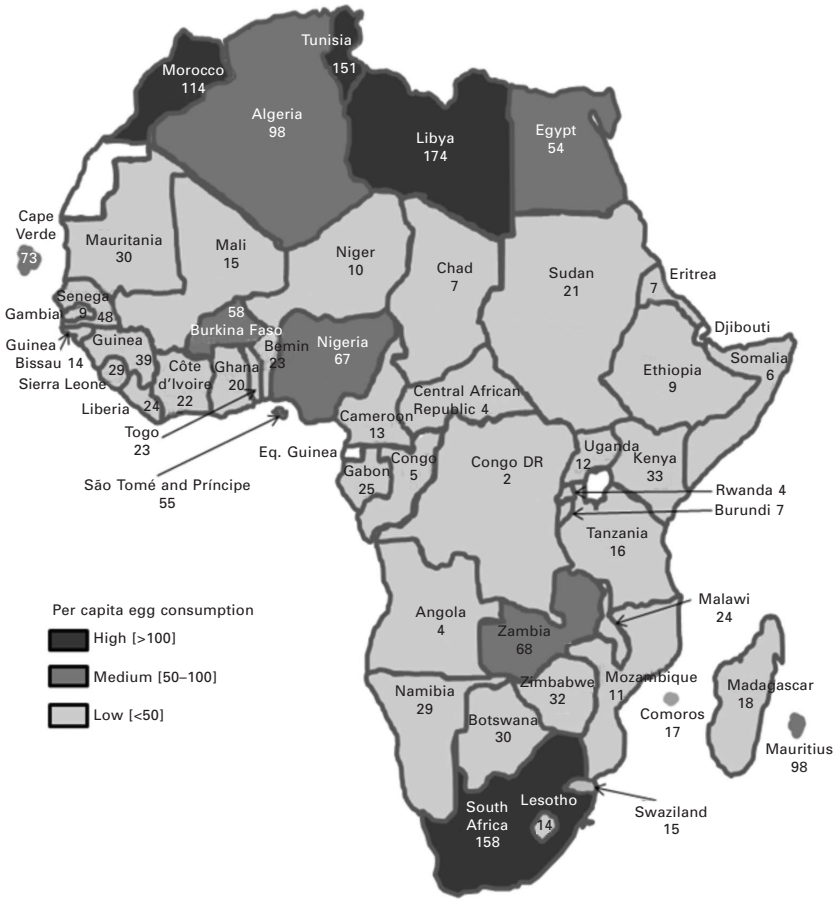

Fig. 3.6 Map of Africa showing per capita egg consumption (no./person/year).

- *Countries with medium per capita egg consumption* – i.e. between 50 to 100 eggs/year. Only seven countries (13% of the number of countries in the continent) fall in this category. They are Algeria (98), Mauritius (98), Cape Verde (73), Zambia (68), Nigeria (67), Burkina Faso (58) and Egypt (54) (Fig. 3.6), with a combined population of 290 million, representing 30% of the population of the continent (Fig. 3.6).
- *Countries with low per capita egg consumption* – i.e. less than 50 eggs/year. Thirty eight countries (73% of the countries in the continent) fall in this category (Fig. 3.6). The total population of these countries is 587 million, representing 60% of the population of the continent. Here, consumption varies from as low as less than ten for Congo DR (2), Angola (4), Central African Republic (4), Rwanda (4), Congo (5), Somalia (6), Burundi (7), Chad (7), Eritria (7) and Ethiopia (9) to over

30 for Zimbabwe (32), Kenya (33), Guinea (39) and Senegal (48), with an average of 18 eggs/country.

Even though egg production in Africa rose by 29% from 1994 to 2007 (with an annual increase of 2.4% per annum), per capita egg consumption increased by only 5.6% (i.e. from 34 to 36 eggs) during the same period. This is mainly due to the increase in population which rose by 25% during the same period (i.e. from 725 million to 962 million).

3.4 The production system

Like in other continents of the world, the domestic chicken is the most widely reared poultry species in Africa. FAO (2009) estimated the total chicken population in Africa at 1.4 billion heads, which varies considerably among countries. Thus, it is as low as 420 000 for São Tomé and Príncipe and as high as 166 million for Nigeria. In general chicken population is related to egg production – i.e. it is higher for the high egg-producing countries and vice versa. These chickens, both the improved exotic hybrids and the local domestic chickens, are reared under different production systems, the characteristics of which are outlined in Table 3.1.

3.4.1 The intensive and semi-intensive systems

On average, 20% of the chicken population in Africa, comprising mainly the exotic hybrid layers and broilers are raised under these systems of management. The proportion of layers seems to vary greatly among countries, as data from eight countries show that it ranges from 2 to 67%. Productivity of birds under this system of management varies between 250 to 300 eggs/bird/year (Pym *et al*. 2010), depending on the intensity of the management system. Farmers operating these systems of management obtain feed from three different sources:

1. *Custom millers*: These are feed companies that mill and market feeds under registered trade names.
2. *Toll millers*: These are individuals who own feed mills located in strategic places in some urban centres. They are patronized by small holder farmers in an effort to reduce the cost of feed. The farmers bring their ingredients to the toll millers who compound the feed for them at a certain price. Some of the toll millers also stock feed ingredients that can be purchased by farmers. By this arrangement, farmers are able to obtain feed at a cheaper cost compared with that sold by the custom millers.
3. Integrated farms own feed mills and produce feed for their own use.

The major ingredients used in poultry feeding in Africa are available

Table 3.1 Characteristics of poultry production systems in Africa

Characteristic	Production system		
	Intensive	Semi-intensive	Scavenging
Breed	Specialized	Specialized and dual purpose	Local indigenous types
Housing	Modern, in some cases with internally regulated environment	Varies from modern to simple types made with locally available materials	Specific housing is rare
Feed	Commercially or on-farm compounded	Commercially compounded/home made mixtures/ scavenging	Scavenging and occasional feeding with home grains and household refuse
Health care	Standard and regular	Disease control and health care programme at varying levels	No regular health care programme or disease control measures
Marketing channels	Well defined (export and urban)	Less defined (urban/rural)	No formal marketing channels (rural/urban)
Infrastructure	Water, electricity and communication available	Moderate infrastructure depending on proximity to urban centres	Underdeveloped infrastructure
Storage and processing of products	Dressed birds (and in some cases table eggs) refrigerated	Minimum refrigeration, occasional dressing of birds	No refrigeration. Sale of live birds and eggs
Technology/ information	Formal training/ extension services mostly available	Moderate formal training and extension services	Use of local knowledge with moderate or no extension services
Food security of owner	High	OK	From OK to bad

Adapted from Kitalayi (1998) and FAO (2009).

locally. The chemical composition of some of these ingredients is shown in Table 3.2. Availability varies among countries, thus there is some level of international trade to balance supply. For example, in 2005, Nigeria imported 23 000 tonnes of soybeans and exported 9000 tonnes of the same feed stuff. Synthetic lysine and methionine (though required in small amounts in poultry diets) are critical ingredients that are not readily available in most African countries. Thus, in 2005, Nigeria imported 828 760 tonnes of these two ingredients from Asia and Europe.

In Nigeria, feed availability is still a problem in some parts of the country. For example, farmers in north-western Nigeria mostly rely on custom millers for feed supply, and the nearest company that services these farms is located in the central part of the country – about 600 km away. Thus delay

Table 3.2 Chemical composition of some locally available feed ingredients in northern Nigeria

Feed ingredient	Dry matter	CP	Nutrients composition (% dry matter)					ME (kcal/kg)
	(%)		Fat	Ash	Ca	P	Fibre	
Energy Sources								
Cereals								
White maize	91.2	8.8	4.3	1.2	–	0.10	10.1	3667
Yellow maize	92.0	10.3	4.9	1.3	–	0.16	10.6	3672
Yellow sorghum	89.6	7.8	3.5	1.7	–	0.27	8.8	3669
Red sorghum	92.0	11.8	3.3	2.3	–	0.47	10.4	3550
Millet (Adamawa)	92.4	13.3	6.3	1.9	–	0.33	8.2	3811
Millet (Sokoto)	91.0	10.9	6.0	1.9	–	0.44	8.4	3794
Cereal by-products								
Maize bran (Sokoto)	91.4	11.1	10.5	3.9	–	0.66	27.9	3023
Rice offal (parboiled)	92.9	5.8	6.0	23.5	–	0.51	56.9	486
Rice offal (non-parboiled)	92.8	6.4	4.9	19.6	–	0.57	50.2	930
Millet bran (processed)	89.5	17.8	8.4	4.9	–	0.23	28.9	2830
Millet bran (unprocessed)	91.7	10.6	7.2	11.0	–	0.49	28.5	2462
Sorghum offal (red)	91.8	18.7	4.1	5.8	–	0.22	37.2	2208
Wheat offal	91.5	16.9	4.3	6.0	–	1.29	44.3	1894
Protein sources								
Groundnut cake (Sokoto local)	95.1	39.5	22.6	5.0	–	0.69	12.4	4231
Groundnut cake (Sokoto industrial)	94.2	45.8	11.6	6.0	–	0.66	15.8	3509
Groundnut cake (Kaduna industrial)	93.4	47.3	14.5	7.5	–	0.69	13.4	3675
Soya bean meal (Kano industrial)	92.7	48.3	3.2	11.1	–	0.68	21.5	2585
Palm kernel meal	93.7	16.3	3.4	8.8	0.21	0.62	67.3	676
Cotton seed cake	94.2	22.8	5.5	6.8	0.25	0.71	53.3	1501
Sources of Ca and P								
Oyster shell	99.9	–	–	99.5	35.0	0.03	–	–
Bone meal (Sokoto)	99.5	–	–	98.2	27.0	13.0	–	–
Bone meal (Kaduna)	98.6	–	–	87.6	26.0	13.6	–	–
Limestone (Sokoto)	98.6	–	–	97.3	35.0	0.18	–	–

in supplies does arise as a result of logistical problems, thus putting farmers under great inconvenience. Feed shortages are also encountered during peak periods of activity – especially during periods of festivities (such as the Eid and Christmas periods) when farmers increase their stocks to meet the high demand.

Although exotic broilers and layers are the two types of poultry species raised under these systems, other poultry species are also reared by farmers (Table 3.3), mostly semi-intensively. A recent survey in north-western Nigeria indicated that these different species of poultry are reared in different combinations (Fig. 3.7).

Table 3.3 Incidence of other poultry species in some African countries

Country	Specie	Occurrence (%)
Morocco	Turkeys	3.5
Guinea	Ducks	2.5
Nigeria	Guinea fowls	5.0
	Ducks	2.4
	Turkeys	0.3
Burkina Faso	Ducks	0.6
	Turkeys	0.1
Tanzania	Ducks	2.0
	Turkeys	0.3
Togo	Turkeys	0.2
Tunisia	Turkeys	7.9
Egypt	Turkeys	0.7
	Ducks	4.7

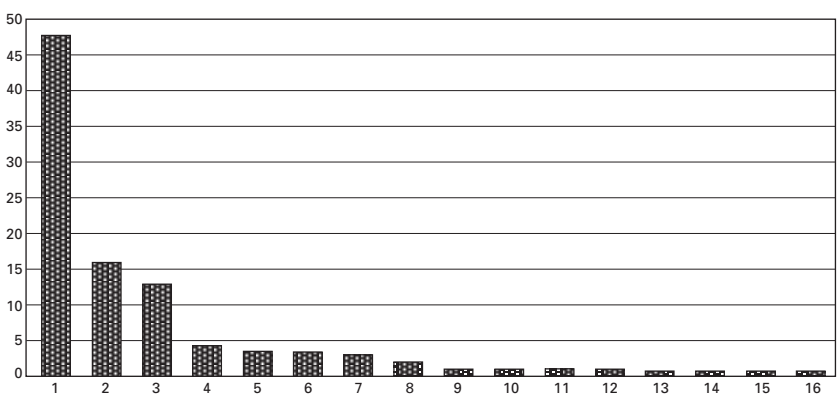

Fig. 3.7 Flock composition (%) of poultry farms in Sokoto and Kebbi States, north western Nigeria: 1 layers and broilers; 2 layers only; 3 broilers only; 4 layers, broilers and cockerels; 5 cockerels only; 6 layers and cockerels; 7 layers, broilers and turkeys; 8 broilers and cockerels; 9 broilers and geese; 10 broilers and turkeys; 11 broilers and pigeons; 12 layers, broilers, guinea fowls and geese, 13 layers and turkeys, 14 layers, geese and ostrich; 15 layers, broilers, turkeys and pigeons; 16 layers, broilers, guinea fowls, turkeys and geese.

3.4.2 The extensive (scavenging) system

This is the predominant system of management in Africa, involving mainly the indigenous domestic fowl, comprising up to 50 local types (Kitalyi 1998). They comprise about 80% of the chicken population in most African countries (Goodger *et al*. 2002). They are dual purpose – i.e. kept for both eggs and meat. The ratio of chicks : growers : adults in the scavenging system

has been estimated to vary from 3:2:1 to 1:1:2, while the ratio of adult males to females is reported to be about 1:3. From these data, Pym *et al*. (2010) estimated that the proportion of adult hens in the indigenous chicken flocks is 25%. These birds lay on average 40–60 eggs per year in three or four clutches. In a typical clutch of 10–16 eggs, only about 2–4 are kept for consumption or sale, because the remainder are set for the hen to hatch. This therefore amounts to 16 eggs/hen/year, compared with 250–300 eggs per hybrid layer (Gueye 1998). Thus egg production of the local chicken is only about 6% that of the hybrid layer (Pym *et al*. 2010).

Even though efforts to introduce high yielding chicken breeds date back to the 1920s, indigenous chickens are still predominant in most African villages, because most local farmers are unable to meet the high input requirements (housing, feed, health care, etc.) of the high yielding exotic breeds (Kaiser 1990; Safalaoh 1997). In addition, the indigenous breeds have been shown to be more disease resistant, more capable of utilizing low quality feeds, and have greater capacity for survival under the scavenging conditions than the commercial hybrids (Horst 1988, Ferrell *et al*. 2000). Improving the productivity of the local chicken will therefore be expected to increase egg supply in Africa. This can be achieved through gradual evolution of the scavenging system to at least the semi-intensive system of management.

3.5 Conclusion

Egg production in Africa is low compared with other parts of the world. With a population of 963 million, the continent accounts for only 3.7% of the global output; while Europe, with a population close to that of Africa, accounts for about 17% of the global egg production. Thus egg consumption in Africa averages 36 eggs/person/year, which is below the world average of 145. Even though egg production in Africa has been on the rise since 1994, per capita egg consumption has remained very low due to the rising population. The low performance of the poultry industry in Africa can be attributed to the inefficient extensive system of management that is predominant among farmers. Improvement can be achieved by gradual evolution from the scavenging management system to a more productive system of management.

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4

Profiling the egg consumer: attitudes, perceptions and behaviours

F. Hansstein, University of Bologna, Italy

Abstract: This chapter aims at a better understanding of consumer attitudes and perceptions toward egg consumption and how they influence purchase behaviour. The chapter is organized in two main sections. The first is based on a reasoned selection of current literature and discusses which factors consumers use to determine the quality and safety of eggs, trying to identify how these could evolve in the light of the recent evolution in European legislative scenarios. The second part of the chapter presents results from a small survey carried out within the framework of the EU project 'Trust' in 2004 and includes, among many others, data on attitudes towards eggs consumption.

Key words: consumer attitudes, egg quality perception, functional foods.

4.1 Introduction

This chapter will explore consumer perceptions and explicit attitudes towards eggs. The *Oxford English Dictionary* defines *attitude* as a 'settled behaviour or manner of acting, as representative of feelings and emotions', while *attitude of mind* refers to a 'deliberately adopted, or habitual, mode of regarding the object of thought'. The surveys presented in this chapter focus on explicit attitudes only. Explicit attitudes, traditionally measured through questionnaires, form the basis of a conscious and deliberate choice. On the other hand, implicit attitudes guide behaviour unconsciously and spontaneously and can be measured only through specific techniques such

as the Implicit Association Test (IAT) (Craeynest *et al*., 2005). This chapter also discusses the roles of food safety, risk and trust, attempting to understand the cues which consumers use to recognize them. When presenting the results of a pilot survey on eggs, a distinction between two types of risk is made. Risk knowledge corresponds to the degree of information consumers think they have about a certain risk: for example, if a food scare occurs, consumers may believe that they would be adequately informed and thus safeguarded. It is evident that risk knowledge is strictly related to the level of consumer trust in institutions and mass media. By contrast, risk perception is related to the degree to which a risk is perceived as involuntary and uncontrollable.

If the concept of explicit attitude can be described as an inner tendency to evaluate an entity with some degree of favour or disfavour (Eagly and Chaiken, 2007), perception is linked to spontaneous brain activity and is often an elaboration of immediate impression. Perception is defined by the Oxford English Dictionary as 'a direct recognition of something, an intuitive insight, an understanding and also an interpretation or impression based upon such an understanding'. Traditionally, when talking about food choice, consumer scientists draw a distinction between experience, credence and search attributes (Nelson, 1970). Experience attributes are those directly experienced by consumers – as taste – and usually occur after the first purchase. If these attributes generate appreciation, they can determine repeated occasional consumption or even become a habit. Search attributes are qualities of goods – like their appearance or price – that consumers may assess before buying. Thirdly, credence attributes are those that are supposed to exist but cannot be directly verified nor experienced, such as quality or traceability. Credence attributes require trust in producers or in food authorities and are becoming more and more important due to an increasing demand for high quality diets and a safer way of eating. When the attribute of interest is food safety (intended as the complementary concept to food risk), it can rarely be classified as an experience attribute, although 'bad' experiences inevitably lead to avoidance and wariness and generate a high risk perception. Instead, food safety as well as nutritional quality are generally classified as credence attributes in the specialized literature, and may become search attributes in specific situations. Consumer choices and attitudes towards food are a weighted combination of the costs and benefits associated with these attributes and vary according to the available information consumers have.

In the second part of this chapter, we present some results from a small survey loosely based on the Theory of Reasoned Action, developed by two American psychologists Icek Ajzen and Martin Fish at the end of the 1970s. According to the TRA, purchasing intentions depend linearly on attitudes and the subjective (referent) norm, where the latter is the importance people give to others' opinions – as parents, friends, colleagues, etc.

4.2 Egg consumption: evidence from the literature

Few surveys have been carried out on consumer perception of and attitudes toward eggs, despite their importance as a nutrient-dense staple food, high in protein, vitamins and an important source of protein for vegetarians, whose number is constantly increasing. Three reasons can be suggested for this lack of attention. The first is the fact that egg consumption has remained not only relevant but also stable over the years in Europe and no significant oscillations have been observed. According to the Food and Agriculture Organization of the United Nations (FAO), average egg consumption in Europe has increased by 0.3% between 1997 and 2007 and it has been estimated that Europeans consume 230–240 eggs per person a year (Pascale, 2009). Secondly, it is difficult to build a brand image for an unprocessed food product through advertising, which makes it less convenient for producers to invest in brand-specific advertising and promotion. Consequently, there is still low interest in profiling egg consumers. Thirdly, as the literature on egg consumption suggests, the great majority of surveys focus their attention only on long-term health risks associated with eggs, such as high cholesterol and foodborne diseases, e.g. salmonella contamination. However, taste, size, nutritional properties and convenience are the traditional determinants of consumers' choice and can be classified as aspects of the experience that people look for when buying eggs, or search attributes.

The following sections identify which are the main tendencies that best describe consumer attitudes towards eggs. From the review of the literature, three main themes are highlighted concerning egg consumption: animal welfare concerns, risk factors such as salmonella and cholesterol, and enrichment of the basic egg nutritional profile.

4.2.1 Animal welfare concerns

Concern for animal welfare is becoming more and more important, and for eggs, it means that consumers take note of and may base their decisions on whether the hens are farmed using battery or free-range systems. When there are labels informing consumers on the type of rearing system, animal welfare can be categorized as a search attribute. A research carried out in 1996 in the south-east of England (Fearne and Lavelle, 1996) pointed out that half of the consumers interviewed claimed to usually purchase free-range eggs and, symmetrically, 44% agreed that modern methods of production are cruel. The authors of this research also identified 'freshness', 'none are cracked' and 'good value for money' as the most important factors taken into consideration by consumers when purchasing eggs, while when asked their reasons for the choice of a specific egg type, the most popular options were 'bird welfare', 'price' and 'tastier'. Respect for animal welfare, in the mind of the consumer, is not only a quality but also a safety cue. As several food scares have shown, safety concerns can lead to a rapid decrease of the level of consumption for certain foods, seriously affecting producers.

Other researchers state that animal welfare is not only important, but even fundamental to the choices of a segment of egg consumers (Grunert, 2005). Why is animal welfare so important to consumers that it can be considered as a search attribute? Beside the increasing institutional attention given to organic production systems, I propose two explanations related to consumer attitudes: a 'psychological' one, and an 'economic' one.

The increasing importance of animal welfare: personal values

The first interpretation – that could be further investigated using the mean-end chains method (Gutman, 1982) – is related to understanding how consumers mentally link product characteristics to more abstract quality dimensions and, from there, to their personal values (Grunert, 2005). Animal welfare is linked to sustainability production and, at a higher level, to the desire of living in a nature-friendly planet, respectful of the environment and of different eco-systems. The proverbial expression 'you are what you eat' seems to perfectly fit in this context. Consumers prefer to buy free-range eggs because they feel they are doing something good and valuable (for the environment but also for themselves).

The increasing importance of animal welfare: willingness to pay

The second explanation is related to the willingness to pay (WTP) approach towards the monetization of quality, that is the translation of the added value of perceived quality into monetary terms based on the amount that consumers are willing to pay. It is often the case that consumers accept the trade of lower price for lower quality, but in the case of eggs, the free-range requirement seems not to be 'tradable', given that egg price is too low to be given up for quality (Grunert, 2005).

Normally, a gap between a positive attitude and a positive behaviour is observed (Lievonen *et al.*, 2004). Although consumers may hold a genuine positive opinion on, for example, sustainability, this does not necessarily generate consistent purchase behaviour of eco-friendly products, owing to factors such as their higher price, lower availability in common market place or a lack of perceived behavioural effectiveness[1]. But, again, this inconsistency does not seem to apply to eggs. Free-range eggs are largely available in supermarkets (people do not need to drive to a farm in the countryside to buy them) and they are always affordable. The lower importance of price in the case of eggs has been also observed in a survey conducted in Germany in 2002 by the Department of Agricultural Economics of Kiel (Röhr *et al.*, 2005). The survey suggested that price is less relevant than the appearance and taste of eggs, citing the fact that 77% of consumers interviewed are prone to pay at least 30% extra for additional egg safety, and 7.3% of consumers

[1]Perceived behavioural control (PBC) is a form of control behaviour and correspond to the extent to which the consumer believes that his/her personal efforts can contribute to the solution of a problem (Vermeir and Verbeke, 2006).

would even accept a doubled price. The study also found that price-sensitivity negatively correlates to safety-sensitive factor. It would be interesting to integrate this evidence with a socio-demographic analysis of the situation.

To conclude the argument on animal welfare, we can argue that it seems to be used by consumers as a proxy for different product attributes: animal-friendly production, safety and impact on human health.

4.2.2 Long- and short-term risks: salmonella and cholesterol

Salmonella

As regards food safety, since the middle of the 1980s salmonella has become a major concern among eggs consumers. Unlike other risk factors, such as bovine spongiform encephalopathy (BSE) or pesticide residue, whose risk perception has decreased over the years, salmonella remains a steady worry (Röhr *et al*., 2005). Despite all the hygiene measures adopted by national and international food authorities, consumers should still be careful how they manage eggs at home. This point is relevant because people can undervalue the role of their own behaviour as a risk factor. For example, a survey carried out in Finland in 2004 pointed out that only 34% of respondents said they always wash their hands after breaking eggs. Furthermore, undercooked eggs are habitually consumed in preparing some dishes (Lievonen *et al*., 2004). A meta-analysis on consumer food safety knowledge and handling practices (Patil *et al*., 2005) observes that consumer knowledge of safe handling practice does not correspond with their reported habits, suggesting that knowledge is a poor indicator of actual behaviour. On average, correct knowledge exceeded correct use by 10% of the total sample and those differences were higher for individuals who had been educated beyond high school, men and middle-aged adults. This is particularly true for eggs. With respect to other undercooked food, more people (47%) consumed raw or undercooked eggs than consumed ground beef (21%), shellfish (12%) and raw milk (2.1%), where 'raw and undercooked eggs' are those eaten whole or as ingredients in other unheated preparations (e.g. cookie dough, egg nog, custard or homemade ice-cream). A tendency to over-report behaviour perceived to be good (the so-called social desirability issue) was also observed and it is thus recommended that additional effort is probably needed to identify reasons other than knowledge that drive safe behaviour of consumers (Patil *et al*., 2005). Further research should investigate the psychological mechanisms that people adopt to make themselves feel 'safer' (with particular attention to the perceived behavioural control, PBC) and the role played by habit. The more a habit is consolidated, the more difficult it is to challenge despite all the reasonable and persuasive arguments that experts or institutions can advance.

Cholesterol

Purchase of eggs is also affected by their cholesterol content (on average 213 mg per 100 g), perceived by buyers as a risk factor for coronary heart

disease (CHD). According to several European public health institutions, consumers have to pay attention when eating too many eggs due to the levels of cholesterol consumed. For example, 'Eat well, be well' – an English campaign launched by the Food Standard Agency – warns consumers of the potential risks related to excessive egg consumption. However, there are also some critical voices on those issues that invite institutions, consumers and experts to take into account the fact that so far only a limited number of studies have specifically explored the relation between egg consumption and CHD. Further research in this area should be encouraged, especially on how egg consumption influences the overall cholesterol ratio (Gray and Griffin, 2009). It will not therefore seem paradoxical that, in the US, there is a research branch that promotes egg consumption as a method of tackling obesity, due to the finding that egg consumption increases the feeling of satiety and thus reduces short-term energy intake (Vander Wal *et al.*, 2005). Interestingly both the American Heart Association (AHA) and the British Heart Association (BHA) have started to promote information on the meaning of a healthy diet without referring specifically to eggs. They assume that it is now known that eggs are high in cholesterol and have chosen a different communication strategy based on the recommendation that people should not consume more than 300 mg cholesterol per day. Lower values are suggested for those who may be susceptible to CHD (Gray and Griffin, 2009).

4.2.3 New opportunities for improving health: enriched eggs

The benefits offered by enriched food form the other side of the coin to consumers' worries for their health, and today this field is moving forward thanks to the advances in technology opportunities. The demand for this type of food is becoming more and more relevant and labels that provide consumers with the relative information belong to the category of search attributes (Degeratu *et al.*, 2000).

Some research was carried out in the US in 2001 on the benefits of so-called 'specialty' eggs, as distinct from the common white ones (Patterson *et al.*, 2001). The term 'specialty' includes eggs produced by hens that are fed a vegetable or nutritionally altered diet, welfare managed, fertile and organically reared. However, Patterson *et al.* pointed out that in some cases specialty eggs have certain added values, for example nutritionally added properties, or provide consumers with specific quality attributes – fulfilling an emotional need or providing a healthy ingredient. However, according to the results of their research (with exceptions for weight and number of cracked eggs), white eggs were statistically superior to specialty ones. This is a sign that further research on 'specialty' eggs is surely needed.

4.3 European legislation between consumers and eggs

Legal measures adopted by the European Union can influence egg production and consumption in both direct and indirect ways. There are measures that directly hit egg producers, like strict controls to avoid food contamination, while others aim at regulating agricultural sector in general and at protecting consumers' health. Food safety is a priority in the EU policy agenda and many programmes are addressed to strengthening it. But what is food safety? If we mean food safety in a broader sense, it is clear that it can be interpreted both as a credence or a search attribute. For example, if we consider the requirements imposed by legislation and communicated to consumers through labels, there is room for food safety to become a search attribute, while if in consumers' minds food safety is related to the origin of a certain product it is classified among credence attributes (Umberger *et al.*, 2003). Together with a strategy aimed at improving consumer self-prevention of risk, the making of informed choices by consumers is the goal the European Union wants to reach through its legislation.

Among the variety of EU legal measures on food, it is worth focusing on three 'hot topics'. The first explores new challenges posed by legislation on nutritional and health claims, the second the requirements for animal welfare improvement and the third will discuss regulation on labelling, with special attention to allergen warnings.

4.3.1 Claims and functional foods

On 20 December 2006 the European Parliament and the Council of the European Union adopted a Regulation on nutrional and health claims made on behalf of foods, which posed new challenges and opportunities for both consumers and producers. By regulating these claims, the commission aims to ensure that they are clear, accurate and substantiated. This has multiple implications, generating a more powerful marketing tool on the one hand, but also introducing a stricter constraint on advertising and challenging brand images when scientific substantiation is rejected by the authorities. According to the classification of attributes we have seen in the introduction, succeeding in this challenge for authorities and (healthy) producers depends on the transformation of health and nutritional claims from credence to search attributes.

Nutritional claims

As reported in the Regulation, *nutritional claims* mean any claim which states, suggests or implies that a food has particular beneficial nutritional properties due to:

- the energy, in terms of calorific value, it (i) provides, (ii) provides at a reduced increased rate, or (iii) does not provide at all; and/or
- the nutrients or other substances it (i) contains, (ii) contains in reduced or increased proportions; or (iii) does not contain.

Health claims

A *health claim* is any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health. Health claims can also refer to the reduction of risk factor in the development of a disease (such as substances that have been shown to reduce blood cholesterol) or to child development and health.

The overall goal, as stated in the EC Regulations, is to ensure a high level of protection for consumers and to facilitate their choices. The European Food Safety Authority (EFSA), and the NDA Panel – Nutrition, Dietetics and Allergens – are in charge of assessing the scientific reliability of general and new functional health claims (1), of claims regarding disease risk reduction and child development (2) and of criteria for setting nutrient profiles (3). The panel's opinions help the European Commission and its member states to decide on the authorization of those health and nutritional claims on food packages. The panel has published two series of opinions on the list of general fuction health claims so far, and its work should be completed in 2011.

Eggs and functional foods: first evidence from the USA

Even if the above regulations are too recent for a thorough assessment, it is still possible to outline some future paths on the basis of US surveys. HealthFocus International (a private consulting firm) has identified five primary benefit platforms for functional and nutritional products (prevention, performance, wellness, nurturing and cosmetics) and analysed how they correlated with general consumption of eggs (Gilbert, 2000). More than half (60%) of respondents among those who are oriented to the 'prevention' platform declared that they eat eggs weekly. For the second and the third platforms – 'performance' (which involves health enhancement) and 'wellness' respectively – more than 30% of respondents were frequent consumers of eggs. About 38% of those surveyed are frequent consumers on the 'nurturing' platform, meaning that the benefits they seek from eggs are growth, development and quality of life. Finally, shoppers oriented towards the 'cosmetic' platform are only moderate egg consumers. The data would suggest that there is a segment of egg consumers that have positive attitudes to functional foods and associate their consumption with a healthy lifestyle. In relation to the source of information, the survey has shown that frequent egg consumers are more likely than non-consumers to watch television, to listen to the radio, to read women's magazines and newspapers for useful health and nutrition information. Non-consumers of eggs put more emphasis on labels and health magazines.

Future trends in egg consumption in Europe

Additional research on functional eggs is needed to assess their acceptability to consumers and their long-term effects on health. Consumer acceptance depends not only on the recognition of health benefits, but also on the

acceptance of paying an augmented price for 'functionality'. Given their general positive attitude to pay extra for animal welfare, we can infer that consumers would probably be well disposed to pay a little more for functional eggs. There is not a huge difference in prices between free-range (or organic) and functional eggs (Surai and Sparks, 2001). As reported in the European Food Information Council (EUFIC) website, despite the cholesterol content, omega-3 fatty acids enriched eggs have been recognized as a functional food because of their benefits in preventing hypertension and to strengthening the lipid metabolism. Commercial table eggs contain a high proportion of omega-6 but are a poor source of omega-3 and, thus, as a matter of fact, eggs could be accompanied by a proper health claim. In Europe it is still too early to discuss consumer acceptance of functional foods as their consumption is restricted to a niche market.

On 26 February 2010, the EFSA opened the guidance on a risk–benefit assessment of foods that could be labelled with a health claim to the public. In May 2010, EFSA published an updated version of the database of claims of general functionality as foreseen by Article 13 of the European commission Regulation of health and nutritional claims. There is room for eggs to be claimed 'functional' when enriched with selenium or, if raw, when the quality of dietary lipids is improved. Although the EFSA has still to express its opinion on some claims, it will be interesting to evaluate if consumers' future attitudes and perceptions towards eggs are influenced by changes in the EU legislation.

4.3.2 Consumer perceptions of animal welfare and laying hens

The EU places great importance on animal welfare, and the health of laying hens was the first aspect of this issue to be regulated in 1986. Since 1999 the Protocol on Protection and Welfare of Animals of the Amsterdam Treaty has set out fundamental principles concerning EU action. Three types of rearing systems are admitted within European borders: enriched cages with at least 750 cm^2 of cage area per hen, non-enriched cages with at least 550 cm^2 and non-cage systems with at least one nest per seven hens and where the stocking density does not exceed nine laying hens per m^2 usable area. European commission taxonomy also recognizes three classes of eggs for human consumption: 'fresh eggs' belong to Class A, 'Certain secondary quality or preserved eggs' to Class B and 'non-graded eggs' to Class C. Consumers are unlikely to be aware of the legal implications of animal welfare regulations. In this setting, it is interesting to reflect on results of a survey founded by the European Commission on attitudes of consumers towards the welfare of farmed animals, carried out in 2005 by the Eurobarometer. Consumers seem to have a general positive attitude towards welfare of laying eggs: almost four citizens in ten surveyed (38%) state that they buy eggs from hens raised in free-range or outdoor production system. Having made just one visit to a farm is a good predictor of a consumer's

positive attitude towards free-range eggs. Among consumers who have never been to a farm, 33% buy eggs from hens kept in a cage, while 29% take no notice of the type of production system. Respondents who have visited a farm three times declared they would accept at least 25% increase in the price of eggs in exchange for stronger guarantees of welfare.

Over 30% of consumers rate the welfare of laying hens as 'very bad' in the Netherlands, Denmark, Germany and Belgium, while the highest percentage of 'very good' ratings has been registered in Malta (19%). Together with the Scandinavians, North Europeans are also particularly vociferous in calling for improvements in rearing conditions and are the most willing to pay an extra price for it. The welfare of laying hens is perceived as worse than that of other farmed animals such as dairy cows and pigs. Regarding socio-demographic characteristics, women are more prone than men to buy free-range eggs (43% against 34%), as are inhabitants of rural villages as opposed to those living in towns (Eurobarometer, 2005). To conclude this brief review on attitudes towards animal welfare, there are two questions that appear to be relevant.

What comes first, attitude or labels?

When asked if they could identify the rearing system, almost 51% of EU citizens stated they can very rarely identify whether the production system was animal welfare friendly or not by only reading the label. One-third of consumers could not do this at all. This is in contrast with the statements of animal welfare supporters. The finding would thus suggest that for consumers who are already concerned about animal welfare it is easier to find the information they want, while for the average consumer it is more difficult.

Is animal welfare a guarantee of a better quality?

Some researchers have observed that quality is surely correlated to the space per hen and to their group size (Elson, 1990). The World Organization of Animal Welfare has also recognized that the link between animal welfare, animal health and food safety is accepted everywhere. However, consumers should bear in mind that information about a production system (e.g. free range) does not necessarily equate to the level of animal welfare. To give a valid rating to a product would require traceability and regular welfare monitoring throughout the animal's life (Sossidou and Elson, 2009).

4.3.3 Food labels and allergens

The EU current legal basis for food labelling is Directive 2000/13/EC, which establishes general requirements for food labelling applicable to foodstuffs and pre-packaged foods. The first legal measures on food labelling, adopted at the end of the 1970s, aimed at guaranteeing free circulation of goods in the community, rather than consumer safety. With the development of consumerism, new rights and needs came out and consumer safety and health became priorities for the European Commission policy agenda. Food labels

include today a huge variety of information, from nutritional profiles to quality assurance schemes. In 2008, the European Commission presented a proposal (EC, 2008) for a Regulation on the provision of food information in order to strengthen the current legislation and to further help consumers in making an informed choice. Given its key role in prevention of health hazards, information on allergic ingredients is one of the main issues that emerged as needing to be most improved, and this should be systematically regulated through mandatory legislation. It has been observed that there are still a number of foods from which information is missing and labels should be provided, not only for pre-packaged foods. Apart from consumers, an information system also has to be implemented for producers, who need to be constantly updated on the food allergen list in order to control all the costs they have to cover (EC, 2008).

Eggs and egg products are listed among the ingredients causing allergies or intolerance, because egg allergy is, together with milk, the most common among children. Anaphylactic shocks are rare and the physical reactions most often registered are wheezing, nausea, headache, stomach ache and itchy hives. Egg allergy leads the immune system to unleash an army of chemicals to protect the body and the release of these chemical substances can affect the respiratory and cardiovascular systems, skin and gastrointestinal tract. Being informed of the risks through labels is of course a good way to reassure consumers and to enhance their trust in institutions and producers.

However, as reported by Cornelisse-Vermaat *et al*. (2007), recent research has shown a negative impact on the quality of life and economic functioning of with allergies owing consumers to the time spent in looking for what they are allowed to eat. Cornellisse-Vermaat *et al*. (2007) conducted research both in the Netherlands and in Greece to investigate how the attitudes of consumers who suffer from allergies (eggs included) are influenced by food labels. All participants reported problems regarding both the readability and the visibility of the information. Terminology was difficult to understand, font size too small and packages overloaded with information. Participants also reported feelings of insecurity associated with the consumption of new products, especially if they were parents of food-allergic children. Young adults and adolescents were more willing to take risks compared with other groups interviewed (Cornelisse-Vermaat *et al*., 2007).

Another study on consumer attitudes has registered a decreasing level of worry among consumers between 2003 and 2006 (Hefle *et al*., 2007). The food labels analysed in this study advised of the presence of peanuts and the consumers interviewed suffered from severe peanut allergy. The results clearly indicated that an increasing number of these consumers are ignoring allergy advisory labels. The researchers pointed out that further studies are needed and that the decreasing trend could depend on the increasing number of information provided to consumers who, as noted in the previous study, are more and more overloaded by warnings. A third survey (Simons *et al*., 2005) arrived at the same conclusion: 16% of people surveyed misunderstood

label indications and called for clearer warnings. A simple solution, already adopted in some European countries, is to use symbols instead of words. The use of figures would thus help consumers not to misunderstand information, to improve their time-efficiency and to avoid confusion caused by the multiple language translations on packages.

4.4 Evidence from the trust pilot survey

This part of the chapter presents the results of a pilot survey that was part of a European-wide investigation (five countries were involved: the United Kingdom, the Netherlands, Germany, Italy and France) on the issues surrounding the food supply chain, focusing on chicken and eggs consumption. The part of the survey concerning consumer attitudes and purchasing behaviours towards eggs was introduced in a pilot study preceding the actual survey, which focused only on chicken (Mazzocchi *et al.*, 2007). Given the limited number of interviews (126), the results have to be interpreted with caution; they are not always of statistical significance, but are helpful to distinguish interesting future paths for research.

4.4.1 Sample and questionnaire

The survey is divided into two main sections, one dedicated to understanding consumers purchasing behaviour towards chicken, and the other on eggs. General consumption habits, purchasing drivers, risk/safety perceptions and trust have been investigated for both foodstuffs. The structure of the questionnaire was loosely based on the TRA, which explains the intention of doing or not doing something as the result of the interaction between attitudes towards the behaviour and the subjective norm. As anticipated in the introduction, the subjective norm is the degree of importance given to the opinions of the relevant social group (as those of parents, fiancé, friends or colleagues). The questionnaire also includes a series of questions aimed at understanding different attitudes towards safety and quality preferences, and also sensitivity to environmental issues.

Concerning the socio-demographic characteristics of the sample (Table 4.1), there was a large prevalence of females (85%), a relatively high level of education (above 60% with high school diploma or higher) and a dominance of married respondents (64%), while other characteristics seem reasonably balanced. The target was the person in charge of food purchases within the household.

4.4.2 Framing the egg purchase

The first step of our analysis was to profile the average egg consumer through quantity and expenditure. According to the pilot survey, the majority of

Table 4.1 Sample description

	N	%
Gender		
Females	104	82.5
Males	18	14.3
Education		
Primary school or less	13	10.3
Lower secondary	32	25.4
Higher secondary	36	28.6
University degree or higher	37	29.4
Marital status		
Single	18	14.4
Married	81	64.8
Other	26	20.8
Household size		
1	25	20
2	41	32.8
3	18	14.4
4	23	18.4
5 or more	18	14.4

Table 4.2 Types of eggs purchased

		Frequency	Percent	Valid percent
Valid	I don't know	6	4.8	6.9
	Value eggs	2	1.6	2.3
	Standard eggs	35	27.8	40.2
	Organic eggs	9	7.1	10.3
	Free range eggs	19	15.1	21.8
	Barn eggs	6	4.8	6.9
	Luxury eggs	10	7.8	11.5
	Total	87	69.0	100.0
Missing	System	39	31.0	
Total		126	100.0	

respondents stated that they buy eggs once a week (38%) with an average expenditure of 1.70€ and with an average consumption of seven eggs per household per week. As we expected, variance is influenced by family size: the bigger the family, the higher the expenditure and the number of eggs eaten. The number of eggs purchased in a week is not significantly related to income. Table 4.2 reports the percentage of each type of eggs that consumers prefer. Standard eggs are eaten by 40% of respondents (missing excluded), while 20% choose free-range eggs and 10% organic ones.

In order to better understand consumer beliefs about eggs, respondents were asked to express their level of agreement over a series on statement using a Likert scale of 7 points. There was strong agreement with the beliefs

that eggs taste good, that they are a good value for money and are easy to prepare. Strangely, both the statements 'eggs are low in fat' and 'eggs are high in cholesterol' have the same (two) modal values, even if their value is low. Of the respondents 15% and 16% respectively claimed to agree completely, while 19% and 13% selected the 'neither' item.

Regarding hen rearing and welfare, general agreement was expressed in relation to the idea that intensive chicken breeding is bad, and that eggs are still produced with little attention to animal welfare.

Associations with safe eggs

Interviewees were then asked for their level of agreement on a 7-point scale in order to understand what attributes they associate with a 'safe egg' (Table 4.3). Answers ranged from 'completely disagree' to 'completely agree' on a seven point scale. The highest means were observed for freshness, clear labelling and free range, attributes that can be considered as principal warranties of safety. This is also consistent with the evidence discussed before. However, there was significant variability between answers: a sign of the persistence of some uncertainty among consumers.

Even if too few observations per country were analysed, the topical differences were identified wherever possible. Comparisons are made between three countries: United Kingdom, Italy and France (Table 4.4). Italian consumers seem a little bit more agreeable with all the statements on egg safety and more convinced than the British of the fact that safe eggs are produced in their own country. Mean comparison tests show that means are significantly different between the three countries over the idea that safe eggs are packaged. French consumers, with a mean that reveals little agreement, are in fact less convinced than Italian and British consumers.

Risk perception towards eggs

Risk perception has been measured through a series of questions presenting different types of risks usually associated with eggs. Some questions – measuring the degree of risk awareness – asked consumers if, in their opinion, a list of food associated risks were more or less knowledgeable (answers options ranged from one to seven from 'not at all' to 'extremely'). Other questions instead asked how dangerous were perceived to be (the 7-points scale ranges from 'negligible' to 'extremely high'). General results are presented in Table 4.5. Not surprisingly, salmonella and cholesterol are the most knowledgeable and the most dangerous perceived risks in all countries. This confirms what has emerged from the available literature presented in the previous section of the chapter: salmonella and cholesterol are perceived as the most risky factors associated with egg consumption.

Between risk and safety: the role of trust

To conclude discussion with safety and risks, we present results of questions on trust. Trust has the function of 'bridging the gap' between risk and safety.

Table 4.3 Safety cues

	Statistics – Safe eggs are...											
	Packaged	Clearly labelled	Brown	From farmer	From supermarket	Produced in Britain	Expensive	Free range	Barn eggs	Organic eggs	Recognizable by colour, taste or smell	Fresh
N Valid	89	88	83	87	88	91	86	88	85	85	86	90
Missing	37	38	43	39	38	35	40	38	41	41	40	36
Mean	4.28	5.7	3.63	4.48	4.60	5.58	3.9	5.7	5.67	5.4	5.13	6.1
Std. deviation	2.0	1.5	2.1	1.6	1.7	1.6	2.1	1.4	1.4	1.6	1.9	1.4
Mode	5	7	1	4	6	7	4	7	7	6[a]	7	7

[a]Multiple modes exist. The smallest value is shown.
Completely disagree = 1
Completely agree = 7

Table 4.4 Mean comparisons between countries on safety cues

Country		Safe eggs are packaged	Safe eggs are clearly labelled	Safe eggs are brown	Safe eggs are from the farmer	Safe eggs are from the supermarket	Safe eggs are produced in Britain	Safe eggs are expensive	Safe eggs are free range	Safe eggs are barn eggs	Safe eggs are organic eggs	Safe eggs are recognizable by colour, taste or smell	Safe eggs are fresh
UK	Mean	3.90	5.43	2.70	4.00	4.31	5.16	3.25	5.17	4.69	5.22	4.89	5.80
	N	30	30	28	27	29	30	28	29	26	27	29	31
Italy	Mean	5.76	6.60	5.17	4.26	5.20	6.43	4.9	6.30	6.24	5.70	5.80	6.80
	N	30	30	28	30	30	30	28	29	29	30	30	30
France	Mean	2.69	5.13	3.23	5.33	4.00	5.16	3.79	6.12	6.10	5.60	4.70	5.86
	N	23	22	21	24	23	25	24	25	25	23	22	23
Total	**Mean**	**3.09**	**4.29**	**2.78**	**3.40**	**3.38**	**4.19**	**2.99**	**4.40**	**4.26**	**4.13**	**3.85**	**4.62**
	N	**83**	**82**	**77**	**81**	**82**	**85**	**80**	**83**	**80**	**80**	**81**	**84**

Table 4.5 Mean comparisons between countries on risk perception and knowledge

Country		Knowledge								
		E. coli?	Salmonella?	Listeria?	Cholesterol?	Allergy from food additives?	Health problems from pesticides?	Health problems from antibiotics?	Health problems from growth hormones?	Health problems from chicken flu?
UK	Mean	3.25	3.87	3.22	4.32	3.87	2.77	3.16	2.70	2.48
	Std. deviation	1.69	1.47	1.66	1.42	1.74	1.49	1.57	1.65	1.80
Italy	Mean	2.82	4.10	2.55	5.20	4.13	3.51	3.93	3.56	5.00
	Std. deviation	1.62	1.56	1.37	1.18	1.73	1.97	2.01	1.88	1.85
France	Mean	1.48	2.96	2.88	3.88	2.96	2.59	2.73	2.62	2.14
	Std. deviation	1.42	2.04	2.08	1.98	2.10	1.92	2.06	1.88	1.48
Germany	Mean	1.53	4.13	1.33	3.40	3.53	3.16	3.60	3.46	2.76
	Std. deviation	1.30	1.70	1.24	2.23	2.09	2.24	2.37	2.17	1.90
Total	**Mean**	**2.33**	**3.80**	**2.48**	**4.16**	**3.60**	**2.95**	**3.31**	**3.04**	**3.04**
	Std. deviation	**1.74**	**1.76**	**1.77**	**1.83**	**1.92**	**1.91**	**2.03**	**1.91**	**2.06**

Country		Perception								
		E. coli	Salmonella	Listeria	Cholesterol	Allergy from food additives	Health problems from pesticides	Health problems from antibiotics	Health problems from growth hormones	Chicken flu
UK	Mean	5.80	6.06	5.58	4.32	3.83	3.87	3.77	3.64	3.20
	Std. deviation	1.88	1.56	2.04	2.18	2.26	2.07	2.01	1.79	2.17
Italy	Mean	4.70	5.13	4.73	5.40	5.25	4.53	4.03	3.67	4.41
	Std. deviation	2.00	2.04	2.20	1.52	1.89	2.00	1.81	1.98	2.71
France	Mean	2.90	4.00	3.69	3.58	3.08	3.12	2.95	2.69	3.22
	Std. deviation	2.18	2.02	1.94	2.12	1.95	2.00	1.94	1.89	2.06
Germany	Mean	1.86	4.90	1.10	3.16	2.83	2.60	2.20	1.96	2.26
	Std. deviation	2.63	2.50	2.04	2.71	2.65	2.14	2.00	1.97	2.42
Total	**Mean**	**3.90**	**5.09**	**3.81**	**4.06**	**3.67**	**3.50**	**3.20**	**2.97**	**3.26**
	Std. deviation	**2.68**	**2.14**	**2.66**	**2.32**	**2.37**	**2.13**	**2.03**	**1.99**	**2.44**

If consumers trust a certain source of information, their beliefs towards a food will be influenced by the way a message is delivered. Questions on trust are valid not only for eggs, but also for chicken consumption (and in general).

Reliability of source was measured in a dichotomous way: respondents were asked to simply say 'yes' or 'no'. Given the limited numbers of observations, the results must be interpreted with extreme caution, but a relatively black picture emerges from the available data. some 55% of people interviewed declared they trusted television news, 40% the Internet and only 29% radio. Government, billboard and flyers were the agents with the lowest percentage of 'yes', where the respective percentages were 18.34% and 5.6%, results that indicate a necessity to strengthen media and institutional devices to reassure citizens.

4.4.3 Determinants of behaviour: attitude and social norms

As anticipated in the introduction, the pilot survey was loosely based on the TRA, where purchasing intentions depend linearly on attitudes and on subjective (referent) norm. Intention has been measured through one question that asked respondents the probability they will buy eggs in the following week. The mean confirms a positive intention, although not very intense (the average answer is 'likely').

To deepen the understanding of factors influencing purchasing behaviour, attitude and subjective norms have been further explored. According to the TRA, attitude and subjective norms toward a certain behaviour can be expressed in two ways: as an overall variable and as a sum of beliefs. If people have a positive (overall) attitude toward a certain behaviour, this is probably because there are a series of attributes related to it that they also connote positively. If I like eating apples it is probably because I like their taste and fragrance, or because I value being healthy and so on. If I value others' opinions, it's because my actions might be influenced by parents, friends or colleagues.

Coming back to eggs, the general attitude towards consuming eggs was measured through the statement 'a good diet should include standard eggs' (overall attitude), for which respondents had to express their level of agreement. Attitude has been then broken into a series of attributes that try to capture the opinion of respondents towards the general act of buying eggs (good–bad, disagreeable–agreeable, convenient–inconvenient, disadvantageous–advantageous, ethical–unethical, wrong–right) and the level of agreement with the statements discussed in the previous paragraph (e.g. 'eggs taste good' or 'eggs are good value for money').

To measure overall subjective norms, respondents were asked if they take the opinion of others into account when making decisions on whether or not buy eggs. Some 44% of respondents stated that they were unlikely to take another's opinion into account. Frequently people tend not to admit (often also to themselves) to rely on others when making decisions.

4.4.4 Determinants of consumer attitudes and country differences

To conclude our exploration of the egg consumer, this section provides some quantitative analysis of the determinants of attitudes, mainly based on consumer evaluations of quality, safety and environment and also of their risk perception and safety.

I have employed principal component analysis (PCA), a data-reduction method widely used in marketing research to combine a set of observed variables into a reduced number of latent factors which are a linear combination of the former. Latent variables cannot be directly observed but are measured through a series of statements with which consumers have to express their level of agreement. For example, the attention to the environment is broken into a series of questions (such as 'when humans interfere with nature, it often produces disastrous consequences' or 'humans have no right to modify the natural environment to suit their needs').

From the analysis, five latent variables (factors) explain more than 50% of the total variability. Table 4.6 presents the component profiles detected with the PCA, together with the average values for the UK and Italy. Despite the

Table 4.6 Consumer profiles as defined by PCA

Consumer profile defined by factors					
	Factor 1 Safety-oriented	Factor 2 Quality-oriented	Factor 3 Nature-oriented	Factor 4 Wary consumer	Factor 5 Wary consumer but optimistic over the environment
Attitudes and perception	Traditional and conservative; concerned about food safety; not opened to ethnic food; not interested in environment; preference for British eggs	Attentive to ingredient; attentive to content in pesticides and antibiotics residuals: willing to pay an extra for better quality; preference for organic eggs	Preference for natural foods; critical over human exploit of environment; worried for pesticides and hormones content	Unfamiliar with ethnic food; pessimistic over the environment worry for all kind of risks	Similar to fourth profile, but more optimistic and oriented toward environment
Percentage of the total of variability explained	27%	12%	10%	5%	5%
Average factor score per country					
UK	–0.36	0.15	–0.66	–0.24	–0.04
Italy	0.29	0.30	0.55	0.41	0.37

low number of observations, it is interesting to notice both the characterization of the latent factors and the major differences between the two countries.

The first component brings together the statements related to concern for safety, but not necessarily for environment. According to component loadings onto the original variables, a consumer with a high score in this component is convinced that safe eggs are those coming from their own country and from barns and is especially careful about cholesterol content. The first component explains more than 25% of variability in the sample. The second component is dominated by the original variables related to quality and to a higher willingness to pay to obtain it. High scores in this component are associated with consumers who are very attentive to food quality cues (e.g. the content of natural ingredients). On average, consumers with a high score in this component perceive eggs to be safe when they are brown, organic and fresh. The third component, explaining about 10% of the variability, places more emphasis on concern and fear. A high score refers not only to a strong preference for foods containing natural ingredients, but also to strong environmental concern. Within this component, perception of egg safety is associated with price and the main concern is about pesticides, hormones and chicken flu. The fourth and fifth segment represent respectively 5% of the total amount of variability and their interpretation is less straightforward. The fourth component expresses lack of familiarity towards ethnic foods and serious concerns (pessimism) towards the environment. The fourth resembles the wary consumer, not familiar with ethnic food and pessimistic over the environment. Consumers with high scores place great attention to safety cues when purchasing eggs and are seriously worried about any risk associated with egg consumption. The fifth component is very similar to the fourth, with the only difference being that it gives a little more emphasis to the environment and relatively less to egg safety and risks.

Italians are more conservative and traditional than the British, and much more scared about ethnic foods. Italian consumers are also more afraid than British (fourth and fifth components) about foods that seem less nature-oriented.

4.5 Conclusion

The intent of this chapter was to outline the state of the art regarding attitudes towards and perceptions of eggs, trying to distinguish the main factors that concern and reassure consumers. Although the trust pilot survey, even though it does not include a large number of observations, it confirms what was observed in the literature. Eggs are favoured mostly because they are a 'versatile' food, good value for money and because of their taste. Comparing our analysis with a previous one carried out in the south-east of England ten years earlier, safety and risk cues for eggs have not changed: freshness and animal welfare on one hand, and cholesterol and salmonella on the other.

Regarding the most problematic issues, the key findings are summarized here. Firstly, as regards research on consumer attitudes and perceptions, additional research evidence is needed, for example to better understand what consumers intend for animal welfare and organic production. Qualitative focus groups have shown there is sometimes overlapping of the concepts, as well as misunderstanding or even confusion (Harper and Makatouni, 2002). Secondly, additional research should be promoted in order to monitor the effect of new legislatory measures and their impact on consumer behaviour. Attitudes towards eggs can be readjusted following changes in the environment such as context or information framing. Consumer attitudes and perceptions are not constant over time; new information (health and nutritional claims) can change existing attitudes (Wilson and Hodges, 1992). Cross-cultural analysis should be encouraged as well.

Policy makers should also pay attention to message perception – or misperception – collected by consumers, monitoring how this influences consumer purchase behaviour and how attitudes and perceptions can change after an informational campaign and legislative intervention. If the effects of public campaigns are not monitored, there is a risk that an unjustified fear of eggs will spread. However, a relaxation on egg policy has been recently observed by some researchers (Gray and Griffin, 2009). As emerged in the pilot survey and confirmed by other research, trust in institutions is not very strong.

Thirdly, labels should be more effective and communicative through, for example, the use of symbols and the establishment of a clear, easy, and credible labelling system helping the consumers to make an informed choice (Sossidou and Elson, 2009).

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5

Egg quality assurance schemes and egg traceability

F. C. Schwägele, Max Rubner-Institut, Germany

Abstract: Egg production is expected to increase to approximately 72 million tons in 2015 corresponding to 1260 billion eggs per year. In the European Union egg producers and marketers are required to follow certain regulations. For success in the marketplace, though, quality assurance systems for eggs have to take into account consumer demands in terms of egg quality as well as regulatory requirements. This chapter discusses the concept of 'quality' and various factors affecting egg quality characteristics. It also reviews traceability in the egg industry. There is a need for fast and reliable systems to enable traceability along the full egg supply chain to provide safe and high quality food for the consumer.

Key words: Egg quality assurance schemes, egg traceability.

5.1 The role of eggs in human nutrition

Since the end of World War II eggs have represented an increasingly important factor in human nutrition. Global egg production increased 2.5-fold between 1960 and 1990 from about 15 million to more than 37 million tons per year. In 2005 the worldwide egg production exceeded 60 million tons (FAO, 2005; WATTAgNet.com). World egg production has quadrupled in the 45 years since 1960 while the world population only increased by just over 2-fold in the same period (Deutsche Stiftung Weltbevölkerung, 2009). An increase in egg production to approximately 72 million tons is expected in 2015 corresponding to 1260 billion eggs per year. Taking this number into consideration there will be an average worldwide egg consumption of

about 170 eggs per head of the world population in 2015. However, there are considerable differences in the annual egg consumption of different countries around the world. The world leader in egg consumption in 2004 was Japan (339 eggs/capita and year), followed by China and the South East Asian countries. The average egg consumption in the EU was at that time 238 eggs per capita and year.

With respect to the different European Union countries egg consumption in 2005 varied considerably: Ireland 120, France 252, Spain 306, Hungary 324, Denmark 327; EU average 229 (eggs/capita and year).

5.2 Egg quality

Today, many people in developed countries talk about quality. In recent years 'quality' has gained more and more importance in many different areas of everyday life: education, housing, sports, travel, automobiles, clothing, telecommunication and last but not least nutrition. In contrast developing countries all over the world are still struggling to provide sufficient food for their people to prevent hunger and starvation. Quantity rather than quality is the more important matter of discussion there: on 19 June 2009 the BBC reported that about 1 billion people were suffering from hunger (BBC, 2009; FAO, 2009). Every year more than 9 million people, mostly children, die from hunger not only in developing and newly industrialized countries, but also in industrialized countries.

But what is quality? It can be defined as 'the sum of the different properties and characteristics of a product or an activity fulfilling the desired requirements' (*Römpp Lebensmittelchemie Lexikon*, 1998). This definition corresponds to the Latin word '*qualitas*'. With respect to an objective evaluation of the quality of a foodstuff, several factors have to be taken into consideration: sensory quality (flavour, taste, colour, etc.), nutritional quality (content and chemical state of nutrients), safety (pathogens), toxicology (toxins, residues, etc.) and process-related factors. Therefore egg quality can be objectively defined as the sum of all sensory, nutritional, safety and process-related characteristics of eggs. In short: 'Egg quality is the sum of all quality factors, which are significant for the usage of eggs as a foodstuff'.

5.2.1 Objective egg quality and subjective appreciation

Beside objective quality aspects of eggs in addition there are subjective aspects of egg quality, which are summarized under the term 'estimation'. The consumer estimates the quality of an egg according to his or her specific subjective demands. The distinction between quality and estimation is as follows: quality is to be found in the egg itself, whereas estimation of the egg is located in the head of the consumer. Furthermore, the way in which we

estimate a product is influenced not only by individual and social factors, such as habits, tradition, image, ecological, ethical, religious and political aspects, but also by prejudice and sometimes by certain fears (Schwägele, 2001). Nowadays egg production in Europe has to operate under EU regulations, and also respond to consumer demands. Quality assurance systems for eggs have to take into account the requirements of both those parties to provide eggs of acceptable quality.

5.2.2 Expectation of the consumer – external and internal egg quality

Systems for quality assurance in Europe always have to be considered in terms of both legislation and the present demands of the consumer. Basic requirements concerning egg quality are provided by European legislation (Council Regulation 2007/1234/EC; Commission Regulation 2008/589/EC) and compliance with the Regulations is supervised by agencies appointed for the purpose in each member state. There are three main questions relating to egg quality: (1) What is a quality egg? (2) Which factors influence egg quality before laying? (3) Which factors determine egg quality after laying? (Preisinger, 2000; Russell and Pattern, 1994). It is necessary to establish a quality assurance system covering the whole egg supply chain starting at the very beginning in the hatchery and ending at the point of retail. Each of the various steps in egg production has to be covered by an integrated hazard analysis and critical control point (HACCP) plan (Lücke, 1998), which helps to exclude poor quality eggs from the supply chain and to meet consumer demands.

External egg quality

The most important quality criterion is the egg weight. The required optimum weight is between 53 and 73 g corresponding to eggs of the sizes Medium (M) and Large (L). Factors influencing the resulting egg weight are genetics, health conditions and hen diet. Genetically identical hens produce eggs of different weight depending upon management. There is a positive correlation between the weight of the hens and the resulting egg weight. Furthermore the weight of an egg can be positively influenced by the intake of linoleic acid, sulphur-containing amino acids, controlled regulated feeding and lighting programmes, which beside the genetics of the hens are also important for the yolk percentage in eggs. Early stimulation results in a reduced egg weight, whereas delaying the onset of sexual maturity (using a step-down lighting regime) results in higher egg weight. Because egg weight also depends upon the age of the hens, it is not easy to produce eggs of the weight grades required in practice. Therefore the most important objectives with respect to egg weight are (a) to realize a fast increase in egg weight (less than 50% small (S) eggs in the age of 22–23 weeks) and (b) to stabilize the weight level beginning with the 45th week (a maximum of 15–20% extra large (XL)

eggs at the end of the laying period). Furthermore, it should always be kept in mind that the demands of the market can change at short notice.

The colour of the eggshell is very important to the consumer, who prefers a uniform white or brown without extreme deviations. The eggshell is expected to be clean, unwashed and unbroken and to have a stability of more than 30 newton. Eggshell colour depends to a large extent upon the health and genetics of the laying hens. Improving egg quality in terms of eggshell colour can be considered a significant objective for breeders intending to satisfy consumer requirements.

Internal egg quality

External egg quality is without doubt of great importance to the consumer, but the internal quality of eggs should not be forgotten. What are the decisive quality parameters for the internal components of hen eggs? The colour of the yolk is of outstanding importance and should always be considered in relation to varying consumer expectations in different European countries. These subjective colour demands vary between a light yellow and a deep orange. Yolk colour is greatly influenced by various components provided as feed ingredients (various types of carotenoids).

More objective methods are applied when determining the freshness of an egg. A small air space (< 2 mm) and relatively high Haugh units (>75) are good evidence that the egg is fresh. Nevertheless freshness is influenced by more than one factor, including genetics and state of health, as well as the treatment of the eggs after laying.

Moreover eggs are expected to be free from blood and meat spots (Thiagarajan *et al.*, 1994), which are caused by particles consisting of either blood or lymphatic tissue from the ovary or the oviduct. Triggers for the inclusion of these foreign elements are stress on the one hand and different toxins or vitamin K antagonists on the other. Reducing the frequency of such spots is one goal of quality assurance.

Extraneous odours are also an undesirable property in eggs, which may be caused by genetic factors, hen health or feed (rape seeds, fish meal, tannins).

5.2.3 Legislation in the European Commission for marketing of eggs

There are two relevant Regulations concerning egg quality. The first deals with 'common marketing organisation of agricultural markets and on specific provisions for certain agricultural products' (Council Regulation 2007/1234/EC), and the second with 'detailed rules for implementing Council Regulation 2007/1234/EC on marketing standards for eggs' (Commission Regulation 2008/589/EC) (Table 5.1).

Regulation 2004/852/EC of the European Parliament and of the Council of 29 April on the hygiene of foodstuffs, and Regulation 2004/853/EC of the European Parliament and of the Council of 29 April 2004 lay down

Table 5.1 Content of the most importanbt articles of the Commission Regulation 2008/589/EC

Article 1	Egg means eggs in shell – other than broken, incubated or cooked eggs – produced by hens of the species *Gallus gallus* suitable for human consumption or preparation of egg products. Various other definitions are made: broken eggs, incubated eggs, marketing, operator, production site, packing centre, final consumer and producer code, according to point 2 of the Annex of Commission Directive 2002/4/EC; industrial eggs not intended for human consumption
Article 2	Quality characteristics of eggs; properties of Class A eggs (not washed or cleaned; no preservation or chilling); Class B eggs do not meet the quality characteristics of Class A eggs
Article 3	Deals with 'washed eggs'
Article 4	Grading of Class A eggs by weight; indicated using corresponding letters
Article 5	Packing centres grade, pack eggs and label the packs; requirements for packing centres
Article 6	Time limit for grading, marking and packing eggs; marking packs
Article 7	Information displayed on transport packaging (without prejudice to Article 18 of Regulation 2002/178/EC)
Article 8	Marking of eggs for cross-border delivery
Article 9	Producer Code (Point 2 of Annex to Directive 2002/4/EC)
Article 10	Indication of Class B eggs
Article 11	Marking of eggs delivered directly to the food industry
Article 12	Marking of packs (packing centre code; Class A or 'A'; weight grading; date of minimum durability; washed eggs in accordance with Article 3; advice to keep eggs chilled after purchase accordance to Article 3(1)(6) of Directive 2000/13/EC; indication of farming method); Class B eggs (packing centre code; Class B or 'B'; packing date)
Article 13	Date of minimum durability; not more than 28 days after laying
Article 14	'Extra' or 'extra fresh'; Class A eggs until 9th day after laying
Article 15	Indication of how laying hens are fed
Article 16	Information on loose egg sales (quality and weight grading; farming method; producer code, minimum durability)
Article 17	Quality of packs (shock-resistant, dry, clean, protection from extraneous odour)
Article 18	Industrial eggs (packaging containers with red band or label indicating 'unsuitable for human consumption'; name and address of operator for whom eggs are intended as well as operator who has dispatched the eggs)
Article 19	Repacking (Class A eggs only by packing centres)
Article 20–23	Records to be kept by producers, collectors and packing centres; time limits for keeping records

Table 5.1 Continued

Article 24–25	Checks and decision of non-compliance
Article 26–28	Tolerance for quality defects, egg weight and marking eggs
Article 29–30	Eggs for export to third countries; imported eggs
Article 31	Reporting of member states on number of production sites, maximum capacitiy and number of birds
Article 32	Notification of infringements
Article 33–34	Exceptions for French overseas departments and certain regions of Finland
Article 35	Evaluation of practices regarding certain voluntary labelling
Article 36	Penalties
Article 37	Communications between member states and Commission

specific hygiene rules for food of animal origin and also apply to eggs. These horizontal regulations should therefore also be taken into consideration.

5.2.4 Factors influencing egg quality before and after laying

Factors influencing egg quality prior to oviposition

Various factors influence egg quality prior to laying. For this reason, quality assurance systems dealing with egg production must cover the necessary requirements for laying hens if they are to improve egg quality at oviposition. Housing and the environmental conditions provided for the hens (Council Directive 1999/74/EC) are very important to the quality of the resulting product (Bartels, 2000). The primary purpose of housing is, therefore, to make a comfortable environment available for the layers to maximize egg production and to optimize egg quality. In such an environment, temperature and light must be controlled, humidity maintained at a moderate level, and circulated air kept free from dust and undesirable harmful substances (Stadelman, 1994). Adjustable lamps and a coloured light spectrum have a sedative effect on the laying hens. Lighting levels can also be adapted to minimize the outbreak of vices such as feather pecking and cannibalism.

Feeding (Regulation 2005/183/EC) is also a decisive factor in egg quality, and should be adapted to the requirements of the hens during the laying period (Pingel, 1993). The quality of the feed is greatly influenced by the components of the raw materials used. Therefore the biological value of different ingredients in feed mixtures has to be guaranteed. Additives in feed stuffs (ethereal oils, omega 3 fatty acids, etc.) offer the potential to change the nutritive value of eggs. Clean fresh water is also critical, and the supply should be free from microbial contamination as well as various undesirable residues (heavy metal ions and organic compounds).

Animal health is another important factor that directly influences egg quality prior to oviposition. A wide spectrum of infectious diseases exists,

which can have deleterious effects on egg production and quality. These diseases can be caused either by viruses (e.g. Newcastle disease, infectious bronchitis, various adenoviruses), bacteria (*Mycoplasma*, *Campylobacter*, coccidia, *Salmonella*, etc.) or parasites (worms), which are disadvantageous to egg quality (Fehlhaber, 1994). Toxic substances can also negatively affect egg quality and production, e.g. metabolites of microbes (mycotoxines), pesticides, fungicides, sulphonamides, specific coccidiostatica and gossypol. Furthermore metabolic diseases such as fatty liver, general liver diseases, or calcium, phosphorus and vitamin D deficiency, can result in poor egg quality. Therefore quality assurance systems must take precautions to reduce or to avoid factors that are detrimental to animal health. The necessary prophylactic steps have to be oriented to a specific established HACCP concept, which is able to recognize the critical points in egg production. The following three points are key to achieving optimal health in laying hens: (a) intensive care for the animals by specially educated personnel, (b) specific vaccination programmes, as well as (c) sufficient care for the laying hens by the veterinary services.

Factors influencing egg quality after oviposition

Numerous factors can influence the quality of eggs after oviposition. The environmental conditions (Council Directive 1999/74/EC; Pingel, 1993) that prevail in the different housing systems are important, e.g temperature, humidity, dust and odours. The conditions eggs experience throughout the supply chain, including transportation, are also critical. For this reason the frequency of egg collection must at least conform to that indicated in Council Regulation 2007/1234/EC and Commission Regulation 2008/589/EC. However, in order to assure high quality, daily egg collection is advisable. This way eggs are removed as quickly as possible from the influences of the housing climate and then stored at a temperature and humidity more suited for conservation of their quality. With respect to the whole process of marketing, beginning at the producer (farm), via collector, packing centre and retail outlet to the consumer, constant refrigeration is recommended and should be assured. Furthermore, it is recommended that eggs be stored and transported in clean, dry premises, free of extraneous odour, and be protected from shocks, weather and light effects.

The requirements for collectors and packing centres must be fulfilled (Commission Regulation 2008/589/EC; Council Regulation 2007/1234/EC) by providing sufficient area, suitable ventilation, and appropriate lighting. Transport facilities and rooms must be properly cleaned, disinfected and protected from temperature variations to avoid both microbial contaminations and reduced internal egg quality caused either by microbial growth or chemical changes. A plan for cleaning and disinfection should be prepared which provides detailed instructions for the responsible personnel. All activities with respect to cleaning and disinfection must be documented. Furthermore, it is also necessary to check the success of cleaning and disinfection by

microbial self controls corresponding to a specific designed plan (Samimi and Ball, 1994).

The origin of eggs must be indicated according to a specific code showing: nationality, type of housing, producing farm and collector/packing centre. This allows the consumer to identify an egg at any time (Commission Regulation 2008/589/EC). Providing this information ensures that consumers are free to choose which product they prefer the most.

5.3 Egg traceability

Until the end of the year 2004, food and feed business operators only had to conform to the traceability directives required by their customers. Large retailers in Europe such as Aldi, Lidl, Real, Metro, and Marks and Spencer were very rigorous in their criteria for traceability. But as of 1 January 2005, the new EU regulations made it mandatory that all food and feed business operators have traceability systems, even when their customers do not require them.

At that time many food and feed companies believed that they already had sufficient traceability procedures in place. However, even if that was the case, there were problems with the detail. The hardest impact of the new regime was on smaller companies, which were not already complying with the traceability requirements of large retail customers.

The General Food Law, i.e. Regulation 2002/178/EC of the European Parliament and the Council published on 28 January 2002:

- outlines the general principles and requirements of food law;
- establishes the European Food Safety Authority; and
- provides procedures in matters of food safety, i.e., among other things the implementation of traceability systems in the food and feed supply chains in Europe.

Article 18 of the regulation referring to traceability has been in effect since 1 January 2005. The following describes the details of the EU legislation on traceability and summarises possibilities for tracing and tracking eggs and egg products.

5.3.1 European legislation on traceability

Article 18 of Regulation 2002/178/EC refers to traceability and consists of five major points:

1. The traceability of food, feed, food producing animals and any other substance intended to be, or expected to be, incorporated into a food or feed shall be established at all stages of production, processing and distribution.

2. Food and feed business operators shall be able to identify any person from whom they have been supplied with a food, a feed, a food producing animal, or any substance intended to be, or expected to be, incorporated into a food or feed. To this end, such operators shall have in place systems and procedures which allow for this information to be made available to the competent authorities on demand.
3. Food and feed business operators shall have in place systems and procedures to identify the other businesses to which their products have been supplied. This information shall be made available to the competent authorities on demand.
4. Food or feed which is placed on the market or is likely to be placed on the market in the Community shall be adequately labelled or identified to facilitate its traceability, through relevant documentation or information in accordance with the relevant requirements of more specific provisions.
5. Provisions for the purpose of applying the requirements of this Article in respect of specific sectors may be adopted in accordance with the procedure laid down in Article 58, paragraph 2, referring to *Committee and Mediation Procedures*.

In particular, Article 58, paragraph 2 of the above Regulation 2002/178/EC says: 'Where reference is made to this paragraph, the procedure laid down in Article 5 of the Council Decision 1999/468/EC dealing with regulatory measures shall apply, in compliance with Articles 7 and 8 thereof'.

Articles 19 and 20 of Regulation 2002/178/EC cover the responsibilities of food and feed business operators respectively, and state that if an operator considers, or has reason to believe, that a food/feed which they have imported, produced, processed, manufactured or distributed is not in compliance with the food/feed safety requirement, they will immediately initiate procedures to withdraw the food/feed in question from the market where the food/feed has left the immediate control of that initial food/feed business operator and inform the competent authorities thereof.

Traceability along the full supply chain

The General Food Law covers the entire supply chain (Regulation 2002/178/EC, Article 18, paragraph 1). In order to be able to trace products and retrieve related information, producers must collect information and keep track of products during all stages of production (primary production, processing, distribution, retailin, and consumer). Traceability can therefore be divided into two key functions: tracking and tracing (Fig. 5.1). Tracking can be defined as the ability to follow the path of an item as it moves downstream through the supply chain from the beginning to the end. Tracing is the ability to identify the origin of an item or group of items, through records, upstream in the supply chain. Methodologies for the analyses of the food and feed materials combined with information

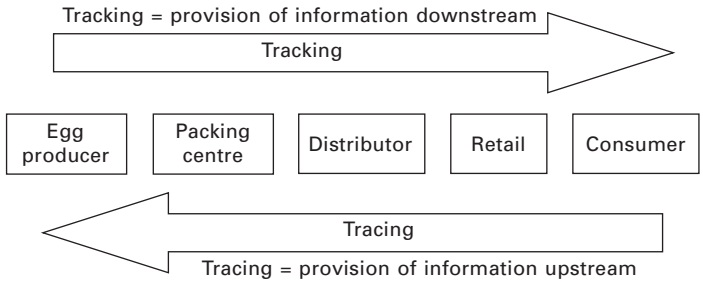

Fig. 5.1 Tracking and tracing along the food chain.

technology systems are essential to delivering a working tracking and tracing system.

Previously, it was sufficient for a processor to be able to identify the source of an ingredient. The processor is now obliged to ensure that the food products meet the requirements of food law. This implies that the source of all ingredients must be traceable and a processor must therefore be able to prove that his supplier can provide full traceability.

If any problem is suspected, tracking must go as far as the consumer. Traceability applies to everything that contributes to food safety, including packaging, closures, seals, jars, etc. Traceability also covers everything that happens to the products before, during and after their production, manufacture, packaging and distribution. This involves ingredients, processes, tests and test results, environment (temperature, time, humidity), resources used (people, machines, etc.), transport methods, timescales, etc.

Implications for egg producers

Traceability regulations have a number of implications for food processors, which they ignore at their peril. More data will have to be recorded on different levels, so who will do this and how will it be done? Data must be kept for extended periods of time. Therefore, storage and accessibility have to be taken into consideration. Gathered data have to be linked for traceability and must be highly accurate, as a data error could result in a whole consignment of products being recalled unnecessarily or even lead to a factory shut down. Data have to be collected and stored quickly. Food processors cannot afford to let data collecting affect their production costs, so all of this needs to be achieved at the lowest cost possible. Food processors cannot rely on paper records, systems that are not linked together or manual data entry. Automated data logging is the only possible option. Food processors will need integrated traceability data through production, storage, selling and quality control. Systems designed to provide instant trace enquiries through highly integrated traceable data will be required. Food processors must have thoroughly tested, proven, infallible systems, which are not as yet always in place for the whole egg chain.

5.3.2 Methods for tracing and tracking

There are several technologies available that can detect certain characteristics of (or elements in) foodstuffs from animal tissue products. Some of these technologies can be used to make definite inferences regarding the foodstuff's origin or history, while others can only be used to confirm the presence of specific components. With respect to traceability along the full supply chain of eggs, the following aspects are of importance: poultry species, origin, authenticity, age, composition and production system (including feed).

Tracking technology

Electronic data management (Automatic Identification and Data Capture [AIDC]) plays an important role in improving operational efficiency and accuracy of information handling in the 'food-to-farm' chain. Since there are no industry standards for handling electronic data throughout the entire food chain, the use of the European Article Numbering Association codes (EAN-UCC, 2002) is proposed to improve data tracking. For successful operation of this technology, the environment in which it operates must be relatively clean, and this is not always achievable on a farm.

Technologies such as RFID (Radio Frequency IDentification) overcome this problem by using radio signals instead of line of sight for identification, and can be integrated into a prototype recording system. However, product identifiers (tags) are not currently in widespread use, and are expensive in comparison to barcodes. Matrix codes are 2D, but information is stored by blanking out areas of a defined array, rather than in bars. These codes are generally only used in specialized applications, including the marking of very small components. Scanners can operate with a 90% success rate where contamination levels are kept below 10% and barcodes are kept clean and undamaged. The performance of the laser scanner is such that any level of contamination will substantially reduce the read success rate. Studies undertaken by Watts *et al*. (2003) indicate that the RFID achieves successful reads over 98% of the time, with unprotected and reused tags.

In electronic tracking and tracing systems, EAN-UCC (2002) is universally accepted as an identification and communication system that facilitates efficient global commerce, and improves the effectiveness of recording and exchanging information between supply chain participants. The system uniquely identifies products, locations, services and assets, and also includes a series of standard data structures known as application identifiers (AIs), which allow secondary information about a product such as batch, expiry and lot number to be encoded.

The EAN-UCC (2002) system consists of three components (Fig. 5.2):

1. *Identification numbers*: used to identify a product, location, logistic unit, service or asset.
2. *Data carriers*: the barcodes or radiofrequency tags used to represent these numbers. The data carriers vary according to the level of information required or the space available. For space-constrained products, the use

Fig. 5.2 Tracking technology using the EAN-UCC system (linear, 2-dimensional and matrix codes).

of reduced space symbology (RSS) barcodes is ideal. For traceability purposes, an EAN 128 barcode is used to encode the identification and supplementary information relating to an item.

3. *Electronic messages*: the means of connecting the physical flow of goods with the electronic flow of information. These technologies have been used in meat traceability, providing a robust tracking system for most elements of the meat chain (harmonized electronic data interchange, HEDI). Such electronic tracking systems play a key role in food labelling.

Authentication and detection of fraud

In order to ensure egg authenticity as well as geographical origin, and to detect fraud, various electrophoretic, chromatographic and molecular biological methods combined with other chemical and physical procedures can be very effectively applied to traceability (Schwägele, 2005). These include the following:

1. *Protected designation of origin (PDO)*. PDO covers the term used to describe foodstuffs which are produced, processed, and prepared in a given geographical area using recognized methodology.
2. *Protected geographical indication (PGI)*. This geographical link must cover at least one of the stages of production, processing or preparation. Furthermore, the product can benefit from a good reputation.
3. *Certificate of specific character (CSC)*. CSC means recognition of all member states of the EU that a foodstuff possesses specific characteristics that distinguish it clearly from similar products in the same category.

Authentification strategies, involving the use of multi-isotopic parameters (^{2}H, ^{13}C, ^{15}N, ^{18}O, ^{34}S and ^{87}Sr) facilitated by increasingly rapid measurement procedures, present a complex analytical challenge because of the many compounding factors, such as imported feed, origin of eggs, and metabolic turnover of tissue-specific substances.

Stable isotope analyses are considered an excellent tool for origin assessment. The ratio $^{13}C/^{12}C$ gives straightforward responses concerning the primary photosynthetic metabolism of feed plants (O'Leary, 1981), and the ratios of the stable isotopes of oxygen ($^{16}O/^{18}O$) and hydrogen ($^{2}H/^{1}H$) are good indicators of environmental conditions, e.g. H_2O (Ziegler *et al.,* 1976) and

enables the tracing of the origin of animal material. The two main techniques used to determine the isotope ratios of natural products are isotope ratio mass spectrometry (IRMS) and site-specific natural isotope fractionation from nuclear magnetic resonance (SNIF–NMR). NMR has the advantage over IRMS in that the natural abundance of ^{2}H isotopomers may be precisely identified in compounds and accurately quantified by SNIF–NMR (Martin and Martin, 1991), whereas IRMS only gives a mean value of the deuterium content of a given chemical species. Both low and high resolution NMR can be used for the detection of plant species and genetically modified plant or animal product material in food, but specific marker components must be isolated prior to analysis.

The geographic origin of a foodstuff can affect its composition and associated foodborne risks to the 'food-to-farm' chain. Also, less expensive ingredients or components of dubious geographical origin may be fraudulently included for monetary gain. A need exists to develop a protocol enabling a foodstuff's geographic origin to be assessed. Certain techniques can be used to 'fingerprint' the geographic origins of certain plant and animal product materials; and these methodologies can form part of a suite of traceability tests (Polychroniadou and Vafopoulou, 1985). Geographical effects arise due to differences in the geological origin of the soils, soil pH, anthropogenic contaminants, atmospheric and climatic differences, and the interaction among certain trace elements. Zoonoses risks can vary considerably from one country to another (e.g. bovine spongiform encephalopathy (BSE) risk in the UK is much greater than in the USA). Trace element analysis by inductively coupled plasma mass spectroscopy (ICP–MS) has been used to determine the geographic origin of soils, plants and fruit (Anderson *et al.*, 1999). Trace element signatures can be used to identify the geographical provenance of a sample because organisms accumulate in their tissues, from the water, food and air, the elements available from the environment in which they live. Differences in the isotope distributions of these trace elements among different geographical locations give different 'signatures' of isotopes in the organic tissues.

Gas chromatography with mass spectroscopy (GC–MS) and liquid chromatography in combination with mass spectroscopy (LC–MS) have been successfully applied to the analysis of organic contaminants (polychlorinated biphenyls (PCBs), dioxins, etc.) in the origin of various feed and food materials.

5.3.3 Vulnerabilities in the egg chain

Risk assessment, according to the Codex Alimentarius Commission, is a scientific evaluation of known or potential adverse health effects resulting from exposure to foodborne hazardous agents. The process consists of four steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment and (iv) risk characterization (CAC/GL 62, 2007). Risk assessment

is mostly directed towards the safety of the end product and consumer protection. During hazard identification the most significant hazards for the end product are identified and addressed within the scope of risk assessment or using a HACCP plan. In most HACCP plans a qualitative approach is used. By using a quantitative approach to risk assessment the hazard analysis can result in a very powerful tool for managing risks. Control measures can be validated and resources can be allocated to minimize the occurrence of hazards, i.e. contaminants at single chain steps as well as in the end product retailed to the consumer.

One of the methods applicable for quantitative risk assessment is failure mode and effect analysis (FMEA). FMEA is a systematic process meant for reliability analysis. It is a tool to assure product quality. It improves operational performance of the production cycles and reduces their overall risk level. The FMEA methodology was developed and implemented for the first time in 1949 by the United States Army. In the 1970s its application field extended to general manufacturing. Today the FMEA method is mainly applied in the industrial production of machinery and electronic components, but also in the food industry (Scipioni *et al.*, 2002). Recently it has been used within the industrial processing of snails (Arvanitoyannis and Varzakas, 2009a), the common octopus (Arvanitoyannis and Varzakas, 2009b), and ready to eat vegetables (Varzakas and Arvanitoyannis, 2009). The EU-Project ΣChain developed a modified FMEA procedure to identify, assess and address vulnerabilities in food production chains such as poultry meat. A clear and specific understanding and description of the products and processes is a mandatory prerequisite for any FMEA application. Thus the poultry meat production chain was exemplarily mapped. Flow charts were designed to identify the single steps in the chain. Following the consideration that a substantial number of contaminants may enter the poultry meat production chain via the feed chain, the latter was mapped, too. A similar study has not yet been performed for eggs. However, the elaborated poultry meat example could be used as a basis for implementing the egg production chain.

Vulnerability within the EU Project ΣChain was defined as a weakness in the system that can result in harm to the system or its operations, especially when this weakness is exploited by a hostile person or organization, or when it is present in conjunction with particular events or circumstances. This definition was applied to the poultry meat production chain in relation to contamination with agents hazardous to human health. Vulnerability was understood as a lack of traceability whereas the implementation of this traceability was understood as a combination of:

- the documentation accompanying the product;
- appropriate physical and electronic tags including the information about their application;
- identification of relevant contaminants;
- occurrence and dynamics of contaminants;

- analytical methods to detect relevant contaminants including information about appropriateness and application.

Vulnerabilities identified were rated according to three criteria, severity, likelihood (of occurrence) and detectability. Severity is the rating of the hazard associated with the vulnerability, in the sense of damage to public health and is rated from 1 (no effect) to 10 (immediate effects and/or serious effect on health). The likelihood of occurrence indicates the frequency of a vulnerability event happening. Likelihood of occurrence is rated from 1 (will not occur) to 5 (occurs on a frequent basis). Detectability or likelihood of detection/recognition refers to whether the vulnerability or event happening will be noticed or detected given the current control measures whereas a rating of 1 was understood as 'likely' and 3 as 'unlikely to be detected'.

For each potentially vulnerable chain step a vulnerability priority number (VPN) was calculated:

$$\text{VPN} = \text{Severity} \times \text{Likelihood} \times \text{Detectability}$$

Thus a prioritization of vulnerabilities or vulnerable chain steps, respectively, was achieved: The higher the VPN, the higher the priority for addressing the vulnerability.

The identified and prioritized potential vulnerable chain steps were addressed by identifying a set of control measures to reduce or even eliminate the vulnerability (reduce the VPN).

The likelihood of occurrence and detectability are understood to be possibly influenced by control measures: thus the likelihood of occurrence can be decreased as the detectability is increased by suitable measures.

It must be kept in mind that vulnerable chain steps and their ranking must be identified and estimated respectively for each individual food business operator and product and a given time. The ranking needs revising and updating regularly. It is not possible to create a generic ranking of vulnerabilities neither for the production of poultry meat nor eggs and egg products, etc. However, an FMEA could equally effectively be carried out for every specific step in the egg production chain to reduce that supply chain's vulnerability to contamination with dangerous agents and substances, and microorganisms.

5.4 Conclusions

Egg production is expected to increase to approximately 72 million tons in 2015. This would correspond to production of 1260 billion eggs per year and an average worldwide egg consumption of about 170 eggs per head of the world population per year. In the European Union egg production, producers need not only to take into account EU regulations, but also consumer demands. With respect to the quality of eggs there are two relevant Regulations: one that

deals with 'marketing standards for eggs' (Council Regulation 2007/1234/EC) and on that deals with 'detailed rules for implementing Council Regulation 2007/1234/EC on marketing standards for eggs' (Commission Regulation 2008/589/EC).

The General Food Law Regulation 2002/178/EC covers the entire supply chain and:

- stipulates that the delivery of safe food and animal feed belongs to specific food and feed producers;
- specifies that foodstuffs, animal feed and feed ingredients must be traceable;
- includes clear procedures for developing food law and dealing with emergencies;
- gives the European Commission new powers to take emergency measures when national authorities are unable to contain an emerging food risk;
- establishes the 'Standing Committee on the Food Chain and Animal Health, in the place of three Standing Committees', bringing together Member States representatives with important roles in decision-making on food safety issues.

In the area of eggs, there is a need for fast and reliable systems to enable traceability along the full chain to provide safe and high quality food for the consumer. Traceability cannot only be viewed as a legislative requirement; it is also in the interests of food business operators to find practicable ways to implement the regulation due to product liability laws. Within the 5th and 6th Framework Programmes, the European Commission has funded various research and development projects (such as ENOSEFOODMICRODETECT, 2003; ENTRANSFOOD, 2003; MOLSPEC-ID, 2004; QUALITYLOWINPUTFOOD, 2005; ∑CHAIN, 2006) dealing with traceability along the food chain. There exists the possibility of applying the results of these studies to various industries including egg production in the future. FMEA can effectively be applied to any specific egg production chain to reduce its vulnerability to contamination with the aim of providing safe and high quality eggs for the consumer.

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6

Egg formation and chemistry

Y. Nys and N. Guyot, Institut National de la Recherche Agronomique, France

Abstract: This chapter provides an overview on the structure, composition and formation of bird eggs with emphasis on chicken, *Gallus gallus*. The first part describes the global structure of the egg and its compartments (yolk, white/albumen, eggshell). The second part reviews their chemical composition in terms of lipids, proteins, carbohydrates, minerals, vitamins and pigments. The last part explains the key steps of egg formation in the liver, ovary and oviduct, providing a broadly based view on the physiology, regulation and kinetics of synthesis or secretion of egg components. It also describes the development, anatomy and role of the genital tract in this process.

Key words: chemical composition, egg, eggshell, hormonal control, oogenesis, ovary, oviduct, oviposition, ovulation, white/albumen, yolk.

6.1 Introduction

Birds are oviparous and produce a cleidoic egg, its environment being almost totally isolated from the exterior. They produce large eggs containing all the essential nutrients for the development of an embryo in an outdoor environment. The egg possesses a physical protection, the shell, but also a complex system of chemical defenses that ensures the survival of the embryo in an environment that might be hostile. The egg is the largest cell in the animal kingdom, resulting from a single cell division and containing a large range of nutrients. The diversity of egg components and their perfect balance to ensure the growth of an animal explain the exceptional nutritional

quality of the egg for humans (Volume 2, Chapter 11). This product is also remarkable because of the diversity of its putative biological functions that are anticipated by the hen to ensure the perfect development of the chick. In addition to the essential nutrients for embryogenesis, eggs contain also many molecules involved in the physical (shell membranes and shell) and chemical (antibacterial, antiviral, antioxidant molecules) protection of the embryo (Chapter 9, this volume; Volume 2, Chapter 16).

The structure of the egg is similar among different species of birds, although the proportions of the various parts described in Fig. 6.1 can vary. Egg components are produced sequentially by two different anatomical structures. The liver produces the egg yolk components that are transported via the bloodstream and deposited in the ovary. During ovulation, the largest ovarian follicle releases a mature egg yolk in the oviduct. Then specialized segments of the oviduct synthesize and secrete the constituents of the outer vitelline membrane, the egg white (albumen), the shell membranes and the eggshell that are sequentially deposited around the egg yolk. This temporal and spatial sequence of egg formation is shown in Fig. 6.2. The ovary, in interaction with the pituitary gland, controls every step of the egg formation by secreting steroid and pituitary hormones. The ovary is also the site of female gametogenesis. In addition to its secretory role, the oviduct ensures the transport of spermatozoa and fertile egg.

Nowadays, the number of eggs produced by a domestic hen is greater than 300 eggs per year. Control of the production level depends on the number of yolks produced in the ovary and on the control of their release (ovulation). This aspect will be discussed briefly in this chapter whose aim is to describe the formation of the egg. Most of studies concerning this area have been conducted on the domestic hen and have already been related in a number of reviews (Romanoff and Romanoff, 1949; Gilbert, 1979; Sauveur and de Reviers, 1988; Nys, 1994; Etches, 1996; Li-Chan and Kim, 2008).

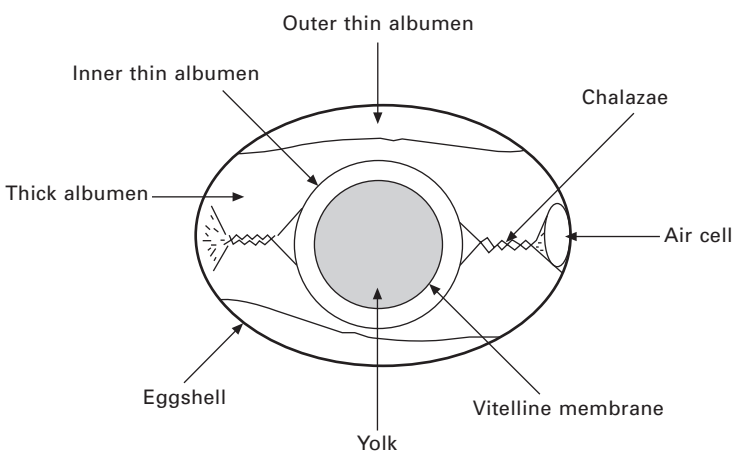

Fig. 6.1 Schematic view of a bird egg (transverse section).

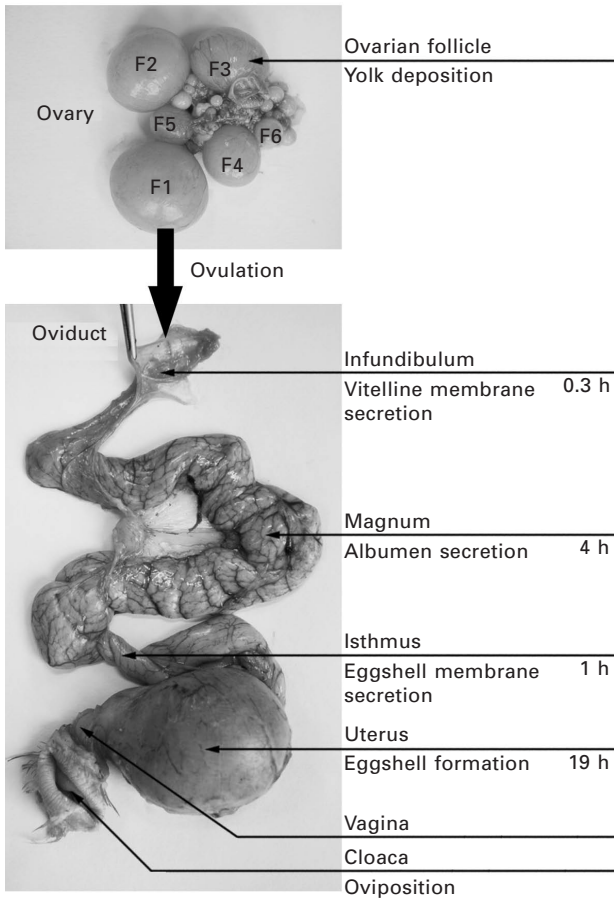

Fig. 6.2 Sequential formation of the egg in the genital tract of hens. Photographs (INRA) represent hen ovary (top) and oviduct (bottom). Phases of egg formation and their durations (in hours) are indicated below each associated site. Ovarian follicles (identified F1 to F6) in the ovary are the pre-ovulatory follicles of the ovarian hierarchy.

6.2 Structure of the egg

6.2.1 General description and characteristics

As seen in Fig. 6.1, the egg is composed of various compartments (from inside to outside): the egg yolk, the vitelline membrane, the egg white or albumen, the shell membranes and the shell. In the domestic hen, proportions of the egg compartments vary depending on the genetic origin of animals, on the egg weight at a given age of the hen, but above all on the age of the hen during a production cycle (Table 6.1). Other parameters can also affect these proportions including the diet, the management of hens (early stage, lighting programme, rearing) and their environment (temperature) (see Chapters 12 and 13).

Table 6.1 Proportion of the different egg compartments in the hen's egg

		% total egg weight	
	Mean weight for a 60 g egg (g)	Mean value	Range of variation (at different egg weights)
Yolk	17.3	29	25–33
Albumen	37	61.5	57–65
Shell membranes	0.25	0.4	
Shell	5.5	9.1	8.5–10.5

Table 6.2 Proportion of the different egg compartments in domestic bird species

Species	Egg weight (g)	% egg yolk	% albumen	% shell, including membranes
Quail	8–10	30–33	52–62	7–9
Muscovy duck	75–85	33–37	50–53	11–13
Khaki duck	55–65	33–36	53–56	9.5–11
Peking duck	92	33	57.2	9.5
Turkey	80–90	31–35	54–58	8.5–10.5
Pheasant	29–32	30–32	52–55	9–10.5
Grey goose	155	30–33	55–58	11–13
Pigeon	18	18–22	65–75	7–9
Guinea fowl	35–45	25–35	50–60	15
Hen (*Gallus*)	50–70	25–33	57–65	8.5–10.5

The structure of the egg is similar whatever the bird species considered. Egg size is usually characteristic of the species, approximatively related to the size of the parents (5 to 15%) and increases with hen age. The weight and size of avian eggs vary within three orders of magnitude, for instance from 1.9 kilograms (ostrich) to 0.5 grams (hummingbird). The extreme in egg size is observed in wild birds, being 25% and 3% of the body weight in the brown kiwi and in the cuckoo, respectively. The largest egg was that of the extinct Madagascan elephant bird (more than 9 kg, 34 × 24 cm!). The proportion of the different egg components is relatively stable among domestic birds (Table 6.2, Chapters 21 and 22), while the egg weight varies from 10 g (quail) to 160 g (goose). Overall, the proportions of egg yolk, egg white and shell vary respectively from 25 to 35%, 50 to 65% and 8 to 14%. The proportion of shell is thus the most variable. Eggs of pigeons (nidicolous species) are, however, a specific case since their yolk is proportionally smaller than the yolk found in other domestic nidifugous species. Across a wide number of bird species, the mass of eggshell has remained proportional to the egg's mass (Ar *et al.*, 1979) representing 10–11% of egg weight with the exception of a few species that have a larger proportion of shell (guinea fowl, Muscovy duck). Also, a strong relationship between shell weight and breaking strength of a whole egg subjected to a static compression test can be observed with the exception of a few species that have a particular crystalline structure of the shell (guinea fowl, duck, goose) (see Chapter 8).

The shape of the egg is usually oval and can be characterized by its shape index: SI = D/L, where D represents the diameter (minor axis) measured at the equator and L is the length (major axis). This index varies mostly between 0.7 and 0.75, the extreme values are ranging from 0.65 to 0.85. A young hen's egg, which is rounder than an egg at the end of production cycle, is more resistant to static pressure.

The surface (S) of an egg can be estimated taking into account its dimensions:

$$S = k_1(\pi LD^2/6)^{0.67}$$

where k_1 is between 4.63 and 5.07, or basically using the weight (W) of the egg:

$$S = k_2 W^{2/3}$$

where k_2 = 4.67, 4.68 or 4.69 for eggs with a weight respectively below 60 g, between 60 and 70 g or greater than 70 g.

The volume (V) of an egg can be calculated taking into account its diameter and its length:

$$V = k_3 \pi LD^2/6$$

where k_3 = 0.85 to 0.99 ; alternatively it can be estimated using the weight W of the egg:

$$V = 0.913 * W$$

6.2.2 Internal structure

The egg contains in its centre the yolk (Fig. 6.1) wrapped with a very thin transparent acellular membrane called the vitelline membrane. The function associated with this protein extracellular membrane is to limit exchanges of material between the egg white and the yolk; it is also the ultimate barrier against bacterial penetration (Burley and Vadehra, 1989). The vitelline membrane consists of three layers (or four according to some authors who include zona radiata in the membrane). The thin continuous middle layer (4 µm thick) is composed of an amorphous material located between two fibrous layers, one in contact with the egg yolk, the other in contact with the egg white. The inner perivitelline layer (4 µm) is a layer of interlaced fibers corresponding to the zona pellucida in mammals. Parts of its constituents are secreted by the granulosa cells (Takeuchi *et al.*, 1999) and the other part by the liver. In this latter case, components are transported by the blood (Bausek *et al.*, 2000). This layer contains a very thin inner layer, called the zona radiata, displaying numerous villi at the blastodisc. The two outer layers of the vitelline membrane (4 µm middle layer and 6 µm fibrous layer) are secreted by the most proximal part of the oviduct, the infundibulum, following ovulation of the yolk into this organ.

At the surface of the yolk, there is a clear disc (3.5 mm diameter) called the blastodisc. Initially it contains the female chromosomes and corresponds to the site of multiplication of embryonic cells when the egg is fertilized in the infundibulum. The female pronucleus is present in a standard physiological medium, different from the lipid globules of the yolk where the blastodisc is floating. In a fertile egg freshly laid, the blastodisc contains 30 000 to 40 000 cells. The size of the blastoderm is approximately 4.4 mm in diameter, its central region (area pellucida) is transparent and surrounded by an opaque area in contact with yolk globules.

In the fresh egg, the yolk is maintained in the center with the help of two spiral filaments called chalazae, which link the two opposite poles of the yolk to the shell at each extremity of the egg. These chalazae coat the yolk and roll up during the rotation of the forming egg to form a spiral junction, linking the opposite sides of the yolk to each pole of the shell. These 'suspensions' are wound clockwise at the 'pointed' end of the egg and counter-clockwise at the 'large' end. These chalazae are the rotation axis of the egg and contribute to keep the blastodisc (containing the germ cell) in a practically stable position.

The egg white (or albumen) is a heterogeneous medium consisting of three parts (in addition to chalazae) that can be differentiated by their viscosity. The inner liquid layer (17% of the total weight of albumen) in contact with the yolk is surrounded by the thick white (57%). This latter viscous albumen is directly in contact with the shell at the small and large ends of the egg, and separated from the shell by the outer liquid layer (23%) in the other areas. The proportions of liquid and viscous albumen vary during storage of the egg. The egg freshness can be measured by assessing the thickness or also by spreading the liquid white on a flat surface (see Chapter 10) as seen in Fig. 6.3. The proportion of each area also varies depending on the weight of the egg. When the egg weight increases during the laying cycle, an increase of the proportion of thick white can be observed to the detriment of liquid white. In contrast, the proportion of outer liquid white strongly increases during egg storage.

The egg yolk and white are surrounded by the shell membranes that determine the shape of the egg in the uterus, before the shell deposition, when egg white proteins are fully hydrated (Fig. 6.4). Between the inner shell membrane and the albumen of the infertile egg or the chorioallantoic membrane at the end of embryonic development, there is a globular nonfibrous layer (less than 190 nm in thickness) called the peri-albumen (Sultana *et al.*, 2003) which may be involved in the limitation of bacterial penetration. The inner (20 μm) and outer (50 μm) shell membranes (Fig. 6.5a) form a layer 70 μm-thick, which consists of an interlacing of protein fibers (Fig. 6.5b). These fibers are composed of a central protein core coated with glycoproteins (0.13 nm thick). The diameter of these fibers is 0.5 and 0.8 nm respectively in the inner and outer membranes. The interlacing density is more important in the outer membrane.

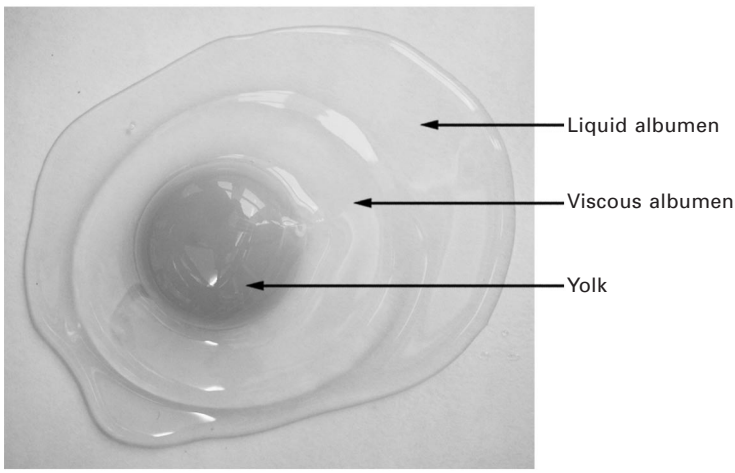

Fig. 6.3 Internal egg material spread on a flat surface (Photograph INRA). Note the difference of density between the viscous and the liquid albumen.

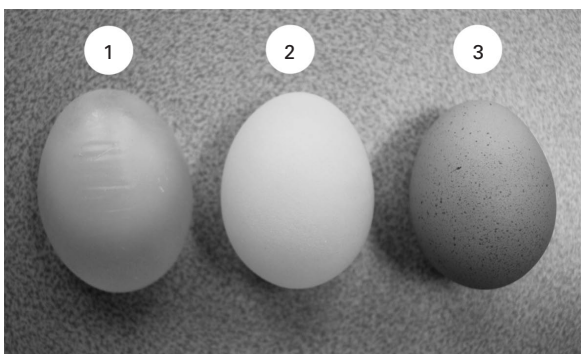

Fig. 6.4 Eggs at different stages of shell formation (photographs INRA); 1, egg in the isthmus (secretion of eggshell membrane); 2, egg in the uterus (calcification of the eggshell); 3, egg after oviposition.

6.2.3 External structure

The existence of a perfectly defined structural polycrystalline organization throughout the calcified eggshell has been known since the earlier studies of Von Nathusius (1821–1899) whose papers were translated and edited by Tyler (Tyler, 1964), and also in numerous reviews (Nys *et al.*, 1999, 2004). A detailed presentation of the eggshell structure is provided in Chapter 8. The shell structure is similar among different species of birds. By scanning electron microscopy, six layers can be observed (see schematic view in Fig. 6.5a).

The inner part of the eggshell comprises two shell membranes consisting of interlacing protein fibers that prevent egg white from spreading out towards

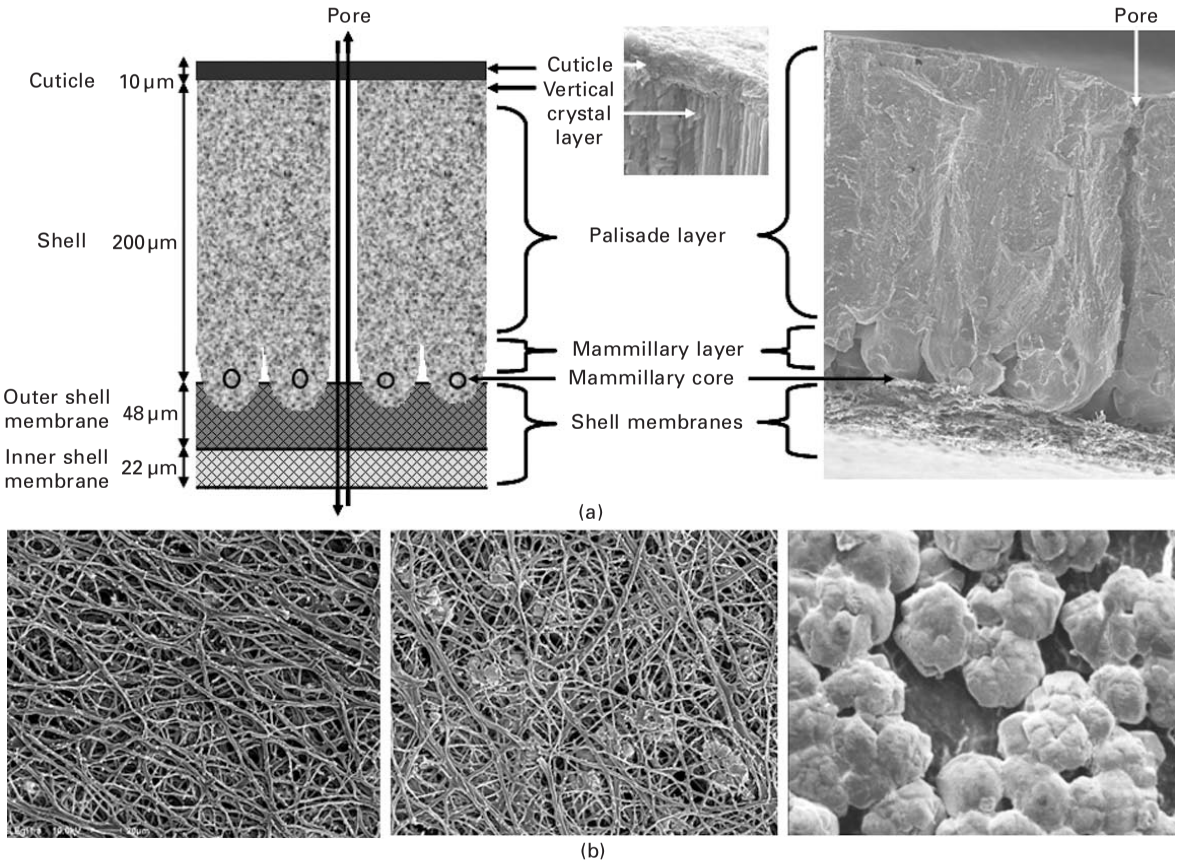

Fig. 6.5 Structure and formation of the eggshell. (a) Transverse section of the eggshell: schematic view (left) and scanning electron photomicrographs of the shell and shell membranes (right) and of the upper area of the shell (center). (Photograph McKee, University of Montreal). (b) Surface of forming egg during eggshell formation observed by scanning electron microscopy: interlacing fibers of eggshell membranes (left), presence of mammillary cores on eggshell membranes (center) and initiation of shell formation by the fusion of calcium carbonate crystals (right). (Photographs McKee, University of Montreal; Solomon, 1991).

the shell. The mineral portion is anchored on nucleation sites, the mammillary bodies, located at the surface of the outer shell membrane (Fig. 6.5b). The multidirectional growth of calcite crystals that are limited toward the inner side by the membrane, is responsible for the formation of inverted cones (mammillary layer) that join to form a compact layer called the palisade layer towards the outer side. This layer is formed by the juxtaposition of irregular columns with diameter ranging between 10 and 30 μm. Its thickness is around 200 μm, corresponding to two-thirds of the eggshell. Its role is crucial in determination of eggshell breaking strength.

The palisade layer consists of columns of rhombohedral calcite crystals of defined sizes being larger toward the eggshell surface. It is thought that the high degree of control of size, shape and orientation of the crystals of calcite in avian eggshells is responsible for its unique ultrastructure and exceptional mechanical properties (in hen, egg breaking strength is 30 N for a mean eggshell thickness of 0.33 mm). The majority of studies indicate two preferred orientations for the calcite crystals (Sharp and Silyn-Roberts, 1984), the crystal orientations being predominantly toward the surface of the palisade layer as compared with regions close to the shell membranes. The orientations 104 and 006 dominate until the 18 h stage of eggshell mineralization; thereafter, the 006 orientation is predominant. (The *C*-axis of the calcite tends to be perpendicular to the eggshell surface; Garcia-Ruiz and Rodriguez-Navarro, 1994). Specific differences in shell ultrastructure in terms of crystallographic texture were observed among domestic bird species (Panheleux *et al.*, 1999). Of particular interest is the eggshell of the guinea fowl because of its exceptional breaking strength relative to that of the domestic hen. Such high mechanical strength results from the larger ratio of eggshell weight to size in guinea fowl and to its particular crystallographic texture. The observation by optical microscopy using thin slices (<30 μm) of radial sections and cross-polarizers revealed that the adjacent calcite crystals in the upper palisade layer are intricately interlaced (Fig. 6.6) in contrast to that of hens where crystal columns remain separated. The palisade layer ends in a thin vertical crystal layer aligned perpendicular to the shell surface. Such a structure is particularly evident using crossed polarizers to reveal different crystallographic orientation of the columns (Fig. 6.6).

The cuticle, an organic layer, is laid on the surface of the egg and contains a large part (two-thirds) of the superficial pigments (Nys *et al.*, 1991). The eggshell is made of calcite but a thin layer of hydroxyapatite crystals has been demonstrated in the inner cuticle (Dennis *et al.*, 1996). About 10 000 pores penetrate the eggshell (200 pores/cm^2) and are plugged by the cuticle. They allow and control the exchange of water and gases during the extra-uterine development of the chick embryo. Many variations in the type, number and thickness of these eggshell layers have been described for taxonomic purposes. A clear descriptive review of these variations as seen by scanning electron microscopy can be found elsewhere (Mikhailov, 1997).

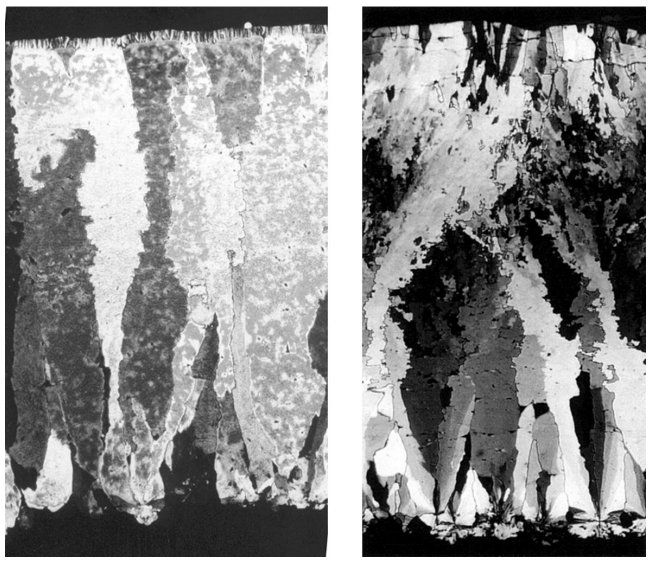

Fig. 6.6 Transverse section of eggshell viewed in cross-polarized light (Photographs J.M. Garcia-Ruiz, University of Granada). Photomicrographs showing the orientation of calcite crystals in the eggshells from turkey (a) and guinea fowl (b) eggs. Note the presence of the thin vertical crystal layer at the top of the turkey shell (a) and the presence of interlaced calcite crystals in the upper palisade layer of the guinea fowl shell (b).

6.3 Composition of the egg

The egg is composed of about 59% white and 31% yolk, contained in a shell corresponding to 10% of the total egg weight. Concerning the edible portion, the whole hen egg contains 74.4% water and two sets of major nutrients: proteins (12.3%) and an equivalent quantity of lipids (11.6%). All the vitamins (except vitamin C) are present, as well as many minerals and trace elements.

Basically, the egg can be considered as a low energy source (148 kcal per 100 g) of proteins that fits the nutritional needs of humans because of its high lysine and sulfur amino acids contents. The egg is also a source of easily digested fats, choline- and cephalin-rich phospholipids, unsaturated fatty acids and cholesterol. It is also an important source of phosphorus, sulfur and many vitamins. Detailed data on the nutritional value of eggs are presented in Volume 2, Chapter 10.

6.3.1 Composition of the egg yolk

The egg yolk represents about 30% of the full egg weight and contains more than 50% dry matter. A mass of 100 g of product supplies 16 g of proteins

and more than twice of this amount regarding the lipids. All egg lipids are contained in the egg yolk where they are bound to proteins to form lipoproteins. Lipids are about 35% of fresh egg yolk and about 65% of its dry matter. Triglycerides (65%) are the main lipids in the egg but phospholipids (31%) and cholesterol (4%) are also present at lower levels.

Egg lipoproteins are produced in the liver, and then transported to the ovary in the form of vitellogenin and very low density lipoproteins (VLDL). These precursors are transferred by endocytosis following their binding to oocyte-specific receptors, without any modification. Therefore, it is not possible to change the global lipid content in egg yolk by modifying the lipid content in the food of hens. However, the profile of fatty acids in egg yolk strongly depends on the food diet (Volume 2, Chapter 14). Although hens are classically fed with wheat, corn and soya, eggs have low levels of saturated fatty acids (about one third). Compared with other lipids of animal origin, egg lipids contain large levels of unsaturated fatty acids.

Proteins

Some free globular proteins, such as livetins, phosvitin and also some minor proteins are present in the egg yolk. Livetins are made up of serum albumin (α-livetin), glycoproteins (β-livetin) and immunoglobulins (γ-livetin), representing respectively 14%, 41% and 45% of total livetins in egg yolk (Jolivet *et al*., 2008). Minor proteins only represent about 2% of egg yolk proteins, but correspond to a large variety of proteins with 86 new proteins having been identified recently by proteomic approaches (Mann and Mann, 2008). However, most egg yolk proteins are bound to lipids to form low density lipoproteins (LDL) and high density lipoproteins (HDL). LDL represents 68% of the egg yolk dry matter whereas HDL amounts are about 16%. The exhaustive proteomic study conducted by Mann and Mann (2008) confirmed the presence of major proteins (serum albumin, apovitellenins, phosvitin, egg yolk glycoproteins, vitellogenins) in the egg yolk, but also vitamin-binding proteins (retinol, vitamin D, biotin, riboflavin), immunoglobulins Y, proteases (nothepsin and thrombin) and antiproteases, antioxidative enzymes, blood serum and albumen proteins.

Lipids

Egg lipids are exclusively linked to egg yolk proteins to form lipoproteins. They are composed of triglycerides (65%), phospholipids (31%) and cholesterol (4%). The stability of the global composition of lipoproteins – reflecting total lipid composition – is in relation to liver synthesis and the transport of their blood precursors (vitellogenin and VLDL) to the ovary. The invariable composition of VLDL (Griffin, 1992), and their small and stable size (30 μm) allow them to cross the basal layer of theca interna of ovarian follicules, before their incorporation into the yolk by endocytosis involving a specific receptor (Schneider and Nimpf, 2003). These physiological mechanisms result in a remarkable stability of the total lipid content in yolks. If hens are

not in a situation of nutrient deficiency, the diet has little effect on the lipid content of egg yolk. Deficiency in linoleic acid (C18: 2) in the hen's food is a long-established case known for its effect since it results in a reduced lipid synthesis and therefore a reduced weight of the yolk.

Considered as a negative aspect of the nutritional quality of the egg, cholesterol content in the yolk is difficult to modify as demonstrated by the number of trials aiming to achieve it. Even if cholesterol can be influenced by the genetic origin of the hen, the proportion of yolk versus white is the most important criterion. Moreover, reducing significantly the cholesterol concentration in the egg is very difficult to achieve without impairing physiological functions of the hen. Indeed, the composition of VLDL, precursor of the egg yolk components, is extremely stable regarding the proportion of its lipid and protein constituents. In addition, cholesterol content cannot be reduced without disrupting the synthesis of yolk constituents in the liver. Cholesterol is a normal constituent of VLDL, its level in VLDL representing 95% of total concentration. The cholesterol content in the egg is therefore dependent on the hepatic synthesis of lipoproteins but not on hen blood cholesterol level (Griffin, 1992). Whereas non-esterified forms of cholesterol bound to phospholipids at the surface of VLDL are predominant (80%), only the remaining 20% that are present in the cores are potentially modifiable.

Carbohydrates

The egg yolk contains 0.7 to 1% of carbohydrates including 0.3% of free glucose. Other carbohydrates are bound to proteins (glycoproteins) or lipids (glycolipids such as cerebroside). Sialic acid (*N*-acetyl-neuraminic acid) is mainly present in the vitelline membrane, but also in the yolk at a concentration of 5.2 $mg.kg^{-1}$ (Koketsu, 1997).

Minerals and vitamins

Egg yolk has high levels of phosphorus. Some 60% of phosphorus is included in phospholipids. This egg compartment also contains most of the iron in the egg, as well as a number of trace elements. Sodium content is low in egg yolk by comparison to egg white.

Egg yolk contains all the fat-soluble vitamins in the egg. It also has high levels of water-soluble vitamins by comparison to egg white, except for niacin and riboflavin.

Carotenoids

The color of egg yolk is due to carotenoids accumulated in the egg (see Chapter 12). The hen does not have the ability to synthesize carotenoids. Therefore all carotenoids exported into the egg come from the carotenoids found in the hen's food. The hen stores preferentially xanthophylls (carotenoids with a hydroxyl group) in its body fat and in the egg yolk lipids.

Colour, intensity, shade and homogeneity of the egg yolk are directly

dependent on the choice of raw materials in the hen's food or on the supplementation by sources with high levels of carotenoids. Yolk colour is carefully controlled by egg producers, since it strongly influences consumer's perception. The acceptability of a product and its desirability strongly depend on its appearance. This is the case of the egg yolk colour which is perceived as one of the priority criteria of egg quality. The preference for a given intensity of yellow varies depending on geographical areas, on consumer's habits which themselves fluctuate in time, with a trend to an intensification of the desired colour.

6.3.2 Composition of the vitelline membrane

The vitelline membrane is the extracellular protein membrane covering the yolk. It is composed of glycoproteins (GPM I, II, III) that might correspond to the major proteins of the zona pellucida (ZP1, ZP3, ZPC, ZPD and other ZP) (Takeuchi *et al*., 1999; Smith *et al*., 2006). ZP proteins play an important role in the interaction with the sperm cell. *In vitro* studies demonstrated that ZPD and dimeric ZP1 are able to induce sperm cell activation while ZPC and monomeric ZP1 have no such effect (Okumura *et al*., 2004). The outer layer contains ovomucin, lysozyme and the vitellin membrane outer membrane proteins (VMO I and II). A study performed on quail eggs suggested that this outer layer of the vitelline membrane corresponds in fact to the innermost portion of the chalaziferous layer surrounding the egg yolk (Rahman *et al*., 2007). Mann previously showed that VMO II corresponds to avian β defensin 11 and that numerous enzymes (ATPases, proteases) are present in the vitelline membrane (Mann, 2008). It has been demonstrated in quail eggs that VMO II (avian β defensin 11) is associated with the vitelline envelopes and mediates the binding of the outer layer (chalaziferous layer) with the inner layer of vitelline membrane via interaction of VMO II with ZP1 and ZP3 (Rahman *et al*., 2009). Among the 137 proteins identified in the vitelline membrane by proteomic tools, Mann (2008) confirmed the presence of proteins firstly identified in the vitelline membrane, including several proteins of the egg yolk (serum albumin, immunoglobulins, apovitellin, apolipoprotein B) and white (ovalbumin, lysozyme, ovomucin). The latter proteins may reflect the presence of chalazae surrounding the egg yolk. This study also demonstrated the presence of additional proteins (mucins, additional ZP, serine protease, Na-K ATPase, ecto-ATP-diphosphohydrolase, ovocalyxins 32 and 36, ovocleidins 17 and 116, olfactomedin I, semaphorin C3, actin, filamin) whose functions remain to be identified.

6.3.3 Composition of the egg white

Egg white is composed of water, proteins and some minerals and vitamins. It also contains free glucose (0.4–0.9%) at a concentration two times higher than that found in blood plasma. Water is the major constituent of egg white;

its content represents 84–89% in this compartment but it varies depending on the egg white area considered (Table 6.3).

Proteins

Proteins represent about 90% of the dry matter of egg white which is composed mainly of globular glycoproteins. Six of them account for 86% of total proteins in albumen. Recently, proteomic analysis revealed a total of 148 proteins in egg white (Mann, 2007; D'Ambrosio *et al.*, 2008) (see Chapter 7). Overall, major proteins are characterized at biochemical and functional levels. The description of their biological activities has given rise to numerous reviews (Rehault *et al.*, 2007; Mine and D'Silva, 2008) (Chapter 9 in this volume; Volume 2, Chapter 16). These proteins are particularly remarkable due to their antimicrobial properties covering a broad spectrum of activities. They play a major role in the innate defense of the egg which is devoid of immune cells. These molecules exert their antimicrobial activity according to four main mechanisms: (1) chelation of vitamins or minerals essential for microbial growth; (2) direct degradation of the pathogen; (3) inhibition of bacterial proteases involved in pathogen invasion; and (4) limitation of adhesion of the pathogen to host surfaces.

Carbohydrates

Egg white contains 0.8% carbohydrates. Half of these carbohydrates are in the free form, mainly glucose at 98% (Li-Chan and Kim, 2008). The other half is composed of monosaccharides, *N*-acetylated amino sugars, uronic acids and sialic acids. Glycans are bound to proteins by an *N*-glycosidic link between the glycan and the amide group of asparagine (ovalbumin, ovotransferrin, ovomucoid and avidin) or by an *O*-glycosidic link between the glycan and the hydroxyl of the alcohol function of serine or threonine (β ovomucin). The gel structure of the egg white is linked to the relative proportions between α and β ovomucins which notably differ by their carbohydrate contents.

Minerals and vitamins

The egg white contains all minerals essential for the development of the embryo. This remarkable diversity is of interest in human nutrition, since the egg covers a significant part of the daily needs in humans, especially regarding

Table 6.3 Water contents in the different egg white areas

	Proportion of total albumen weight (%)		Water contents (%)
	Mean values	Extreme values	
Outer liquid white	23.2	10–60	88.8
Thick white	57.3	30–80	87.6
Inner liquid white	16.8	1–40	86.4
Chalazae	2.7		84.3

phosphorus, potassium and some trace elements (iodine, selenium) (Volume 2, Chapter 11). However, egg contents are low in sodium and calcium. Owing to its mineral composition, egg white is more like an intracellular liquid than an extracellular liquid. Whereas trace-element content can vary depending on food diet, macro-element content is stable (Volume 2, Chapter 15). In contrast to sodium and potassium contents that are stable within the different compartments of egg white, contents of divalent cations (Ca^{++} and Mg^{++}) linked in part to proteins, particularly to β ovomucin, are about two times higher in thick egg white than in liquid egg white. These divalent cations are thought to play a role in the viscosity of the white. Egg white contains low levels of vitamins. Moreover, it contains no liposoluble vitamins, but only hydrosoluble vitamins belonging to the B group.

6.3.4 Compositions of the shell membrane and the egg shell

The shell contains 1.6% water, 3.3–3.5% organic matrix when eggshell membranes are included and 95% inorganic minerals. It is mainly made of calcium carbonate (94% of the eggshell, 98.4% of its mineral part). The cone and palisade layers are pervaded by an organic matrix corresponding to 2.3% of the shell. The amount of calcium in the shell is 37.5% (2.3 g for an eggshell of 6 g) and that of bicarbonate is 58%. There are low levels of numerous minerals and trace elements. The phosphorus is concentrated in the upper surface of the palisade layer and in the cuticle. Other minerals (magnesium, manganese, copper, zinc) are present throughout the shell. Manganese (7 $mg.kg^{-1}$) is known to influence the mechanical strength of the shell (see Chapter 12).

The fibrous material of the eggshell membranes was initially identified as ovokeratin, but the amino acid composition and the use of specific antibodies did not support this hypothesis (Leach, 1982; Chowdhury, 1990). Identification of desmosine and isodesmosine suggested the presence of elastin but this did not agree with the low glycine content (Chowdhury, 1990). Collagen was identified because of the presence of hydroxylysine and the observation of digestion of eggshell membranes by collagenase, and finally was revealed by immunochemistry using antibodies against types I, V and X collagen (Wong *et al*., 1984; Arias *et al*., 1997b; Wang *et al*., 2002). The amino acid composition of the membranes differs from that of collagenous tissues, suggesting that collagen is not predominant. It is therefore likely that a unique protein containing lysine-derived cross-links may be present as initially suggested by Leach (1982). It is noteworthy that intact eggshell membranes are a prerequisite for shell calcification in laying hens, as shown by the detrimental effect that disruption of eggshell membrane cross-linking by copper deficiency or aminoproprionitrile has on shell structure (Chowdhury, 1990; Arias *et al*., 1997a). Nucleation sites are present on the surface of shell membranes (Fig. 6.5b) and contain some muco-polysaccharide proteins. Keratan sulfate proteoglycan is present in these sites where the first crystals

appear (Fig. 6.5b) and their secretion coincides with the formation of the mammillae 5.15 h post-ovulation (Fernandez *et al.*, 1997), suggesting their involvement in the initiation of eggshell mineralization. Lysozyme (Gautron *et al.*, 1997) and ovalbumin (Hincke, 1995) have also been revealed in these sites.

The composition of the organic matrix of the calcified shell and the implication of these proteins in the control of the process of mineralization are described in detail in Chapter 8 of Volume 2. Briefly, eggshell matrix proteins can be subdivided into three groups: proteins that are observed in other tissues of the body, egg white proteins, and uterine proteins unique to the process of eggshell formation.

The ubiquitous proteins are widely expressed in various organs. Osteopontin is a phosphorylated glycoprotein higly expressed in the uterus during the calcification of shell (Pines *et al.*, 1995). This protein inhibited calcium carbonate precipitation *in vitro* suggesting a putative role at the completion of calcification. Clusterin is a secretory disulfide-bonded heterodimeric glycoprotein present in the eggshell matrix (Mann *et al.*, 2003) which might act as an extracellular chaperone in the uterine fluid to prevent premature denaturation of eggshell matrix proteins.

Some egg white proteins (e.g ovalbumin, lysozyme, ovotransferrin) are also found in the eggshell. Ovalbumin is localized in the mammillae of the eggshell (Hincke, 1995) and secreted in abundance in the uterine fluid at the initial stage of eggshell formation (Gautron *et al.*, 1997). Lysozyme and ovotransferrin (Hincke *et al.*, 2000; Gautron *et al.*, 2001b) are also present mainly in the basal parts of the shell (eggshell membranes, mammillae) and modify *in vitro* the calcite morphology.

The third group is made of avian proteins only secreted by the uterus where eggshell formation takes place. Ovocleidin-17, a 142 amino acid phosphorylated protein with a C-type lectin domain (Hincke *et al.*, 1995; Mann and Siedler, 1999; Reyes-Grajeda *et al.*, 2004) is present throughout the entire calcified part of the shell and modifies *in vitro* the morphology of calcium carbonate crystals. Ovocleidin-116 (Hincke *et al.*, 1999) is a 80 kDa protein abundant in the palisade layer and secreted in the uterine fluid during the active calcification phase of the shell. It corresponds to the protein core of the major proteoglycan of the eggshell (Carrino *et al.*, 1997; Fernandez *et al.*, 2001). This eggshell dermatan sulfate proteoglycan alters the morphology and size of calcite crystals *in vitro* (Fernandez *et al.*, 1997). Ovocalyxin-32 is secreted in the uterine fluid during the terminal phase of calcification and is localized in the uppermost part of the palisade layer, and in the cuticle (Gautron *et al.*, 2001a). Ovocalyxin-36 (Gautron *et al.*, 2007) is abundant in uterine fluid and is strongly upregulated during eggshell calcification. Its similarity with proteins associated with the innate immune response suggests a role in the chemical protection of the egg against pathogens. Additional matrix proteins are described in Chapters 8 and 9.

6.4 Formation of the egg: an overview

Birds are homeothermic and oviparous animals characterized by internal fertilization but external development of the embryo. Birds, therefore, exhibit many particularities. The oocyte formed in the hen's ovary contains a large amount of reserves in its cytoplasm, corresponding to the yolk. After ovulation, proteins of egg white and shell are successively deposited around the yolk in the oviduct. The egg, which is in fact a large-sized germ cell rich in nutritional reserves, is telolecithal, meaning that there is uneven distribution of yolk in the cytoplasm of the ovum. This set of nutrients packed in a shell contains all the nutritive elements needed by the embryo for its development. During the incubation period, the incubation of eggs by the hen is a specific animal behaviour, providing warmth to the eggs which is crucial for embryonic development. Another feature of birds is the fact that the female is heterogametic, unlike mammals. The sex chromosomes, called Z and W in birds, are carried by the hen, while the chromosomes WW are carried by the rooster. Unlike mammals, sperm can be stored for several weeks in the uterovaginal glands, ensuring fertilization in the proximal part of the oviduct for several weeks. During fertilization, many sperm (4 to 10) enter the oocyte and become male pronuclei (polyspermic fertilization). However, only one pronucleus derived from one sperm participates in the fusion with the female pronucleus in the oviduct before the first cell multiplications that will lead to 30 000 to 50 000 cells when oviposition (egg laying) occurs 24 h after ovulation. If the hen has not been fertilized by a rooster, the unfertilized egg is formed similarly to the fertilized egg, but without any development of the oocyte, which then contains only the female pronucleus (Etches, 1996; Gautron *et al.*, 1997; Sauveur and de Reviers, 1988; Jamieson, 2007).

6.4.1 Anatomy of the female reproductive system

The female reproductive tract in birds (Figs 6.2 and 6.7) is composed of two parts: the ovary (site of sex steroid synthesis, gametogenesis and yolk formation) and the oviduct (organ receiving the egg yolk during ovulation and successively depositing the white, the shell membranes and the shell). In birds two ovaries are initially present in the embryo, but among most species including the chicken, only the left ovary and its associated oviduct develop and are present in the laying adult. Although the right ovary is conserved in birds of prey and in the kiwi, the presence of the right oviduct is very rare. In birds, the unequal growth of gonads results in an asymmetry of the reproductive tract. The anatomy and structure of the reproductive system in birds, especially in chickens, have been described previously by many authors (Gilbert, 1979; Sauveur and de Reviers, 1988; Nys, 1994; Etches, 1996; Jacob and Bakst, 2007).

During the first 3 days of embryonic development, accumulation of primordial germ cells occurs on the left and right sides of the embryo, after which it preferentially occurs on the left side. In addition to these primordial

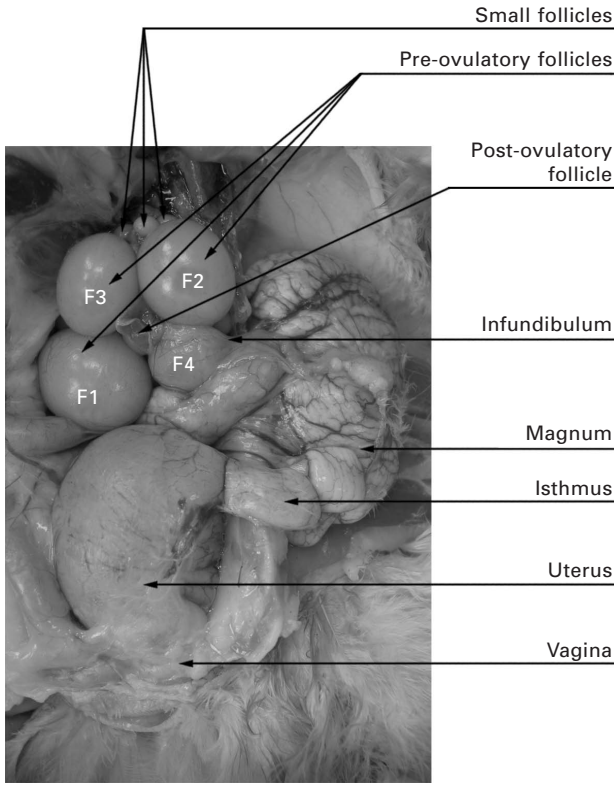

Fig. 6.7 View of the ovary and the oviduct *in situ* in the hen (Photograph INRA). Note the presence in the ovary of a post-ovulatory follicle between the pre-ovulatory follicles F1 to F4 of the ovarian hierarchy. The post-ovulatory follicle is a follicle that has released its oocyte (yolk) into the distal part of the oviduct (infundibulum).

germ cells, cells of mesodermal origin are also present in the embryo and will differentiate into granulosa cells. At day 7, the male/female differentiation of the gonad is not definitive. During gonadic development, the right gonad displays male characteristics. In newly hatched chicks, appearance of the right gonad is usually like a residue of medullary tissue, because of its regression due to the effect of steroids produced by the left gonad. In adult hens, natural or induced cessation of egg production induces a regression of both ovary and oviduct in a few days.

Structure of the ovary

The ovary is located in the medio-ventral area of abdomen. In newly hatched chicks, the ovary is a flat triangle of 6–7 mm, attached to the anterior lobe of the left kidney and weighing 300 mg. At this stage, it is mainly composed of connective tissue perfused by blood sinuses, but also interstitial cells capable of secreting steroid hormones. Three months post-hatching, the ovary is

about 1 cm in size. It consists of a highly vascularized central area (medulla) and a peripheral area (cortex) which presents a granular aspect as early as 5 weeks old due to the progressive development of follicles. The ovary grows rapidly between 16 and 20 weeks of age. Its weight increases from 5 to 60 g during this period and can reach up to 120 to 150 g in breeder hens fed *ad libitum*. The granular aspect of the ovary becomes more marked, and finally gives rise to a characteristic hierarchy of follicles. Four to six yolk-filled follicles, 2 to 4 cm in diameter, are connected by a pedicle to the ovary (Figs 6.2 and 6.7). During follicle maturation, the overall color of ovary turns from light gray to strong yellow a few weeks before sexual maturity. It is located on the dorsal left side of the abdominal cavity, in contact with the kidney. The size differences observed between follicles are marked in laying hens, whereas they are less pronounced in breeding hens especially in the situation of *ad libitum* feeding.

In the mature hen, the ovary is located in the upper part of the abdomen, under the aorta and superior vena cava, in contact with the kidney and the lung. It is very close to the left adrenal gland and both these organs are fixed to the dorsal surface by a peritoneal fold. Venous drainage of the ovary is ensured by two ovarian veins linked to the vena cava.

The adult ovary takes on the 'bunch of grapes' appearance (Figs 6.2 and 6.7) which is characteristically observed in birds. In addition to the 4–6 very large yolky follicles, numerous follicles (6 to 12 mm) in which yellow yolk deposition has been initiated and also white follicles (<6 mm) can be observed on the ovary. The largest follicle will be the next to ovulate.

Finally, thousands of other immature follicles are present on the ovary, but only a few of these follicles will generate a yellow yolk. The central area of the ovary is composed of a vascularized medulla and a cortex containing the small follicles which are oocytes covered by the follicular epithelium. The growing follicle has a characteristic structure, described in Fig. 6.8.

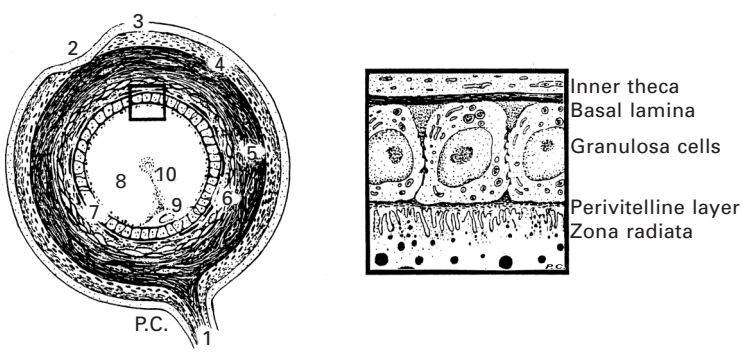

Fig. 6.8 Schematic representation of a growing follicle and a magnification of its follicular wall; 1, pedicle; 2, stigma; 3, epithelium; 4, connective tissue layer; 5, outer theca (*theca externa*); 6, inner theca (*theca interna*); 7, granulosa layer; 8, oocyte (yolk); 9, germinal disc; 10, latebra (Gilbert, 1979).

Inside, the oocyte is limited by the acellular perivitelline layer and successively surrounded by the progesterone-producing granulosa cells (single layer), the basal lamina, the inner and outer theca in which are the interstitial cells synthesizing estrogens and testosterone, and finally the conjunctiva, absent in the stigma to facilitate ovulation. All these elements are covered with an epithelium. The ovary receives its blood supply from the ovarian artery, which arises from the left renolumbar artery or from the dorsal aorta (Hodges, 1965). Arteries pass through the pedicle and thecae to form a dense capillary network in close contact with the basal lamina. Thecae are vascularized by veins and his vascular network is absent in the stigma. The blood flow is the highest in the largest preovulatory follicles.

In terms of a nerve network, follicles are also profusely innervated by both adrenergic and cholinergic fibers (Gilbert, 1979). Neurons are mainly present within the thecal layers of the largest follicles. They provide to the follicle numerous neurochemicals (catecholamines, neurotropins, vasoactive intestinal peptide, calcitonin gene-related peptides) (Johnson and Whittow, 2000; Onagbesan *et al.*, 2009).

Structure of the oviduct

The development of the oviduct is concomitant to the development of the ovary since it is influenced by ovarian steroid secretions. The oviduct is derived from the left paramesonephric (Müllerian) duct (Jacob and Bakst, 2007). In immature pullets, the oviduct is visible as a thin, pale narrow tube stretching from the ovary to the cloaca. It initially weighs a few milligrams. Its development and cellular differentiation occur mainly at sexual maturity, 2 to 3 weeks before the production of the first egg. Oviduct weight increases from less than 1 g to 40 g in 2 weeks and its size from 12–15 cm to 70 cm. At maturity, the oviduct appears as a long gray to pale pink tube. One of its ends is in contact with the ovary, while the other end opens into the cloaca. The oviduct fills a large proportion of the volume of the abdominal cavity, under the kidney. It is suspended dorsally along the ventral surface of the left kidney by a double-layered sheet of peritoneum which divides at the oviduct into a dorsal ligament attached to the dorsal wall of the coelomic cavity and a ventral ligament forming two suspensory ligaments. The oviduct, including the uterus, is vascularized at four levels from the general arterial system; innervation of the distal part of the oviduct is highly developed (Gilbert, 1979). The oviduct has a total length of 70 cm and consists of five distinguishable regions described in Figs 6.2, 6.7 and 6.9.

The infundibulum (10 cm) (Fig. 6.9a) is located in the proximal part of the oviduct. Its first function is to engulf the oocyte liberated by the largest follicle and to transport it to the next region. It is a wide-mouthed, thin-wall flexible funnel, located near the ovary. The infundibulum activity appears to be under the control of a combination of its muscles, the engorgement of blood vessels and the activity of the ligaments. Several cell types are present in the inner mucous membrane of infundibulum. They have either a secretory

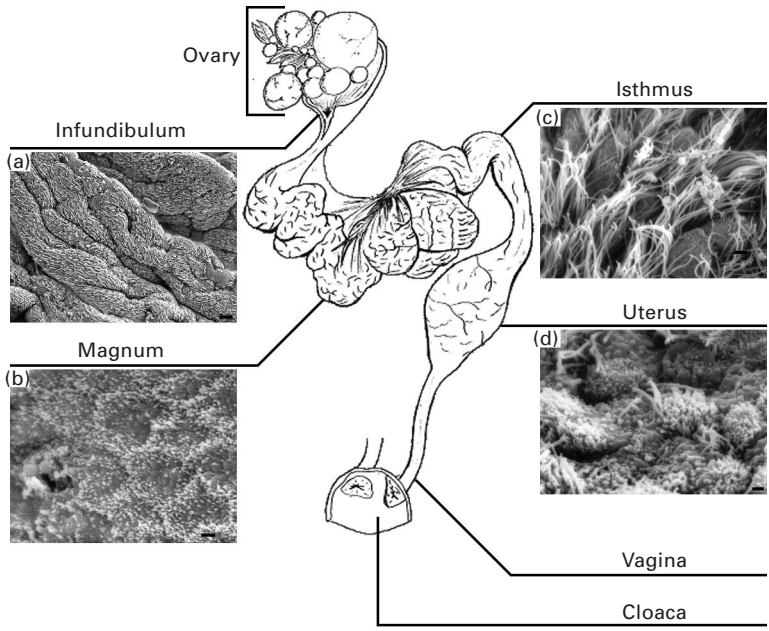

Fig. 6.9 Internal surfaces of the hen oviduct. (a) Scanning electron micrograph of proximal infundibulum. Note the predominant ciliated cells and the areas of non-ciliated cells. × 400. Scale bar represents 20 µm. Egg in the isthmus. (Chousalkar, 2008). (b) Scanning electron micrograph of surface of a mid magnum. Note the bulging of non-ciliated cells on the surface and ciliated cells embedded in between non-ciliated. Also note the secretory granules at the glandular opening. × 6000. Scale bar represents 5 µm. Egg in the top magnum (Chousalkar and Roberts, 2007, 2008). (c) Scanning electron micrograph of isthmus. × 6000. Note secretion on the surface of the ciliated and non ciliated cells. Scale bar represents 2 µm. Egg in the egg shell gland pouch (Chousalkar, 2008). (d) Scanning electron micrograph of shell gland pouch. Note the bulging of non-ciliated cells on the surface and ciliated cells embedded in between non-ciliated cells. × 6000. Scale bar represents 1 µm. Egg in the isthmus (Chousalkar, 2008).

function because of their role in outer vitelline membrane synthesis, or a function of sperm storage (infundibular sperm-host glands). The infundibulum is the site of fertilization which occurs before the covering of the oocyte by albumen.

The magnum (35 cm) (Fig. 6.9b) is the longest region of the oviduct. It is a thick, milky-white, distensible tube with well-developed folds (4–5 mm in height). The muscle layers are well developed in the magnum to facilitate the transport of the egg. This region of oviduct contains the largest number of secretory cells in both the epithelium and tubular glands. These cells contain large amount of proteinaceous material stored as granules (Fig. 6.10) which are discharged with the passage of the egg. Its internal surface is light gray

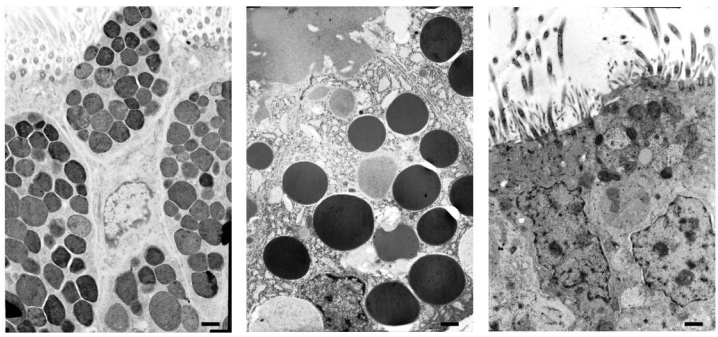

Fig. 6.10 Observation of magnum cells by transmission electron microcopy. (a) Transmission electron micrograph of mid-magnum of a hen. × 6000. Scale bar represents 1 µm. Egg in the top magnum (Chousalkar and Roberts, 2007). Note the bulging non-ciliated secretory cells. (b) Transmission electron micrograph of gland cell type A in the mid magnum. × 6000. Scale bar represents 1 µm. Egg in the top magnum (Chousalkar and Roberts, 2008). (c) Transmission electron micrograph of epithelial lining of mid-magnum. × 6000. Note the ciliated and non-ciliated cells (with microvilli). Scale bar represents 1 µm. No egg in the oviduct (Chousalkar, 2008).

colored, more or less transparent depending on the elapsed time since the last egg passage and the associated protein secretion. The distinction between the magnum and the following region, the isthmus (10 cm) (Fig. 6.9c), is marked due to a substantial narrowing of its diameter and the presence of a narrow translucent zone devoid of tubular glands. This magnum–isthmus junction is involved in the secretion of the peri-albumen layer (Sultana *et al.*, 2003). The tubular glands are more numerous in the isthmus than in any other oviduct region. Epithelial cells are also present. The tubular gland is responsible for the formation of the membrane fiber core before the deposit of the mammillary knob in the red isthmus (tubular shell gland), a tube-shaped portion connected to the pouch-like section of the shell gland (uterus). This region might indeed be part of the shell gland rather than part of the isthmus and numerous authors logically recommend calling this segment tubular shell gland because of large similarities in folding and epithelial structure.

In its distal part, the oviduct expands to form the uterus (10 cm) (Fig. 6.9d), involved in hydration of the forming soft egg and in eggshell deposition. This part has a more rounded shape and is also known as the shell gland. The uterine wall is thick and contains a well-developed muscular layer. The uterine mucosa is dark red coloured and has internal leaf-shaped folds. It differs from the utero-vaginal mucosa by the lack of direction of folds.

The vagina is a narrow, relatively short and muscular duct, often strongly curved, making artificial insemination difficult. It leads from the uterus to the cloaca at a narrow opening in its middle left part. The vaginal mucosa is devoid of secretory glands and displays long and narrow longitudinal ridges with conspicuous secondary folds which thicken in the utero-vaginal

region. In this utero-vaginal junction (1–2 cm), located at the cranial end of the vagina adjacent to the uterus, are the sperm-host glands which play a key role in the prolonged storage of sperm (Bakst *et al.*, 1994). These glands are branched tubular structures in the lamina propria of the mucosal folds with a larger diameter than the tubular glands of the uterus. The vagina is closely bound to the uterus by a thick fibrous structure and has a flared shape that is narrower in its distal part. The vaginal wall is composed of well-developed circular muscle with reduced longitudinal muscle as well as a narrow band of connective tissue in contact with the mucosa. This particular structure allows the vagina to be highly distensible for the passage of the rigid shelled egg.

The wall of the oviduct consists of a series of layers which are present throughout the organ, although mineral or protein secretions are specific in each region. The wall structure is shown in Fig. 6.11. The secretory epithelium of the oviduct is a single cell layer composed of ciliated cells and goblet or

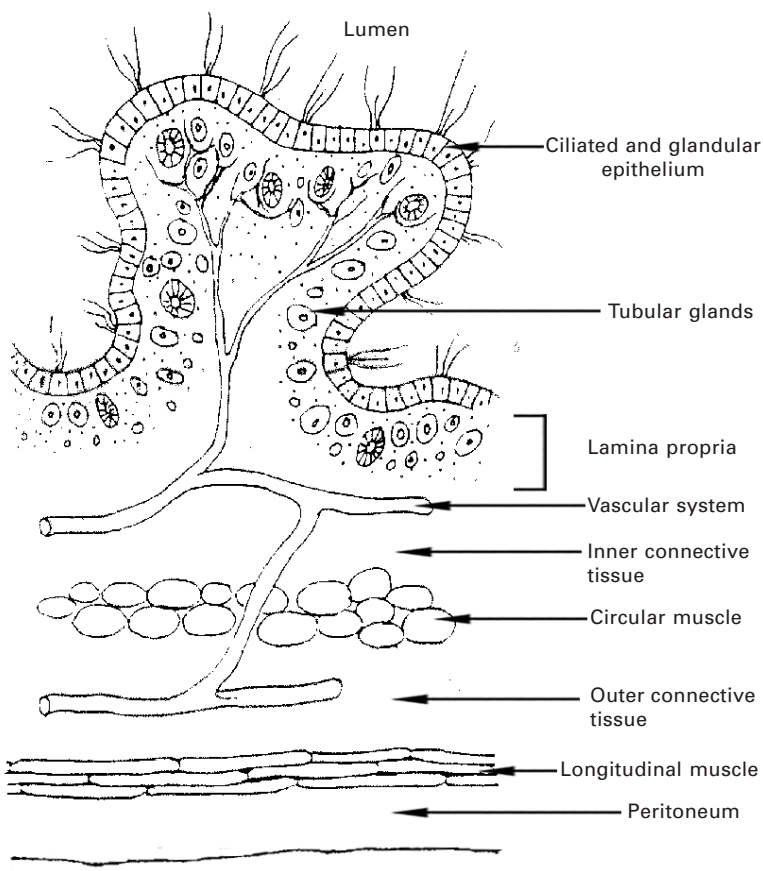

Fig. 6.11 Schematic representation of the oviduct wall (Gilbert, 1979).

mucous cells in various proportions depending on the segment (Fig. 6.9). In the layer called the lamina propria, tubular gland cells form invaginations from the surface which are absent at the magnum–isthmus junction. Only goblet cells and tubular gland cells produce secretions in the oviduct. Ciliated cells are thought to participate in sperm trafficking along the oviduct. The vascularized inner layer is separated from a first circular muscle layer by an inner connective layer and from the external longitudinal muscle layer by additional connective tissue. These muscles allow the circulation of egg and its rotation in the oviduct. At the external surface, the oviduct carries a serous membrane: the peritoneum.

As mentioned previously, the oviduct has two storage sites for sperm: one is proximal (infundibulum), the other is distal (utero-vaginal junction) (Bakst *et al.*, 1994). Sperm can survive in tubular glands (consisting of a single cell layer) and retain their fertilizing abilities in these two compartments for a period of several days to several weeks depending on the bird species. This period of fertility is up to 10 days in quail, 12 days in ducks, 21 days in chickens and 70 days in turkeys. In chickens, there are 2000 to 3000 glands in the utero-vaginal junction that store less than 1% of sperm cells. These sperm cells are strictly selected in the vagina by trapping sperm cells with low motility, or by an immune mechanism recognizing and eliminating about 90% of sperm cells carrying immunoglobulins A or G. This selection helps to maintain a sperm subpopulation of quality, favorable to good fertility and embryonic survival.

6.5 Formation of the egg yolk in the ovary

Egg yolk is an emulsion of water, triglyceride-rich lipoproteins and proteins (mainly lipovitellin and phosvitin). In addition, it contains many constituents present at low levels: serum albumin, immunoglobulins and proteins binding various vitamins (thiamin, riboflavin, biotin, vitamin D) or minerals. Two-thirds of the dry matter of egg yolk is composed of triglyceride-rich lipoproteins. During a year of production (300 eggs), a total of 1.5 kg of triglycerides and 0.75 kg of proteins are exported in the yolk.

Yolk precursors are not synthesized in the ovary but produced by the liver and then transported in the blood to the oocyte. In hens, hepatic lipogenesis and lipemia are increased, respectively, 15- and 20-fold at sexual maturity. This stimulation of protein and lipid synthesis in the liver is dependent on estrogens.

6.5.1 Formation and development of the oocyte

Female gametogenesis

Oogenesis (formation and development of the ovum) starts at day 7 of incubation of the embryo. The primordial germ cells (PGC) differentiate into

oogonia and multiply actively by mitotic divisions up to day 14, and then they differentiate into diploid primary oocytes. Primary oocytes are not renewed during the life of hen, and therefore constitute the final stock. Between days 16 and 18, these oocytes begin meiosis which stops after hatching in prophase (pachytene) of the first meiotic division. The diplotene stage of meiosis will be progressively reached by oocytes, which are maintained at this stage for months or years, up to 24 h before ovulation. The reductional division occurs in the mature follicle few hours before ovulation. The restart of meiosis is triggered by the pre-ovulatory release of LH (luteinizing hormone) by the pituitary gland, approximately 6 h before ovulation (Etches, 1996). This reductional division leads to the haploid secondary oocyte (*n* chromosomes). At this stage, the sex of the future embryo is determined since the hen is heterogametic. After the extrusion of the first polar body (2nd maturation division) in the infundibulum just before ovulation, the oocyte is arrested in metaphase of second meiotic division (anaphase). The penetration of the sperm cell occurs within 15 minutes following ovulation, by the fusion of its plasma membrane with the vitelline membrane. At the same time, the middle layer and the extravitelline membrane are progressively secreted by the infundibulum preventing the penetration of further spermatozoa. If fertilization occurs, the oocyte begins the second meiotic division (equational division), characterized by the extrusion of the second polar body. A male pronucleus moves toward the oocyte, in the center of the germinal disk, while the others move to the periphery. Within 4 h of fertilization, male and female pronuclei fuse and initiate the first mitotic divisions in the zygote.

In most birds, reproduction is sexual. However, parthenogenic reproduction exists in the turkey and this feature has been successively selected in some lines to lead to the development of an embryo in 50% of unfertilized eggs (Etches, 1996). A few of these eggs hatch, but this reproductive mode gives birth to males only. In commercial flocks, 5–12% of unfertilized turkey eggs initiate the development of an embryo, but none of these embryos hatches. It is also possible that part of the early embryonic mortality observed in inseminated turkeys is due to this process if parthenogenesis is initiated before egg fertilization.

Development of the oocyte

At hatch, several thousands of oocytes (about 12 000) are present in the ovary. During the production cycles (7 to 10 cycles possible), only a few (less than 2000) of them will form a yolk. Based on its size, development of the egg is divided into several phases, but without any real discontinuity between them (Table 6.4).

The initial slow growth phase affects all oocytes. It corresponds to an individualization of oocytes associated with development of the follicular epithelium during the first weeks after hatching and to a protein accumulation from granulosa or perivitelline fluid. Many of these primordial follicles are quiescent during the following months or years. A number of them disappear

Table 6.4 Follicular growth of the oocyte

	Growth phase			
	Initial	Intermediate		Rapid
Duration	4 to 5 months	60 days		6 to 14 days
Diameter (mm)	< 1	2 to 4	4 to 8	8 to 40
Weight (g)	0.001	0.01 to 0.03	0.3	20
Number/ovary	> 1000	10 to 40	6 to 25	5 to 8
Appearance	White	Pale yellow		Yellow
Nature of the deposit	Proteins	Proteins		Proteins and lipids
Origin	Ovary			Liver

by atresia at this stage or at a later stage of development. A limited number of follicles grow by accumulating lipid and protein compounds. The normal progress of follicular development requires the selection of follicles and their differentiated growth. It leads to the display of five to seven follicles of gradually increasing size at the surface of the ovary at a given time (Figs 6.2 and 6.7). This hierarchy of follicles results from the sequential development of oocytes selected at daily intervals. Those follicles rapidly accumulate proteins and lipids that are synthesized by the liver.

Determination of the selection of oocytes and in this follicular hierarchy is dependent on hormones from the hypothalamic-pituitary axis of the central nervous system such as neuropeptides, gonadotropins and gonadoliberins and on ovarian regulators such as steroid hormones and peptide factors from the germinal disc (Etches, 1996; Johnson and Whittow, 2000; Bentley *et al.*, 2007; Johnson and Woods, 2007). These factors interact with each other and their secretion is modulated during the intermediate phase of follicular growth or during the rapid growth phase controlling the follicular hierarchy. These exocrine and paracrine regulations are only partially understood. After removal of the pituitary gland, supplementation with pituitary hormones recreates or enhances the number of follicles in the hierarchy. Gonadotropins (luteinizing hormone, LH, and follicle stimulating hormone, FSH) are considered the main extra-ovarian hormones in ovarian and follicular development and in the activation of ovarian steroid secretion (estrogen, testosterone and progesterone), via stimulation of the expression of the enzyme P450scc. FSH is thought to mediate initial recruitment and growth of follicles. Daily injections of FSH increase the number of small yolky follicles and reduce the number of atretic follicles, with no effect on the follicular hierarchy. FSH and LH stimulate the proliferation of granulosa cells *in vitro* and stimulate the production of progesterone by these cells. FSH is active only on follicles in the initial phase of rapid growth, while LH exerts its effect on the pre-ovulatory follicle. The response of pre-ovulatory follicles (especially granulosa cells) to FSH and LH varies depending on their phase of development: they are more sensitive to FSH during the initial phase whereas they become more sensitive to LH during follicle maturation.

A recent work suggests that testosterone plays a role in the initiation of the pre-ovulatory release of progesterone in F1 follicles (Rangel *et al.*, 2009). Testosterone is indeed able to stimulate expression of P450scc and LH receptor in granulosa cells. However, it is supposed that, in maturing follicles, the stimulatory paracrine effect of testosterone, which is locally produced by theca cells, is counterbalanced by the inhibitory paracrine effect of estrogen whose production decreases in F1 follicle. Furthermore, the importance of oocyte-derived factors produced by the germinal disc in the regulation of the follicular hierarchy has been demonstrated (Hernandez *et al.*, 2002). A model has been established suggesting that extra-ovarian hormones FSH and LH modulate the proliferation of granulosa cells and their progesterone synthesis, respectively, in fewer (F5 to F3) and more (F1 and F2) mature pre-ovulatory follicles.

Intra-ovarian hormones (epidermal growth factor, EGF; growth promoting factor, GPF; and other regulatory peptides) produced by the germinal disc and granulosa cells stimulate follicle growth and reduce their atresia, particularly by favoring the maintenance of granulosa cells. Some growth factors produced by the follicular envelopes are also involved in this regulation. They affect the responsiveness to gonadotropin hormones and the transfer of yolk precursors. Small follicles contain high concentrations of activin A, a factor promoting responsiveness to FSH and reinforcing junctions between granulosa cells by stimulating the synthesis of occludin. At this stage, the penetration of yolk precursors is therefore difficult. Activin A content decreases progressively when follicle size increases up to 9 mm. During this period, follicular envelopes have increasing levels of inhibin B. In the same time, occludin production decreases thus promoting penetration of yolk precursors as a result of partial disintegration of the junctions between granulosa cells.

In a follicle with a size corresponding to F3 and F4 stages, activin A levels become higher than inhibin B levels, and then decrease again in follicles that are in terminal growth phase (F1 and F2) while inhibin A (activin A antagonist) levels increase. Inhibin A neutralizes activin A effects by reducing responsiveness to FSH. Balance between these factors is thought to modulate responsiveness of the ovary to FSH and LH and may explain the temporal modulation by these two hormones of progesterone synthesis by granulosa cells. It is also likely that additional factors (IGF-I, BMP and TGF) are involved in these complex regulations. Some studies underlined the paracrine regulation of the function of granulosa cells by bone morphogenetic proteins (BMPs). Indeed it has been demonstrated that BMP-6 produced by theca cells but not by granulosa cells can stimulate secretion of inhibin A and progesterone by granulosa cells derived from F1, F2 and F3/F4 follicles (Al-Musawi *et al.*, 2007). Moreover, BMP-6 can act synergistically with LH or FSH to enhance secretion of progesterone by these cells (Al-Musawi *et al.*, 2007). In contrast, BMPs may also exert opposite effects. For instance, BMP-15 can inhibit gonadotropin-induced secretion of progesterone (Elis *et al.*, 2007).

Some 98% of egg yolk is linearly deposited during the final 7–11 days prior to ovulation. The deposition is interrupted 2–3 h before ovulation. The duration of this rapid growth phase (7 days) is shorter in the early production period, but stabilizes 3 months later (10 or 11 days). The amount of yolk deposited in each egg increases considerably during the year of production (from an average of 12 g to 25 g), while the number of large follicles simultaneously present on the ovary decreases (from 7–8 to 5–6).

6.5.2 Synthesis and transfer of egg yolk constituents

Hepatic synthesis

All yolk proteins (except immunoglobulins), but also triglycerides, phospholipids and cholesterol, are synthesized by the liver in the hen. Sexual maturity coincides with the stimulation of protein synthesis pre-existing in the immature hen and with the induction of new components specific to the yolk. This synthesis of yolk constituents is under the control of estrogens that are produced by theca cells of small ovarian follicles that do not accumulate yolk. Testosterone is mainly produced by follicles in the rapid growth phase, particularly the F3 follicle, but its production is considerably reduced in preovulatory follicles. The production of estrogen and testosterone increased considerably 2 to 3 weeks before the production of the first egg, promoting the development of reproductive organs, secondary sexual characteristics and synthesis of the egg constituents.

Vitellogenin

Vitellogenin consists of a combination of one phosvitin and two lipovitellins (Deeley *et al.*, 1985). This molecule can dissociate into these three proteins by proteolytic cleavage mediated by cathepsin D, after its transfer to the yolk. Cathepsin D is synthesized by the oocyte and stored in specialized organelles located at the surface of the egg where yolk precursors are integrated. Phosvitin and lipovitellins α and β, which are the major phosphoproteins in the egg yolk, are present in the yolk spheres. Vitellogenin is not present in the immature hen. Its synthesis is highly stimulated just before the egg production begins, induced by estrogens in immature birds. This estrogen-dependent synthesis has been a widely used as a model to understand the mode of action of steroids on protein synthesis (Deeley *et al.*, 1985). Estrogens induce the gene transcription of vitellogenins I, II and III (Evans *et al.*, 1987; Cato *et al.*, 1988). The expression of the vitellogenin II gene is dominant since mRNA levels of vitellogenin II are 100-fold higher than mRNA levels of vitellogenin III in hen liver. The induction of transcription follows the standard sequence observed for steroids: association of estrogens with a nuclear receptor, binding of the hormone–receptor complex to the palindromic DNA sequence HRE (hormone response element) and interaction with the gene promoter (Deeley *et al.*, 1985). The 13 bp DNA binding sequence for the estrogen–receptor complex (ERE, estrogen response element) is found

at four sites. Two of them are functional and located upstream from the promoter of the chicken vitellogenin II gene. These sites have a key role in the specific expression and the preferential accumulation of vitellogenin mRNA in a tissue. Binding sites for progesterone are also found upstream from this gene (Cato *et al*., 1988). It is thought that they are at the origin of the additive effect of this hormone on the production of vitellogenin.

Estrogens also promotes vitellogenin synthesis by considerably increasing ($\times$ 30) the stability of its mRNA (Nielsen and Shapiro, 1990). This protein undergoes post-translational modifications before its secretion in plasma: phosphorylation on serine residues that are largely present in phosvitin, glycosylation of phosvitin (11 sites) and lipidation of lipovitellins. Vitellogenin also binds to calcium through sites present in phosvitin. This protein is thus responsible for the increase in plasma calcium concentration observed when egg production is initiated.

Triglyceride-rich lipoproteins

The major fraction of egg yolk (plasma) contains most of the yolk lipids (95%) and is composed of LDL. This fraction results from the transfer of hen-specific unmodified lipoproteins in the yolk – VLDL. These triglyceride-rich proteins are carried in the plasma after their synthesis in the liver (Nimpf and Schneider, 1998).

VLDL are present in immature hens, but their production in the liver increases considerably when animals reach sexual maturity: VLDL level in blood plasma is lower than 1 $g.L^{-1}$ in immature hens and increases up to 10–20 $g.L^{-1}$ in mature hens. These plasma precursors exhibit characteristics appearing at hen sexual maturity. The highly homogeneous size of VLDL in egg-producing hens is remarkably stable and reduced to a diameter of 30 $\pm$ 5 nm. These characteristics facilitate VLDL transfer into the ovarian follicular wall. VLDL have also a standard structure consisting of a core of triglycerides and cholesterol esters surrounded by a surface layer composed of phospholipids, cholesterol and apoproteins. However, the proportion of these different components is different in young immature pullets and adult hens: increased proportion of triglycerides in hens (56% in hens versus 47% in pullets) and concomitant decreased proportion of cholesterol (7% versus 24% respectively). Other important particularities of hen VLDL are the presence of an apoprotein (apo-VLDL-II) present only in egg-producing hens, and privileged accumulation of this protein and apolipoprotein B (apo B) to the detriment of other apolipoproteins present in the immature hen (apo-AI, C-type derived apolipoproteins). The catabolism of VLDL in hens is limited before their transfer into the yolk, so that they remain similar to their native hepatic form. The blood levels of LDL and HDL, which normally derive from VLDL by hydrolysis, are therefore very low. Most of the lipids are transported by VLDL, whose composition formed in the liver determines the lipid profile in the yolk. Therefore, any changes in the lipid composition of yolk, especially the profile of fatty acids, are the result only from

alterations in VLDL composition during their synthesis in the liver (Griffin, 1992).

The synthesis of apo-VLDL-II, present only in hens, is strictly dependent on estrogens (Deeley *et al*., 1985). Estrogens stimulate gene transcription of apo-VLDL-II, which is not expressed in broilers or in immature hens and enhance the stability of mRNA (7 to 8 times) (Nielsen and Shapiro, 1990). This mRNA therefore becomes extremely abundant in the liver. Apo-VLDL-II is synthesized as a pre-apolipoprotein of 106 amino acid residues, including a peptide of 24 residues which initially prevents the binding of apo-VLDL-II to membrane lipids. Apo-VLDL-II is present as a dimeric form (19 kDa) in VLDL. It is thought that this protein functions as an inhibitor of lipase lipoprotein and protects VLDL during their transport in plasma (Nimpf and Schneider, 1991). However, it is likely that this protein is involved neither in VLDL binding to oocyte receptors nor in their intra-follicular transfer, contrary to the initial hypothesis. Apo B is the major protein of VLDL. It is present in the immature pullet, but its synthesis in the liver is highly stimulated by estrogens at sexual maturity, whereas its concentration in intestine and kidney remains stable. It is a large (500 kDa) hydrophobic protein composed of a single unit. Apo B undergoes proteolytic cleavage during its transfer into the yolk, leading to the formation of several fragments. Nine fragments have recently been identified by mass spectrometry in purified egg yolk LDL (Jolivet *et al*., 2008). Hen Apo B is thought to share the predicted pentapartite structure of the human apolipoprotein B-100 precursor which is composed of three amphipathic α-helical domains (α1, α2, α3) and two amphipathic β-strand domains (β1, β2), in accordance with the following organization: NH2-α1-β1-α2-β2-α3-COOH. Two distinct regions of hen apo B that are not accessible to proteolysis may represent areas firmly associated with lipids, presumably the two β-strand domains (Jolivet *et al*., 2008). Apo B participates in the stabilization of VLDL. VLDL are successively assembled in hepatocytes by the association of apo B with phospholipids in endoplasmic reticulum, and by the association of this complex with triglycerides and apo-VLDL-II in Golgi apparatus. This group is secreted by vesicles in the blood plasma.

The higher synthesis of VLDL in the liver is directly dependent on increased supply of fatty acids which are *de novo* synthesized from carbohydrates or derived from the feed intake (Hermier, 1990). Studies on the mechanisms leading to the high stimulation of triglyceride synthesis are scarce. The injection of estrogens into immature hens increases hepatic levels of phospholipids and triglycerides by stimulating fatty acid synthesis and incorporation of circulating fatty acids (Courtney *et al*., 1988). The blood fatty acids may result directly from feed fatty acids since dietary lipids are transported in birds from the intestine to the liver through the portal vein. Indeed, no lymphatic system is present in birds. Dietary fatty acids are transported by portomicrons or associated with albumin. Unlike VLDL, portomicrons are rapidly hydrolyzed by lipase lipoprotein, because of their high affinity for

this enzyme and their ability to activate it in mature and immature hens (Hermier, 1990). Fatty acid plasma levels are low in hen, but increase after the intake of a lipid-rich diet. The rapid degradation of fatty acids in the liver is likely to be responsible for the modifications in the nature of yolk fatty acids which are incorporated in VLDL before the transfer into the yolk.

Other yolk proteins

Some proteins are present in egg yolk at low concentrations. Some are specifically induced at sexual maturity, such as proteins binding biotin (Murty and Adiga, 1985), thiamine, riboflavin, vitamin A (Durgakumari and Radhakantha, 1986) and vitamin D (Nys, 1990). In contrast, the synthesis of serum albumin (α livetin) is not influenced by estrogens.

Cholesterol

Yolk cholesterol is transported by VLDL and to a lesser extent by vitellogenins (Hermier, 1990; Griffin, 1992). Therefore, levels of cholesterol in yolk depend on its concentration in VLDL, not in blood plasma. The presence of free cholesterol in the surface layer is essential for the establishment of the structure and properties of VLDL. Therefore, very little variation is observed for this free cholesterol. In contrast, esterified cholesterol present in the core is more variable. Indeed, high dietary supply of cholesterol increases the esterified fraction and the cholesterol concentration in the egg. Conversely, a reduction in dietary cholesterol has only a limited effect on its concentration in the egg since the proportion of the esterified form is low (20% of total egg cholesterol). Reducing egg cholesterol levels by genetics also appears difficult to achieve. Indeed, modification in VLDL structure will be a prerequisite for reducing VLDL and yolk cholesterol but hen VLDL structure is quite stable due to tight physiological controls associated with their formation.

Transfer and deposit of plasma precursors in the yolk

Yolk lipid precursors are transported in the follicular wall by the blood and are released near the basolateral membrane through highly fenestrated capillaries (Gilbert, 1979; Etches, 1996). The presence of large gaps in the endothelial wall is peculiar to this region. These gaps allow the passage of VLDL and vitellogenin that circulate between granulosa cells but prevent the passage of large portomicrons (150 nm). Penetration of specific yolk precursors (VLDL and vitellogenin) is ensured through a process of endocytosis induced by the receptor LR8. These two components bind to the LR8 receptor which is present at high levels in the oocyte membrane (Nimpf and Schneider, 1998). Apo B of VLDL and lipovitellin are responsible for the receptor binding. A group of VLDL and vitellogenin associates with the membrane to form a pit leading to the formation of a vesicle internalized by endocytosis. The vesicle internalization is facilitated by the presence of endophillin III in the oocyte (Hirayama *et al.*, 2003). Apolipoprotein

VLDL-II (apoII) is the second major protein of VLDL. It is involved in the massive transport of triglycerides, particularly by inhibiting lipase lipoprotein. As measured *in vitro*, the concentration of circulating VLDL is about 5 times the maximal binding capacity of receptors for vitellogenin and VLDL. The number of receptors might be the factor limiting the transfer (Griffin, 1992). The existence of a single receptor for the two main precursors rules out the possibility of regulating yolk composition by the proportion of receptors in the oocyte membrane. Therefore, the capacity of hepatic synthesis is crucial.

The receptor LR8 has the ability to bind VLDL and vitellogenin, but also other minor components of the yolk. This receptor can bind the protein α2-macroglobulin that has the ability in mammals to bind and remove proteases from plasma through a process of endocytosis in the liver. This protein may promote the transfer of proteases into the oocyte. Moreover, LR8 facilitates the transfer of riboflavin into the egg yolk. Riboflavin is associated with a transport protein in the blood and forms a complex with vitellogenin. This group is internalized into the yolk after its binding to LR8 (MacLachlan *et al.*, 1994), thus explaining the fact that concentrations of riboflavin and its transport protein are 6 times higher in yolk than in plasma (White *et al.*, 1986). Yolk vitamin A is associated with a 21 kDa transport protein present in plasma, but no specific accumulation has been measured (Durgakumari and Adiga, 1986). The primary role of LR8 is confirmed by the existence of hens with a mutation in the gene encoding this receptor (present on the Z chromosome) and expressing a substitution of one amino acid residue at position 682. These hens have hyperlipidemia and the oocyte is then unable to accumulate yolk. Follicles do not undergo maturation, leading to the sterility of these hens.

Immunoglobulin receptors are present in the oocyte membrane. Yolk accumulates significant amounts of IgY immunoglobulins (livetin) (equivalent to mammalian IgG): up to 100 mg per egg, or 17 to 35 g of IgY per hen per year, 1–10% being specific to an antigen. This property can be used to produce antibodies of economic interest in human or veterinary medicine, immunotherapy, prophylaxis to combat bacterial or viral infections or toxins (Schade *et al.*, 2007) (Volume 2, Chapter 17). These antibodies can also be used in diagnostics or in histochemistry to quantify or localize proteins, peptides, hormones or enzymes. Many examples are of interest in veterinary prophylaxis to prevent bacterial intestinal infections in pigs or fish, but also in human medicine (intestinal infections, dental caries) or to neutralize poisons in humans.

6.5.3 Ovulation

Hormonal control

The ovulation of the oocyte occurs 24–25 h before the oviposition of the egg containing the corresponding yolk and the interval between oviposition and

the subsequent ovulation lasts 15 to 45 min (Sauveur and de Reviers, 1988; Etches, 1996). The duration of the ovulatory cycle (ovulation–oviposition interval) is 24 to 27 h in hens with low egg production, but is about 24 hours for current commercial lines of hen with high egg laying intensity. In addition, these hens might occasionally ovulate before expulsion of the egg. Most frequently, hens lay within the first hours of the photophase; ovipositions and thereby ovulations occur only during a limited period of the day, specific to each species, for example, 0 to 7 h after lights on in domestic hens receiving 14 h of light. On successive days, oviposition occurs later with a progressive shift (lag in hours between successive ovipositions) until a day on which no egg is laid. The number of eggs laid within this interval is called a sequence (or clutch, which is mainly used for wild species) and each sequence is therefore separated by a pause day. The length of a sequence corresponding to daily ovulation is directly associated with the laying intensity (when four eggs are laid for a period of 5 days, the egg production is 80%) and can last more than 30 consecutive days of laying at the production peak of hens laying over 300 eggs per year. The rhythm of ovipositions is efficiently synchronized by the light: when the lighting programme is modified by reversing the day and night, hens synchronize the oviposition timing in less than one week.

The almost daily production of an egg is feasible due to the simultaneous development of a series of follicles spread on the ovary according to a defined hierarchy (Figs 6.2 and 6.7), leading to the regular presence of a single follicle ready to ovulate. The acquisition of ovulation ability corresponds to the maturation of the follicle. The preovulatory follicle on the ovary contains the largest oocyte and its maturation corresponds to a modification in the nature of steroid secretion of follicles in the rapid growth phase (Etches, 1996; Johnson and Whittow, 2000; Hernandez *et al.*, 2002). The day before ovulation, secretion of progesterone by granulosa cells becomes predominant in this mature follicle (F1).

The chronology of the ovulation–oviposition sequence during a specific time of the lighting period (open period) results from the coordination of two rhythms of different duration. The first controls the secretion by the pituitary gland of the hormone which is triggering ovulation. This hormonal secretion is influenced by the nycthemeral rhythm of hen lighting (classically 24 h). The second depends on the endogenous rhythm of follicular maturation in the ovary, the duration of which is longer than 24 h. The daily lag of oviposition results from the difference between these two rhythms which initially delay ovulation. Ovulation is controlled by a pituitary hormone (LH) (Johnson and Whittow, 2000; Bentley *et al.*, 2007). A large secretion of this hormone occurring 6 h before ovulation elicits ovulation. This LH secretion is controlled by a positive feedback of the progesterone secreted by the maturing preovulatory follicle and by the concomitant increase in its plasma level. Progesterone positively influences the hormonal secretion of LH by the posterior pituitary. The production of progesterone by the ovary

is dependent itself on circulating LH. This positive feedback between the ovary and the central nervous system ensures synchronization between the degree of maturation of the follicle and the initiation of ovulation. Ovulation results from the progressive amplification of the synthesis of both hormones: secretion of follicular progesterone by granulosa cells and pituitary LH leading to the ovulating release of LH. This positive feedback is possible only during an open period of 6–7 h occurring just after the end of the light period in the case of high producing hens or a bit later (4 h) in low producing hens. The daily lag of ovulation, and thereby the lag of oviposition are caused by the delay between, on the one hand, the nycthemeral rhythm of LH secretion initiated at the hypothalamic-pituitary axis by cutting the light off, and on the other hand the endogenous rhythm of follicular maturation, lasting more than 24 h. When the desynchronization passes the duration of an open period, the initiation of ovulation ceases. After one pause day, a follicle is ready again to initiate a new sequence.

Release of the egg yolk

The pre-ovulatory follicle increases considerably in size during the rapid growth phase of the oocyte, then it ruptures at the stigma (Fig. 6.8) during ovulation. These changes require a continuous adaptation of the follicular wall and a local rearrangement of the tissue to allow the release of the oocyte at ovulation (Jackson *et al*., 1993; Etches, 1996). They are facilitated by the absence of connective tissue in the stigma (Fig. 6.8), by the specific organization of collagen fibers that are parallel in this area whereas they are interlaced in other parts of the follicular wall and by the ability of fibroblasts to secrete proteases. The remodeling of the follicular wall is controlled by proteases: collagenase hydrolyzing collagen fibers, and a serine protease as activating factor of plasminogen into plasmin. It is thought that this latter enzyme is the cause of tissue remodeling and activates the collagenase. The production of plasminogen activating factor seems to depend on the region adjacent to the germinal disc (both granulosa and theca) which may regulate this activity during follicular maturation. Furthermore, fibroblasts of the theca produce cathepsins that hydrolyze fibronectin, a protein associated with collagen which reinforces adhesion between the components of the follicular wall.

During the pre-ovulatory phase initiated by the release of LH (6 h before ovulation), the connective tissue, composed of collagen and interfibrillar matrix of dermatan sulfate-rich proteoglycans, is significantly altered in the stigma area of the follicle (Fig. 6.8). Collagen, at low concentration in this region, is partially degraded and interfibrillar links are digested, as demonstrated by the accumulation of dermatan sulfate in the milieu. This phase ends when the yolk is released by the local rupture of the follicular wall (Fig. 6.7).

6.6 Formation of the egg white and the shell in the oviduct

6.6.1 Sequential deposition along the oviduct

During ovulation, the yolk released from the largest follicle is captured by the oviduct and undergoes successive deposits in different parts of the tissue according to a predetermined and definite sequence (Fig. 6.2) (Romanoff and Romanoff, 1949; Gilbert, 1979; Sauveur and de Reviers, 1988). The duration between the yolk entrance and the egg expulsion (oviposition) from the oviduct is about 24–26 h.

The penetration of the yolk into the oviduct is facilitated by the infundibulum, a funnel-shaped structure undergoing muscle contractions during ovulation and capturing the oocyte liberated by the mature follicle. In breeders, fertilization of the oocyte by sperm previously stored in the sperm glands occurs at this site before completion of the vitelline membrane. The outer layer of the vitelline membrane is deposited on the perivitelline layer. This thin membrane (10–12 µm thick) limits exchanges between the yolk and the white. Its completion precedes the secretion by the magnum of albumen proteins which are accumulated beforehand in the epithelial and tubular cells. The deposition of these proteins takes about 3.25–3.5 h. Egg white is partially hydrated in the magnum. It has a wrinkled aspect when it enters the isthmus, 4 h after ovulation. The perialbumen layer is secreted by the magnum–isthmus junction. The isthmus is the site where the two shell membranes are secreted. In its terminal portion, the isthmus deposits the organic sites for the nucleation of calcium carbonate crystals at the surface of the outer shell membrane (Fig. 6.5b). Then the egg enters the uterus and stays in this compartment for nearly 20 h. First, the egg undergoes the second phase of hydration (plumping) which gives the final ovoid shape to the egg (Fig. 6.4) and puts it in close contact with the uterine mucosa. At the same time, the mineralization of the shell starts on the expanding egg (Fig. 6.4). This nucleation phase corresponds to the deposit of the first calcite crystals on the organic nucleation sites (Fig. 6.5b). From 10 to 22 h after ovulation, a large quantity of calcium carbonate is quickly deposited, together with a small proportion of proteins composing the organic matrix of the shell. This linear deposit stops about 2 h before egg expulsion (oviposition). During this phase, the shell is covered with the cuticle containing most (two-thirds) of the superficial pigments (porphyrins) of brown shells.

6.6.2 Formation of the egg white

The albumen is an aqueous solution (88% water) of protein (90% dry matter), minerals (6% dry matter) and free glucose (3.5% dry matter). No lipid is present in the albumen. Unlike the yolk constituents, all albumen proteins are synthesized and secreted locally by the magnum (Fig. 6.2). Secretion of water and minerals continues also in the distal parts of the oviduct (Romanoff and Romanoff, 1949; Sauveur and de Reviers, 1988; Etches, 1996).

Protein synthesis: site and timing

Protein synthesis is continuous in the magnum, regardless of the presence or absence of an egg in the oviduct. However, it is likely that protein synthesis is accelerated during the passage of the egg in the magnum, since the mRNA concentration in this tissue and the formation rate of albumen are higher during this phase of egg formation, according to Muramatsu *et al.* (1991). These authors estimated that the protein amount in the magnum wall corresponds to that of two eggs. The synthesis of the egg white proteins is a much faster process (<2 days) than the synthesis of the egg yolk constituents (8 to 10 days). Thus, a deficiency in hen's diet will affect the albumen much faster than the yolk.

Proteins synthesized in the magnum are stored in granules adjacent to the lumen (Fig. 6.10) and are secreted during the passage of the egg. The formation of these granules follows the standard process of secretory cells: proteins are synthesized in the endoplasmic reticulum at the basal region of the secretory cells, then they are packed in the granules generated by the Golgi apparatus inside a trilamellar membrane, the initial vesicles fuse into large granules that migrate into the apical region of the cell and are then extruded from the cell after the fusion of their membrane with the cell membrane.

The synthesis of egg white proteins is cell-specific (Gilbert, 1979; O'Malley, 1984). The major egg white proteins (ovalbumin, ovotransferrin, ovomucoid and lysozyme) are synthesized in the tubular glands and contribute to at least 80% of the weight of albumen proteins. The goblet cells (or mucous cells) that are epithelial cells, contribute to the synthesis of avidin and ovomucin. Owing to the role of ovomucin in the gel structure of the egg white, a dysfunction of goblet cells results in the formation of liquid egg white as it is the case when laying hens have infectious bronchitis.

Protein synthesis: hormonal control

The development of the oviduct and the synthesis of egg white proteins are strictly dependent on sex steroids (Gilbert, 1979; Nys, 1994; Johnson and Whittow, 2000; Bentley *et al.*, 2007). Both processes are induced rapidly *in vivo* or *in vitro* by steroid treatment. That is why this model has been widely used to investigate the hormonal regulation of protein synthesis (O'Malley, 1984).

Sex steroids induce both development and cell differentiation of the oviduct. This transformation is initiated in the embryo between 8 and 9 weeks by a slow cell proliferation. Cell proliferation accelerates dramatically between 16 and 18 weeks, before the phase of tissue differentiation. Estrogens induce cell proliferation, the formation of ciliated cells from the undifferentiated epithelium and the evagination of tubular glands (Pageaux *et al.*, 1984; Joensuu *et al.*, 1990). Moreover, they stimulate the synthesis of receptors to progesterone, testosterone and glucocorticoids, allowing the expression of their biological effects. Progesterone is involved in the differentiation of

goblet cells but has prior inhibitory effects on the cell proliferation and cell differentiation of the oviduct.

Sex steroids stimulate protein synthesis in the magnum according to a defined sequence (O'Malley, 1984). The oviduct contains specific receptors for estrogens, progesterone, testosterone and glucocorticoids. In the presence of the hormone, the hormone–receptor complex migrates into the nucleus, binds to DNA and induces gene transcription and production of the mRNA of the protein (for example ovalbumin, ovotransferrin). Steroids have also the capacity to increase the stability of the mRNA (Rories and Spelsberg, 1989).

This control has been demonstrated for the major egg white proteins ovalbumin, ovomucin, ovotransferrin and lysozyme. The sequencing of the genes coding these proteins and the sequencing of the nearby regions (Chambon *et al.*, 1984) demonstrated the presence of specific binding sites for hormone–receptor complexes upstream of the coding sequence.

In the case of ovalbumin, a labile protein factor induced by estrogens seems to be an intermediary necessary for the induction of the gene transcription (Nordstrom *et al.*, 1993). In addition to this feature, another complexity of control of the gene expression resides in the multi-hormonal regulation of albumen protein synthesis. Ovalbumin and ovotransferrin are induced by estrogens, but it has been clearly demonstrated *in vitro* that this induction is also triggered by progesterone (Chambon *et al.*, 1984), glucocorticoids or testosterone in an oviduct experimentally induced in immature birds by estrogens. Thus, there is a synergistic effect of these various hormones secreted in laying hens. However, the hormonal control mediated by estrogens and progesterone is predominant because concentrations of these hormones are strongly stimulated in the laying hen compared to the immature hen. The synergy between the sex steroid hormones results more particularly from the induction of their receptors (progesterone and testosterone receptors induced by estrogens). In contrast, avidin synthesis is strictly dependent on the progesterone (Joensuu *et al.*, 1990).

Protein secretion

Proteins synthesized in the magnum by tubular glands and epithelial cells accumulate in the cytoplasm in the form of secretory granules (Fig. 6.10) and in the ducts of tubular glands before the passage of the egg (Etches, 1996; Nys, 1994). The distension mediated by the passage of the forming egg induces a rapid secretion of these proteins. The amount of released proteins corresponds to about 50% of total synthesized proteins (Muramatsu *et al.*, 1991). The accumulation of total proteins of albumin, ovomucoid, ovotransferrin and lysozyme is linear during the 4 hours of transportation of the egg along the magnum. It is proportional to their respective concentration in the egg. It seems that protein secretion is not dependent on nervous or hormonal control, since the introduction of an artificial egg yolk induces the protein secretion whatever the period of the ovulatory cycle.

The peri-albumen layer present at the surface of the albumen is thin. It is composed of globular proteins that are secreted by epithelial cells in the magnum–isthmus junction, before the formation of shell membranes in the isthmus (Sultana *et al.*, 2003).

Formation of chalazae

The chalazae layer covers the egg yolk and has a thickness of 40 μm. It is therefore in close contact with the vitelline membrane of the yolk. At the external extremities, the filaments penetrate the thick egg white at the small and large ends of the egg. Chalazae secretion is initiated in the infundibulum. The proteins of chalazae are produced by secretory cells of the surface epithelium and tubular glands (Rahman *et al.*, 2007). Initially, chalazae result from fibers anchored on the opposite poles of the egg yolk. These fibers wind during the egg rotation in the magnum. They are anchored in the thick white and migrate outwards during the hydration of the egg white, the progressive appearance of the liquid and thick albumen parts showing different viscosities. The continuous rotation of the egg in the uterus facilitates the winding of chalazae and a lower water content. Both chalazae are spiral filaments attached to the shell membranes and connected to the yolk, thus maintaining the yolk in a central position.

Hydration of the egg white

The structure of the egg white is characterized by the presence of four (including chalazae) distinct physical areas that are of liquid or viscous consistency (Romanoff and Romanoff, 1949; Gilbert, 1979). The viscous consistency of the thick egg albumen and chalazae is due to an ovomucin enrichment of these areas which contain four times more proteins than the liquid egg white. Moreover, physicochemical characteristics of ovomucin are not identical between the thick egg white area and the liquid egg white area (levels of glycosylation).

The structuring of the egg white occurs progressively during the hydration of the albumen. At the distal part of the magnum, egg white is only partially hydrated (3.5 g water per g of dry matter) and has a wrinkled aspect. After the deposition of shell membranes, the water content in albumen is doubled during the first 6–7 hours of presence of the egg in the uterus (plumping phase). Egg rotation contributes to the formation of chalazae and to the formation of the inner and outer thin egg white, by the expulsion of water from the thick egg albumen during the torsion of the protein fibers.

The secretion of minerals in albumen occurs at the same time as the secretion of water by the magnum and uterus. It ends during the calcification of the shell. The magnum secretes 80% of sodium, 60–70% of calcium and magnesium, and 50% of chloride contained in the egg white. Most of the potassium is, however, secreted in the uterus. During the shell formation, there are partial reabsorption of sodium and secretion of potassium (Sauveur and de Reviers, 1988).

6.6.3 Formation of the egg shell

The process of shell mineralization is described in detail in Chapter 8 of this volume.

Site and timing of shell formation

Eggshell membrane fibers (Fig. 6.5b) are synthesized and secreted by glandular cells of the isthmus (Nys *et al.*, 1999, 2004), the organic components of mammillae (nucleation sites) are laid down on the external shell membranes (Fig. 6.5b) in the distal red isthmus. The first calcium crystals may be deposited on these sites before migration of the wrinkled egg into the uterus. The progressive hydration of egg albumen dilates the egg, creating its ovoid shape and allowing close contact with the uterine wall about 10 h after ovulation. Active secretion of calcium, carbonate and organic precursors over 12–14 h contributes to the rapid and linear deposition of the shell which ends with cuticle secretion about 1.5 h before oviposition.

The uterus has a similar cell population to that of other oviduct regions; a surface epithelium with subjacent glandular glands which secrete the mineral precursors of the shell. These glands contribute also to organic component secretion (ovocleidin 17, ovocalyxin 116) but additional matrix proteins are secreted by epithelial cells (ovocalyxin 32).

Eggshell formation is the longest step of egg formation as it lasts 20 h if the initial phase of shell nucleation is included. It is initiated at 4.5 h after ovulation and ends 1.5 h before oviposition. Shell formation occurs in three periods, the nucleation phase, the rapid deposition of shell material and the termination of mineralization when considering the kinetics of deposition and the composition of the uterine fluid where shell mineralization takes place.

Duration of shell secretion remains stable throughout the laying year even if the interval between two ovipositions increases throughout the egg laying period when hens age. In contrast, hens submitted to continuous light or to an ahemeral light cycle longer than 24 h increase concomitantly the period between two ovopositions and the duration of shell mineralization (Nys *et al.*, 1991).

Duration and secretion of shell precursors are regulated by undetermined factors. Dilation of the shell gland due to the presence of the egg contributes to, but is not enough to induce, shell precursor secretion. This ability of secreting mineral and organic shell material is present for a particular period of the ovulatory cycle and the cessation of shell mineralization occurs before egg oviposition. Mechanical retention of the egg in the shell gland does not increase the shell weight in contrast to a retention elicited by sex hormones (progesterone or testosterone) which interact with the maturation of the follicles on the ovary (Nys, 1987).

Shell mineralization

The process of shell mineralization is described in Chapter 8, therefore only the main features are described here. The eggshell of the hen (Fig. 6.5a)

is a porous ceramic formed at low temperature and exhibits remarkable mechanical properties. Its mean breaking strength is 30 N for a mean thickness of 0.33 mm. The eggshell consists basically of calcite, the most stable polymorph of calcium carbonate at ambient temperature and pressure. The crystals are anchored on eggshell membranes at specific sites, the mammillae then grow rapidly to form the compact palisade layer (Fig. 6.5). The shell shows a perfectly defined structure when observed with a scanning electron microscope or under polarized light with an optical microscope (Fig. 6.6). The shell is formed by precipitation of calcium carbonate onto the eggshell membranes (Figs 6.4 and 6.5). It is thought that the high degree of control of size, shape and orientation of the calcite crystals in the hen's eggshell is due on one hand to competition for space between crystals growing from adjacent nucleation sites (Garcia-Ruiz and Rodriguez-Navarro, 1994) and, on the other hand, to the interaction of calcium carbonate with the organic matrix during the nucleation and growth phases (Arias *et al.*, 1993; Nys *et al.*, 1999, 2004; Hincke *et al.*, 2008) (see Chapter 8). The resulting crystallographic organization is considered to determine the mechanical properties of the eggshell.

Origin of mineral precursors: calcium

No calcium storage occurs in the shell gland before shell formation (Sauveur and de Reviers, 1988; Nys *et al.*, 1999). Calcium is directly provided by ionic blood calcium. The amount needed for forming a shell (2 g of calcium) is very large; therefore the pool of blood ionic calcium is renewed every 12 min. The hen exports, during one yearly cycle, more than 300 eggs corresponding to more shell (1.8 kg) than its body weight!

The calcium is initially provided by the hen's diet (3.5% calcium; see Chapter 12). About two-thirds of calcium deposited in the uterus is directly supplied by the hen's diet, one-third (30–40%) by mobilization of bone calcium. Bone calcium mobilization is needed because of the desynchronization between food intake during the day and egg formation which mainly takes place during the night. Therefore, large dietary particles of calcium sources, which provide calcium for a longer duration in the intestine, will reduce the bone mobilization for shell formation. Conversely, a hen constrained to a low dietary level or deficiency of calcium, has the ability to mobilize up to 58% of the bone calcium. This daily resorption of bone is facilitated in hens due to the presence of medullary bone which represents about 11.7% of total bone calcium. The medullary bone, as with other secondary sex parameters, is induced two weeks before the onset of egg production in immature pullets by estrogens and testosterone, as shown by experimental treatment of immature pullets and the observation of increased estrogens levels at sexual maturity. During shell formation, bone resorption is increased 9-fold; however, osteoblastic activity reflecting bone accretion is also activated (x2) to renew the medullary bone. Hens also express a particular appetite for calcium just a few hours before shell formation favouring its dietary supply (see Chapter 12).

Origin of mineral precursors: carbonates

The shell contains 60% of carbonate which originates from the blood CO_2. The carbonic anhydrase enzyme present in the tubular gland is crucial for hydration of CO_2 to produce bicarbonate in the uterine fluid. The precipitation of calcium carbonate provides H^+ ions which are reabsorbed by the shell gland. The metabolic acidosis due to acidification of uterine fluid and plasma during shell formation is corrected in hens by respiratory hyperventilation and by an increased renal excretion of hydrogen.

Ionic transport in the shell gland during shell formation

The eggshell contains mainly calcium carbonate ($CaCO_3$) deposited as the calcite polymorph by coprecipitation of Ca^{++} and HCO_3^-. Both ions (Ca^{++} and HCO_3^-) are not stored in the uterus before eggshell calcification but are continuously supplied during eggshell formation via the blood plasma by important trans-epithelial transport through the uterine endothelium. Changes in ions concentrations of uterine fluid throughout the stages of shell formation (Nys *et al.*, 1991) and in ions transfer *in vitro* and *in vivo* when ionic transport inhibitors are introduced (Eastin and Spaziani, 1978a, 1978b; Pearson and Goldner, 1973) allowed a model of ionic transfer describing various putative transport proteins to be built. Calcium secretion in the shell gland occurs against the concentration gradient. It is highly reduced in the presence of inhibitors of the sodium pump or carbonic anhydrase, suggesting numerous interactions between minerals and presence of cotranporters of calcium, sodium and bicarbonate. These observations allowed the construction of a hypothetical model regarding ion transport pumps or exchangers between blood and uterine fluid (Sauveur and de Reviers, 1988; Nys *et al.*, 1999). The use of proteomic (Mann *et al.*, 2006) and transcriptomic approaches (Jonchère *et al.*, 2010) revealed numerous novel genes in the uterus with putative ionic transport functions. A range of candidate proteins has been selected in these lists by analogies with protein sequences of transporters previously described in mammalian tissues (intestine, kidney). The identification of genes coding proteins which might be involved in uterine trans-epithelial ion transport facilitated the updating of the initial model of ion transport through the uterine wall for supplying mineral precursors of the shell in the uterine fluid (Jonchère *et al.*, 2010) (Fig. 6.12).

Calcium secretion through glandular cells involves the TRPV Ca^{++} channel (intrusion into the cell), calbindin 28K (intracellular transfer), sarcoplasmic Ca^{++} pumps (intrusion into the reticulum) and inositol triphosphate receptors (extrusion from the reticulum). Calcium is then extruded from the cells against its concentration gradient by membrane Ca^{++} pumps and Ca^{++}/Na^+ exchangers. The sarcoplasmic Ca^{++} pumps, inositol triphosphate receptors and calbindin 28K contribute to maintain a low calcium concentration in the cell essential for cellular survival. Sodium transport implies a Na^+ channel (intrusion into the cell), Na^+/Ca^{++} exchangers (intrusion into the cell), and the Na^+/K^+ pump (extrusion from the cell). Bicarbonates are mainly produced by

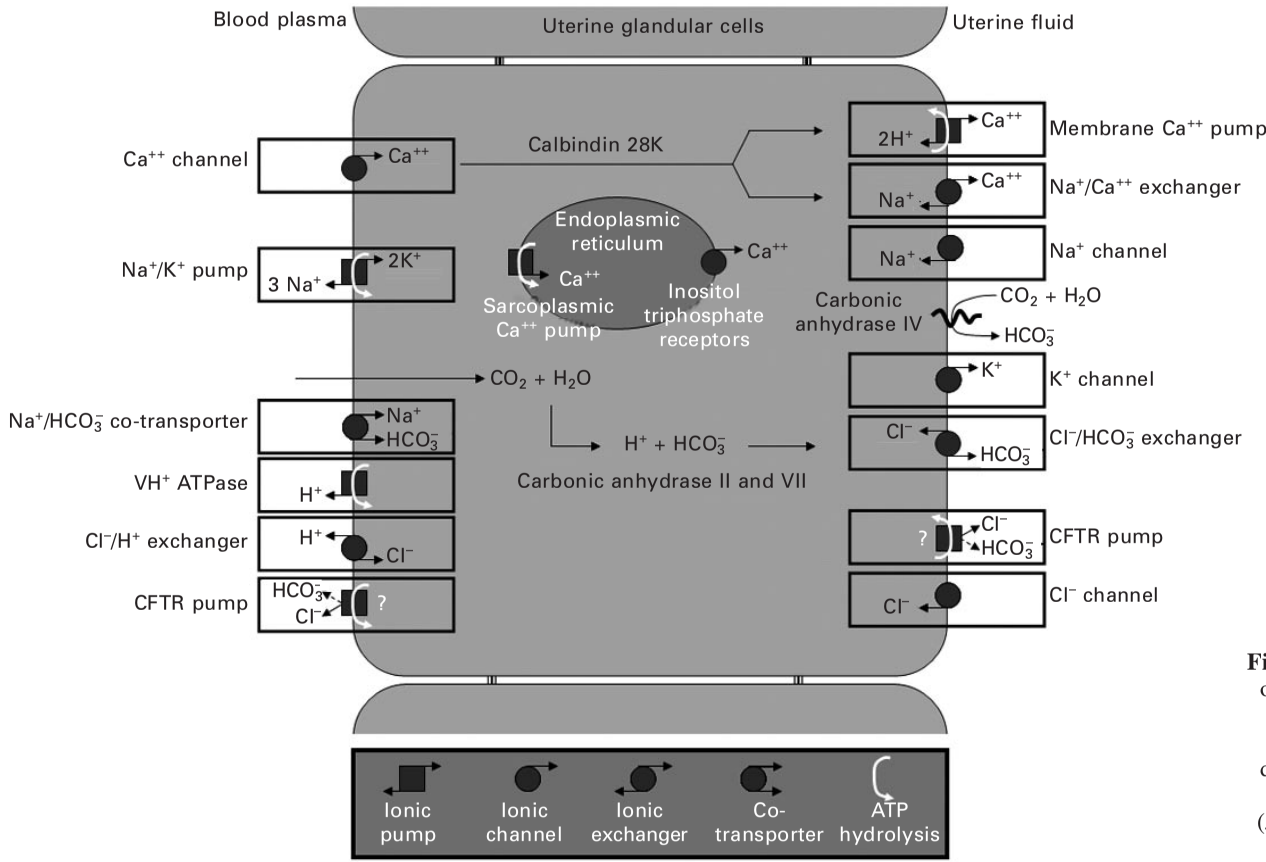

Fig. 6.12 Model of ion transport through the uterine wall during eggshell mineralization (Jonchère *et al.*, 2010).

the carbonic anhydrases (type II, IV and VII), and might be provided, at a low level, from plasma through the Na^+/HCO_3^- co-transporters. Bicarbonate is exported from the cell through the HCO_3^-/Cl^- exchanger and possibly the CFTR pump. Bicarbonate synthesis in the cell and coprecipitation of HCO_3^- with Ca^{++} in the uterine fluid generate the production of two H^+ which are exported via membrane Ca^{++} pumps, the VH^+ ATPase pump and a H^+/Cl^- exchanger. These pumps and exchanger control the pH balance and therefore contribute to maintaining acid–base equilibrium in hens. Chloride ions in the uterine fluid are reabsorbed by a Cl^- channel (intrusion into the cell), HCO_3^-/Cl^- exchanger (intrusion in the cell) and the cystic fibrosis transmembrane conductance regulator (CFTR) pump (extrusion from the cell). Finally, potassium is secreted by the Na^+/K^+ pump (intrusion into the cell), and several K^+ channels (extrusion from the cell). The presence in the uterus of Na^+/K^+ pumps and Na^+ channels, and Cl^- channels is essential to maintain the membrane potential.

It is also well established that uterine calcium secretion is tighly and quantitatively associated with the level of a cytosolic protein with calcium affinity, calbindin, present in the cells of the tubular gland involved in calcium transfer. The synthesis of this protein is induced by the active metabolite of vitamin D in the intestinal epithelium, in contrast to the uterus (Nys, 1993; Bar, 2008). The experimental supply of this active metabolite vitamin D or increased endogenous level by introducing low dietary calcium does not alter uterine calbindin level or shell deposition. The level of uterine calbindin is likely to be controlled by the calcium flux or an intracellular factor induced by this flux.

6.7 Oviposition

The expulsion of the egg (oviposition) by the oviduct is coordinated by physiological factors eliciting uterine muscle contraction and relaxation of abdominal muscle and of the sphincter between the uterus and vagina (Shimada, 1988; Johnson and Whittow, 2000). The process lasts a few minutes. Most eggs (90%) form in the oviduct with the sharp end oriented toward the vagina but at least 20–30% of eggs are laid blunt end first so must be rotated end for end while passing the posterior part of the oviduct (Romanoff and Romanoff, 1949).

In hen, contraction of the uterine muscle increases greatly at the timing of each oviposition, even when no egg is present in the uterus as a result of an experimentally induced premature oviposition or when the first ovulation of an egg sequence occurs. The timing and process of oviposition depend on a double hormonal control: on one hand the neurohypophyseal hormones, arginine vasotocin and oxytocin, and on the other hand prostaglandins secreted mainly by the ovary but also, at a lower magnitude by the uterus (Saito *et*

al., 1993). The control by the ovary is crucial as shown by the observation that ablation of the pre- or post-ovulatory follicles prevents oviposition.

The method of control through the neurohypophyseal hormones was established at the onset of the 20th century. It has been supported since by numerous experimental observations. The blood level of arginine vasotocin is highest during oviposition. Receptors for arginine vasotocin have been revealed in the shell gland. Finally, contraction of the uterus and premature expulsion of the egg occur following injection of arginine vasotocin (Saito *et al*., 1993). However, removal of the neurohypophysis fails to delay the timing of oviposition. The contraction of the shell gland associated with premature egg expulsion elicits a release of arginine vasotocin and administration of indomethacin (an inhibitor of prostaglandin synthesis) prevents oxytocin-induced oviposition. These observations support the hypothesis that prostaglandins (PGs) have a preponderant role in controlling oviposition.

Exogenous administration of PGs (PGF 2α) elicits uterine contractility and induce premature oviposition (Shimada and Asai, 1979). PG receptors are present in the shell gland which has the ability to synthesize PGs. Indomethacin, which inhibits PG synthesis, decreases the peak of prostaglandins in the pre and post-ovulatory follicle, suppresses uterine contraction and delays oviposition (Hertelendy and Biellier, 1978). Both prostaglandins E and F induce shell gland contractility, and PGE in addition induces relaxation of the vagina. Blood levels of PGE and PGF are highest at oviposition but only PGF level increases in the inner theca of pre-ovulatory and post-ovulatory follicles. PGF secreted by ovary is therefore considered as responsible for eliciting uterine contractility, which then stimulates the release of arginine vasotocin. The combination of these neurohypophyseal peptides and of prostaglandins issued from the ovary or uterus are considered to induce the oviposition. In addition to PGs, other factors such as oestradiol-β17 and progesterone (Takahashi *et al*., 1994), acetylcholine (Shimada *et al*., 1987), angiotensin II (Goto *et al*., 1986) and calcitonin (Nakayama *et al*., 2010) can also cause a release of arginine vasotocin from neurohypophysis.

6.8 References

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7

Use of high-throughput technology to identify new egg components

J. Gautron, S. Réhault-Godbert and Y. Nys, Institut National de la Recherche Agronomique, France, K. Mann, Max-Planck-Institut für Biochemie, Germany and P. G. Righetti, Politecnico di Milano, Italy

Abstract: The development of molecular biology in the late 1980s, the recent publication of the chicken genome sequence and the development of new bioinformatics tools were major scientific advances leading to the characterisation of several egg components previously unidentified. Using recent data on the characterisation of egg proteins, the chapter describes the most recent developments in egg biochemistry (proteomics), and molecular biology (transcriptomics). The newly identified molecules will stimulate research in understanding avian biology and identifying biologically active compounds of potential interest to the pharmaceutical, cosmetic and food industries.

Key words: egg, proteins, transcriptome, proteome, secretome, function annotations, biological activities.

7.1 Introduction

The chicken egg is formed in the hen's left ovary and oviduct. The ovary supports the accumulation of egg yolk proteins and maturation of the ovum. After ovulation, the yolk enters the oviduct, where albumen, eggshell membranes and the eggshell are sequentially deposited in the different segments of the hen's reproductive tract (magnum, white isthmus and uterus, respectively). The biological role of the chicken egg is to be a natural container of nutrients and bioactive molecules for the extra-uterine development of the embryo. This means that the egg must contain all components that are essential for the development of a reproductive cell into a mature chick

(Anton *et al*., 2006; Mine and Kovacs-Nolan, 2006; Rehault *et al*., 2007). The egg contains the vitamins and proteins (egg white and yolk), the lipids (yolk), and the mineral (eggshell) necessary for embryonic development. The egg is also a basic food for humans all around the world. The egg is of high nutritive value because it is a well-balanced source of amino acids that are easily assimilated (Nys and Sauveur, 2004; Seuss-Baum, 2007).

To face physical and microbial attack, the egg possesses two major protective systems. The first natural defence of the egg is the eggshell, which acts as a physical barrier against bacteria as long as it remains intact. The eggshell is a highly ordered structure made of calcium carbonate and of an organic matrix. The chicken eggshell matrix is a complex mixture of proteins and polysaccharides (Gautron and Nys, 2007a; Nys *et al*., 2001; Tullet, 1987), which plays a crucial role in the control of mineralisation and in determining the mechanical properties of the shell (Gautron and Nys, 2007b; Hincke *et al*., 2008; Nys *et al*., 2004). The second natural defence of the egg is a chemical protection system consisting of yolk, egg white and eggshell proteins with antimicrobial properties (Anton *et al*., 2006; Hervé-Grépinet *et al*., 2009; Mine and D'Silva, 2008; Rehault *et al*., 2007). Furthermore, the egg compartments contain molecules with a broad range of biological activities of major interest for several industrial areas, including pharmaceutical, cosmetic and food industries. Thus, the chicken egg is a major source of active molecules such as anti-hypertensive, anticancer, antioxidant, cryoprotective, immunomodulating and anti-adhesive components (Anton *et al*., 2006; Mine and D'Silva, 2008; Rehault *et al*., 2007). These remarkable properties mainly rely on proteins present in egg yolk, egg white and the eggshell. Consequently, the characterisation of egg proteins and the study of their functions is an important challenge that stimulates egg science.

The fractionation of the proteins of white and yolk was initiated more than 50 years ago. The major proteins of albumen and yolk were separated and purified using ammonium sulphate precipitation, chromatographic methods and electrophoretic techniques (Li-Chan *et al*., 1995). This catalogue of classical biochemical techniques was extended by molecular biology tools in the 1980s. Despite these efforts, the composition of the egg was still not completely understood. Only the major proteins were identified and many minor egg proteins remained to be discovered. During the last ten years, the results of functional genomic studies have dramatically transformed biology and biotechnology, including egg science. The recent development of high-throughput methods used in combination with the newly available chicken genomic sequence (International Chicken Genome Sequencing Consortium, 2004) and the development of bioinformatics tools to predict functions generated new insights for the characterisation of new and minor egg components (Gautron *et al*., 2007b). Some of these molecules may be a source of active compounds with specific properties beneficial to human and animal health. In this chapter, we describe how these high-throughput technologies were used in recent contributions to enable major advances in the characterisation of egg proteins.

7.2 Functional genomics generated new insights for the characterisation of egg proteins

Since the year 2000, a number of groups have characterised chicken (*Gallus gallus*) gene transcripts. cDNA libraries were prepared by isolating the mRNAs from the tissues and organs of chicken at different physiological stages. Reversed transcribed mRNAs, containing the information coding for proteins expressed in particular tissues, were inserted into bacterial vectors to produce large amounts of protein for experimental characterisation. Furthermore, sequencing of cDNAs yielded short nucleotide sequences (200–500 bp), known as expressed sequence tags (ESTs), representing a comprehensive catalogue of global or tissue-specific mRNA sequences expressed in the chicken. Scientists working at the University of Manchester (UK), have carried out a research programme allowing the identification of about 300 000 ESTs (http://www.chick.manchester.ac.uk/index.html) (Boardman *et al.*, 2002). Additional EST libraries originated from the University of Delaware (http://cogburn.dbi.udel.edu/index.html) (Carre *et al.*, 2006), and from the French programme 'analysis of breeding animals' genome' developed by INRA (http://www.inra.fr/agenae). The combination of projects allowed the characterisation of 600 414 EST sequences (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html) (release 030510), almost corresponding to an important amount of translated transcripts for *Gallus gallus*. With the aim of having an organised view of the chicken transcriptome, these sequences were assembled into 260 460 contigs in the SIGENAE database (http://public-contigbrowser.sigenae.org:9090/Gallus_gallus/index.html), to yield a genome-wide non-redundant catalogue of 32 566 different mRNAs (UniGene) (http://www.ncbi.nlm.nih.gov/UniGene/UGOrg.cgi?TAXID=9031). With the development of next generation sequencing (NGS) technologies, several tens of millions of chicken transcript fragments are now available, and contribute to improve our knowledge of the chicken transcriptome (NCBI Sequence Read Archive, http://www.ncbi.nlm.nih.gov/sra/).

The publication of the chicken genome sequence was another major advance to identify and characterise chicken genes (International Chicken Genome Sequencing Consortium, 2004). The publication of the genomic sequence, the existence of a large number of ESTs and the use of high-throughput technologies (proteomics and transcriptomics), provided a major advance to identify egg proteins.

7.2.1 Egg proteome

Proteome refers to the complete set of proteins present in a given cell or organism under defined conditions. Proteomics is the study of the proteome, with the goal of identifying and quantifying all proteins, elucidating their interactions, and determining their post-translational modifications. The study of proteomes usually involves mass spectrometry-based high-throughput

methods for protein identification (Steen and Mann, 2004). Typically, the proteins of a given cell or tissue are extracted and degraded by specific proteases. This highly complex mixture is then introduced into a mass spectrometer for determination of peptide masses as precisely as possible. For the identification of proteins, the masses of these peptides, and of fragments produced from peptides by collision-induced dissociation (CID) in the mass spectrometer, are compared to the theoretical masses predicted by dedicated computer programs from *in silico* digestion and fragmentation of the entire sequences stored in databases. Such an approach of course, depends on the availability of databases containing the sequences of the proteins to be analysed. The recently assembled databases containing a large number of chicken gene transcripts and proteins sequences enabled the exploration of egg compartment proteomes, using mass spectrometry-based high-throughput methods. Recent analysed proteomes are the organic matrix of the chicken calcified eggshell layer proteome, the egg white proteome, the egg yolk proteome and the vitelline membrane proteome (Table 7.1).

Egg proteomics is a very efficient way to identify egg proteins, the synthesis of which is specifically induced at sexual maturity for egg formation. However, the proteome survey also revealed numerous minor components which may originate from decaying cells and basement membranes lining the oviduct. Some of these may therefore not be actively secreted by the tissue which secretes the egg compartment and may not have a function in the egg compartments in which they were identified. These proteins may have been passively incorporated into the egg compartments merely due to their presence in the forming milieu. Therefore, the study of the expression of genes coding for egg proteins in the various parts of the oviduct secreting respectively the egg white (magnum), eggshell membranes (isthmus) and eggshell (uterus) constitutes a complementary approach which revealed in addition all proteins involved in regulation and process of the egg molecule synthesis.

7.2.2 Egg transcriptome

Analysis of the transcriptome is a way to reveal the genes specifically expressed in the tissues responsible for the production of an egg compartments. The

Table 7.1 Number of egg proteins identified using proteomic facilities

Egg compartment	Number of identified proteins	References
Eggshell	528	Mann *et al*. (2006, 2007), Miksik *et al*. (2003, 2007)
Egg white	148	Raikos *et al*. (2006), Guérin-Dubiard *et al*. (2006), Mann (2007), D'Ambrosio *et al*. (2008)
Vitelline membrane	137	Mann (2008)
Egg yolk	316	Mann and Mann (2008), Farinazzo *et al*. (2009)

transcriptome quantifies all mRNA molecules (or transcripts) in one cell type or a population of cells at a stage defined by a particular set of physiological conditions. Transcriptomics determines the expression level of genes, using techniques capable of screening thousands of different mRNA molecules at a time. This technique uses DNA arrays, which are a large collection of identified microscopic DNA spots attached to a solid surface, such as glass or nylon membranes, forming an array. DNA arrays provide a medium for matching known and unknown DNA samples based on base-pairing rules. DNA arrays can be used to compare differential gene expression under different conditions such as different tissues or different physiological stages. The result is a list of genes differentially expressed in one cell type or tissue under particular conditions. Next, statistical and bioinformatic tools are used to link the expressed genes to the identified clones present on the arrays. Differentially expressed genes are then classified according to known functions of the encoded proteins, to predict a potential biological role for them.

The chicken reproductive tract is a well-adapted model for a transcriptomic approach. Egg formation in the oviduct occurs daily by sequential secretion onto the yolk of the various compartments of the egg. Each part of the oviduct, magnum, isthmus and uterus has a very specific role in the synthesis of egg compartments. The entire oviduct originates from the same population of cells, which specialise at sexual maturity into specific regions responsible for the deposition of egg white (magnum), eggshell membranes (white isthmus) and calcified shell (uterus). Consequently, the comparison of gene expression in the various segments of the oviduct is a way of revealing genes encoding proteins synthesised and secreted in specialised region of the oviduct for constituting each egg compartment. Two recent reports have analysed global gene expression in hen's reproductive tract (Dunn *et al*., 2009; Jonchere *et al*., 2010). Dunn *et al* (2009) compared oviduct gene expression in mature versus juvenile birds, and the over-expressed genes were related to the dramatic changes due to the sexual maturity and the onset of egg production. Jonchere *et al*. (2010) compared gene expression in the uterus where the eggshell is formed with two other segments of the oviduct (magnum and white isthmus) to reveal genes encoding uterine proteins involved in supplying mineral and organic precursors contributing to eggshell formation. Additional transcriptomic studies are currently in progress to determine gene expression profiling in the magnum (egg white proteins) and in the isthmus (eggshell membranes) (Gautron *et al*., 2009).

The transcriptomic approach allows a screening of genes encoding proteins of the oviduct that might be molecules deposited in the egg, or might be involved in the cellular mechanisms producing egg components. Genes coding for oviduct proteins can be divided into two groups: (i) intracellular proteins involved in oviduct metabolism in egg molecule synthesis and secretion, and (ii) extracellular proteins, which are secreted and are deposited in the egg and consequently might have a function in the egg.

One way to discern extracellular from intracellular-related proteins is to determine the oviduct secretome specific for oviduct sections and egg compartments. The over-expressed genes, which were identified using a transcriptomic approach in the various regions of the oviduct, are translated into putative proteins. The protein sequences are then compared to the proteins already identified in the corresponding egg compartment by proteomic methods. In a second step, the protein sequences can be analysed to determine the presence of a signal peptide sequence that is required for secretion. This approach has already been used for the eggshell and allowed the identification of 54 eggshell proteins secreted to be deposited in the eggshell (Jonchere *et al.*, 2010).

7.3 Newly identified egg proteins

In 2006, the total number of identified proteins in the egg was about 50. However, the sequences in databases lead the use of high-throughput techniques and allowed the identification of hundreds of egg proteins in a very short period of time.

7.3.1 Eggshell proteins

The eggshell is made of 95% of calcium carbonate and 3.5% of organic macromolecules, known as organic matrix. The organic matrix plays a crucial role in shell assembly and in determining eggshell mechanical properties (Gautron and Nys, 2007b; Hincke *et al.*, 2008; Nys *et al.*, 2004). Matrix proteins were traditionally studied using a variety of biochemical and molecular methods. These classical approaches have allowed the identification of 10 major eggshell matrix proteins which were classified in three groups. The a first group includes proteins previously identified in egg white. These are ovalbumin, lysozyme and ovotransferrin (Gautron *et al.*, 2001b; Hincke, 1995; Hincke *et al.*, 2000). The second group contains ubiquitous proteins such as osteopontin and clusterin (Mann *et al.*, 2003; Pines *et al.*, 1994). The last group is composed of proteins unique to shell calcification and only secreted in regions of the oviduct where eggshell calcification takes place (red isthmus and uterus). These eggshell specific proteins are ovocleidin-17 and 116 (Hincke *et al.*, 1999a, 1995), and ovocalyxin-32, 36 and 21 (Gautron and Nys, 2007a, Gautron *et al.*, 2001a, 2007a). A major advance came in 2006, with the use of mass spectrometry-based proteomic analysis of peptides obtained from enzymatic cleavages of the acid-soluble eggshell organic matrix. This method identified a total of 528 different proteins as constituents of the eggshell matrix (Mann *et al.*, 2006, 2007, 2008).

The abundance of identified proteins was estimated by calculating the exponentially modified Protein Abundance Index (emPAI), which estimated the protein amount by relating the number of identified peptides o the number

of theoretically possible peptides of a protein (Ishihama *et al*., 2005). The emPAI was used to rank proteins according to their abundance and to discern major from minor proteins. However, it has to be noted that a protein of low abundance may have an important biological role mediated by enzymatic activity, antimicrobial action, or as a component of a signal transduction chain. The high-abundance group of eggshell proteins is composed of 32 proteins representing 6% of the total proteome. Except for osteopontin, all the previously known matrix proteins were identified in this group. The eggshell matrix protein inventory contained many egg white proteins, extracellular growth factors and signalling molecules, other signal transduction chain components, components involved in protein folding, stabilisation and degradation, lipid-binding proteins, immune system-related and antimicrobial proteins. It also contained many proteins previously identified in body fluids, such as serum albumin, hemopexin and vitamin D-binding protein. This study also identified some proteins of unknown function, which were not present in other egg compartments and were thus predicted to be new specific eggshell matrix proteins.

The insoluble fraction of the eggshell matrix was also investigated (Miksik *et al*., 2003, 2007). Only proteins already identified in the acid-soluble shell matrix were identified. These were the four eggshell-specific matrix proteins, ovocleidin-116 (Hincke *et al*., 1999a), ovocleidin-17 (Hincke *et al*., 1995), ovocalyxin-36 (Gautron *et al*., 2007a), ovocalyxin-32 (Gautron *et al*., 2001a), the ubiquitous extracellular chaperone clusterin, which is also present in egg white (Mann *et al*., 2003), and the egg white protein ovalbumin (Hincke, 1995).

It is not clear whether all the proteins detected have any function related to the eggshell formation. The eggshell proteome identified proteins that are secreted by proximal segments of the oviduct not involved in shell formation. These proteins may have migrated through the uterus together with the unfinished egg. Other identified molecules may have been released by decaying cells lining the oviduct, or as by products of previous secretion processes. These proteins are passively incorporated into the eggshell, just because they are present during mineralisation and are likely to have no biological significance. Proteins specifically produced by eggshell gland cells (uterus) are more likely to have a specific role in the eggshell formation. In such a context, the study of specifically expressed genes in the tissues responsible for the deposition of specific egg compartments using transcriptomics, is a valuable complementary approach to identify genes coding egg proteins. Using cDNA microarrays, large changes were observed in uterine gene expression relative to other parts of oviduct (Jonchere *et al*., 2010) reflecting the spatial sequence of egg formation throughout the oviduct. The uterine transcriptome was composed of 605 activated genes, which correspond to 469 different genes and 437 proteins potentially related to eggshell deposition and associated cellular pathways. The putative functions of the 605 uterine transcripts were investigated using gene ontology (GO) annotations. This

classification shows an over-expression of genes encoding proteins involved in mineral transport and ion transfer to provide eggshell mineral precursors. Another group of importance is composed of calcium binding proteins, which are essential for the mineral phase interactions during eggshell calcification. The transcriptional analysis has identified proteins previously suggested as transporters (Nys *et al*., 1999), and also revealed new ionic transporters possibly involved in supplying minerals needed for building the eggshell (Jonchere *et al*., 2010). Fifty-four proteins were predicted to be secreted by uterine cells in the uterine fluid (Jonchere *et al*., 2010). These proteins were classified according to their biological function in the eggshell.

A first group contains proteins potentially involved in the biomineralisation of the shell. This group contains ovocleidin-116, ovocalyxin-36 and 21, uterine proteins which were reported to be eggshell-specific matrix proteins (Gautron and Nys, 2007a; Gautron *et al*., 2007a; Hincke *et al*., 1999b). Also included in this group is osteopontin, which was also described as an eggshell matrix protein (Pines *et al*., 1994). Calcium-binding properties often are a prerequisite for matrix proteins involved in calcium biomineralisation. Calcium binding proteins could interact with calcium to favour crystal nucleation or to determine the morphology of crystals by interacting with particular faces of growing calcite crystals. The ordered deposition of calcium carbonate, under the control of organic matrices, determines the texture of mineral in the eggshell (Nys *et al*., 1999). The presence of calcium binding proteins is reported in the secretome of uterine cells. It included endoplasmin, SLIT2, SLIT3, nucleobindin-2, follistatin-related protein-1 and FK506-binding protein 9. All contain calcium-binding EF-hand domains. Calcium is also a ligand of calsyntherin-3 and mannose-binding protein C, which could also interact with calcium during eggshell fabrication. Another interesting secretory protein is podocalyxin, a sialoprotein which was first identified in the renal glomerular podocytes. Because of its high net negative charge, podocalyxin could interact with calcium carbonate during the calcification of the eggshell. Dentin matrix protein-4 was also identified as a secreted uterine protein. This calcium-binding protein plays a role in dentin mineralisation. However, the hypothesis of the involvement of these proteins suggested by the presence of calcium binding domains need to be confirmed *in vitro* using calcium carbonate crystal growing test (Hernandez-Hernandez *et al*., 2008), to confirm such interactions between proteins and mineralisation.

A second functional group corresponds to proteins secreted in uterine fluid to play a role in proper folding of the eggshell matrix proteins. Ovocalyxin-21 contains a brichos domain and, consequently, could play a role as a molecular chaperone. A similar role is also proposed for endoplasmin, a protein of the heat shock protein 90 family. Several additional proteins involved in protein folding were identified among the 54 proteins possessing signal peptide sequences. These, including ICOS ligand, neuroplastin, beta-microglobulin and butyrophilin subfamily 1 member A1, were previously identified in the eggshell proteome (Mann *et al*., 2006). These four proteins contain immunoglobulin-

like (Ig-like) domains, which is one of the most common protein modules found in a variety of mammalian proteins including sandwich-like proteins (Potapov *et al.*, 2004). Lysosomal alpha mannosidase (MAN2B1) also plays a role in protein folding and its mRNA is the most abundant over-expressed uterine gene product detected in the uterine transcriptome (Jonchere *et al.*, 2010). In the eggshell proteome survey (Mann *et al.*, 2006) five proteins identifiers correspond to MAN2B1. MAN2B1 is a glycoside hydrolase that participates in the metabolism of glycoproteins, maturation of N-glycans and in protein folding (Herscovics, 1999). Its role is related to calnexin, an acidic protein (pI = 4.46) also identified as a putative uterine secretory proteins. CANX is a molecular chaperone which assists in protein folding. CANX binds only glycoproteins that have been folded by an enzyme (i.e. MAN2B1). Consequently, these two proteins are suspected to be involved in metabolism of glycoprotein and proteoglycan of the eggshell matrix. Proteoglycans are negatively charged and consequently are thought to interact with the mineral phase and to influence the texture of the mineralised shell (Gautron and Nys, 2007b; Nys *et al.*, 2004).

A third group is made of uterine proteins with potential antimicrobial activity. This role is suspected for ovocalyxin-36, a specific eggshell matrix protein with analogies to LBP (lipopolysacharide binding proteins), BPI (bactericidal permeability-increasing) proteins and palate, lung and nasal epithelium clone (Plunc) family proteins. These proteins are well known in mammals for their involvement in defence against bacteria (Gautron *et al.*, 2007a). Additional antimicrobial proteins, particularly proteins that contain Ig-like domains (ICOS ligand, neuroplastin, beta-2-microglobulin, butyrophilin subfamily 1 member A1), which are related to the immune responses, are also secreted by the uterus. Of particular interest are amyloid beta A4 protein and beta-amyloid protein 751 isoform, which contain an amyloid extracellular domain and a heparin-binding domain. Heparin-binding proteins have basic domains that could interact with bacterial lipolysaccharide (Andersson *et al.*, 2004). Avian β-defensin 9 is also over-expressed in uterus. The avian β-defensins (AvBDs) are small cationic non-glycosylated peptides (1–10 kDa) with a three-stranded β-sheet structure and a β-hairpin loop which exhibit activity against Gram-positive and Gram-negative bacteria (Hervé-Grépinet *et al.*, 2009; van Dijk *et al.*, 2008). Mannose-binding protein C, which contains a C-type lectin like domain, could also have an antimicrobial role, as this role was ascribed to ovocleidin-17 and its goose orthologue ansocalcin, two major eggshell-specific proteins containing C-type lectin-like domains (Wellman-Labadie *et al.*, 2008).

Finally, the last group of proteins secreted by uterine cells is related to proteases and proteases inhibitors, which are involved in blood coagulation, cell migration and proliferation, innate defence and gamete maturation. The study identified three proteases: cathepsin A, glioma pathogenesis-related protein 1 and beta-secretase 2. Previous work has shown that the proteolytic activity present in uterine fluid varies according to the stage of the

calcification (Réhault-Godbert *et al*., 2008). Proteases could have a specific and controlled role during the calcification process, by either degrading proteins or regulating processing of precursor proteins into mature forms. The study also identified seven genes encoding uterine antiproteases. They are amyloid beta A4 protein, follistatin-related protein 1, tissue factor pathway inhibitor 2 and beta-amyloid protein 751 isoform, all of which contain a Kunitz/bovine pancreatic trypsin inhibitor domain. In addition there are Alpha2-antiplasmin, which belongs to the serine protease inhibitor (or serpin) family, BMP-binding endothelial regulator protein which contains a trypsin inhibitor-like cysteine rich domain, and tissue metalloproteinase inhibitor 2, which belongs to the tissue inhibitor of metalloproteinase (TIMP) family. Protease inhibitors could locally regulate the proteolytic activity of the uterine proteases or have an antimicrobial action by inhibiting bacterial proteases (Mine and Kovacs-Nolan, 2006). Besides their potential role in defence of the egg, the proteases are likely to have a role in embryonic development in participating to a gradual dissolution of matrix and membrane proteins, and allowing the mobilisation of calcium and other molecules from the eggshell to ensure bone formation.

7.3.2 Egg white proteins

Until 1989 only 13 proteins were identified in the egg white (Li-Chan and Nakai, 1989). This was mainly due to the unfavourable composition of the albumen with six major proteins constituting about 86% of the total protein content, and to its high viscosity (D'Ambrosio *et al*., 2008). For hen egg white proteomic analysis, different strategies and methods have been applied to overcome some technical problems due to egg white consistence. Using 2D electrophoresis and matrix-assisted laser desorption/ionisation–time of flight (MALDI-TOF) mass spectrometry (MS), Raikos *et al*. (2006) identified five proteins. Three of them (ovalbumin, ovotransferrin, clusterin) had already been identified previously, and the other two were activin receptor IIA and the hypothetical protein FLJ10305. Guérin-Dubiard *et al*. (2006) separated egg white proteins by chromatography combined with 2D electrophoresis. The 69 excised protein spots from the 2D gels were cleaved in-gel with specific proteases and the masses of the peptides were measured using MALDI-TOF and liquid chromatography (LC)-MS/MS. Altogether 16 proteins were identified, two of which, Tenp and VMO-I, had not previously been identified as egg white proteins. Both were also detected in the eggshell matrix (Mann *et al*., 2006). Many of these 16 proteins were present in more than one spot in 2D gels, possibly due to differences in post-translational modifications.

Major advances came recently from the Mann and Righetti laboratories, which were able to identify a total of 148 proteins in the egg white (D'Ambrosio *et al*., 2008; Mann, 2007). Mann analysed egg white proteins using LC-MS/MS and MS^3 of peptide mixtures prepared by in solution cleavage of egg white proteins or 1D electrophoresis separation followed

by in-gel digestion and slice by slice analysis. This method allowed the identification of 78 proteins, 54 of which were identified in egg white for the first time (Mann, 2007). This egg white proteomic study also contributed to a better characterisation of some previously known egg white proteins. Thus, it identified the complete ovomucin β-subunit and the N-terminal sequence of the ovalbumin gene X product. Among the newly identified proteins is gallin, a basic protein related to avian β-defensins (Gong *et al.*, 2010; Hervé-Grépinet *et al.*, 2009). A new 7 kDa egg white protein consisting of a single secretoglobulin sequence was also identified and named as ovosecretoglobin. Several new egg white proteins potentially of interest were related to antibacterial defence. A protein that was annotated as 'similar to acyloxyacyl hydrolase' shared 62% identity with mammalian acyloxyacyl hydrolases, which are known to cleave acyl chains from bacterial lipopolysaccharides. A similar antibacterial function was also suggested for a 74 kDa protein (IPI0058627.1), predicted to contain several BPI domains. This domain binds to bacterial LPS and thereby kills the bacteria. Such domains were already reported for Tenp (Guérin-Dubiard *et al.*, 2006) and for ovocalyxin-36, a new specific eggshell matrix protein (Gautron *et al.*, 2007a). Lymphocyte antigen 86 (MD-1), a protein involved in cellular response to LPS, was also present in egg white and eggshell. Additionally, avian beta-defensin 11 and histones H2A, H3 and H4 were identified as novel egg white components with possible antimicrobial activity.

D'Ambrosio *et al.* (2008) explored the chicken egg white proteome using combinatorial peptide ligand libraries. This method reduces the concentration of high-abundance proteins while simultaneously accumulating the low-abundance species (Thulasiraman *et al.*, 2005). This technology used a combinatorial library of hexapeptides. Using 20 different amino acids, the number of possible different ligand structures was several millions. The separation procedure is based on affinity chromatography and use the ability of a protein to interact with other molecule having complementary structures. The different ligands are attached on beads and then the protein mixture is exposed to the ligands. Abundant proteins will quickly saturate their corresponding ligand and the excess will not be bound on the beads. In contrast, a minor protein will not saturate the binding capacity and the total amount of this trace protein will be progressively accumulated on the ligand. The adsorbed proteins are then eluted from the beads and analysed by MS technologies. This method enabled the identification of 70 additional egg white proteins not identified in the previous studies. Peptides derived from 15 of these new egg white proteins did not match chicken proteins, but proteins from other species. Five are of human origin, six are from mouse (*Mus musculus*), three from rat (*Rattus norvegicus*) and 1 from *Cavia porcellus*. The chicken counterparts of these proteins were probably missing in the still incomplete chicken database. These proteins contained conserved domains between many species and were identified due to identical sequences occurring in a general database and did not imply that proteins from other

species are present in chicken. The function of these 70 additional egg white proteins has not been explored.

cDNA microarrays have been used to determine the global gene expression profiling of magnum tissue involved in the egg white synthesis and deposition and allowed the identification of 828 magnum-specific genes (Gautron *et al.*, 2009). Bioinformatics analysis is in progress to establish their potential biological function and should provide complementary data to egg white proteomes.

7.3.3 Vitelline membrane proteins

The chicken egg yolk is separated from the egg white by the proteinaceous extracellular vitelline membrane, which forms the last barrier to microbial infection. The vitelline membrane is composed of the inner layer facing the yolk, the intermediary and the external outer layer in contact to the egg white. The inner layer components are secreted by granulosa cells surrounding the developing oocyte in the follicle. After the ovulation of the yolk-enriched egg cell, the external layer components are deposited onto the egg in the infundibulum part of the oviduct. Proteomic analysis of the chicken egg vitelline membrane identified 137 proteins (Mann, 2008). Only 13 were previously known to be components of the vitelline membrane. Most of the components were already identified in eggshell, yolk and egg white. Because egg white and yolk both border, and in part overlap, the vitelline membrane layers, it is difficult to determine which proteins are really constitutive of the membranes. Nevertheless, this study is a major advance as it revealed 124 newly identified proteins in vitelline membranes. The vitelline membrane outer layer II (VMO-II) was fully characterised and found to be identical to avian β-defensin-11. Proteins related to BPI proteins and to immunoglobulins were also characterised as potential antimicrobial proteins.

Another group of interest concerns proteins involved in the function of reproduction, such as zona pellucida (ZP) proteins. The inner vitelline membrane layer corresponds to the ZP of mammals and contains avian equivalents of mammalian ZP proteins (ZP3/ZPC, ZP1 and ZPD). The vitelline membrane proteome survey showed the presence of eight ZP proteins, five of which had not been previously identified in egg compartments. The vitelline membrane proteome also identified other proteins known to be involved in early embryonic development. Olfatomedin-1 is both identified in egg white and vitelline membranes (Mann, 2007, 2008), and plays a role in the regulation of ectodermal development (Sakuragi *et al.*, 2006). Slit-2, known to be involved in neuronal migration (Wong *et al.*, 2002), is present in vitelline membrane and in eggshell (Mann, 2007, 2008), and is specifically espresssed by the uterus to be secreted in the eggshell-forming milieu (uterine fluid) (Jonchere *et al.*, 2010). CEPU-1, a cell adhesion molecule, which is expressed in the early chick embryo (Jungbluth *et al.*, 2001) and semaphorin C3, which plays a role in the development of tissues (Yazdani and Terman, 2006), are part of

the vitelline membrane proteome. ATPases were also detected, confirming previous studies indicating the presence of ATPase activity in the vitelline membrane (Etheredge *et al.*, 1971). Unexpectedly, the vitelline membrane proteome demonstrated the presence of specific eggshell matrix proteins (ovocleidins-116 and 17, ovocalyxins-32 and 36), which were thought to be specifically expressed in the eggshell gland (uterus). However, ovocleidin-116 was recently detected in skeletal tissues (Bardet *et al.*, 2010; Horvat-Gordon *et al.*, 2008) indicating a more widespread distribution of these proteins. It is noteworthy that an antimicrobial role was shown for ovocalyxin-32 and 36 (Gautron *et al.*, 2007a; Xing *et al.*, 2007).

7.3.4 Egg yolk proteins

Yolk constituents are mainly synthesised in the liver and transported as plasma very low density proteins (VLDL), to the ovary where they are incorporated in the developing egg yolk. Using 1D electrophoresis and LC-MS/MS, Mann and Mann (2008) identified 119 egg yolk proteins, 86 of them not previously identified in this egg compartment. The most abundant proteins are serum albumin, vitellogenin cleavage products, apovitellin, IgY, ovalbumin and a 12 kDa serum protein with cross-reactivity to β2-microglobulin. The proteins identified can be classified in various groups. The first group is constituted by the vitellogenin-derived yolk proteins and apovitellenins. Vitellogenins are constituents of blood high density lipoproteins, which are cleaved in yolk to yield the mature proteins. The yolk proteome also contained other lipid-binding proteins, vitamin- and cofactor-binding proteins, proteases and protease inhibitors, serum proteins and proteins previously identified in egg white.

The chicken egg yolk cytoplasmic proteome was also investigated using combinatorial peptide ligand libraries (Farinazzo *et al.*, 2009), as previously reported for egg white (D'Ambrosio *et al.*, 2008) (see Section 7.3.2). This approach enabled the identification of 255 new yolk proteins with 54 in common with the previously determined yolk proteome (Mann and Mann, 2008). Altogether, a total of 316 proteins were identified in egg yolk. More work will be necessary to determine the function of these proteins.

7.4 Conclusion

The recent development of high-throughput technologies boosted by the availability of ESTs, the chicken genomic sequence and the development of *in silico* functional annotations, allowed the identification and characterisation of several new egg components and of proteins involved in the process of egg components synthesis or secretion. It also provides powerful tools for exploring the regulation of this process. The challenge is now to explore this important amount of data to functionally clarify the biological role of

these proteins as an egg component involved in the biology of egg formation and embryonic development. Indeed, transcriptomics should contribute in the near future to analyse regulation of ovogenesis by exploring differential gene expression at various physiological stages. On the other hand, screening of egg components by proteomics and transcriptomics has been an efficient method of identifying novel molecules in the egg components. The next step is to predict function from protein sequence and to purify proteins of interest to confirm predicted functions by *in vitro* tests or analysis. In addition, the availability of software that allows large amounts of information contained in databases to be interrogated, either using data from literature (text mining), or classifications using protein sequences in different ontologies or pathways, will allow a broader view of the functions and of the biological relevance of the detected proteins. This approach has recently been performed on egg yolk and egg white proteomic data (D'Alessandro *et al.*, 2010). Data from the literature have been regrouped and elaborated for network and pathway analysis, and these analyses highlighted a role for egg proteins involved in cell development and proliferation, cell to cell interaction, haematological system and cell migration. However, the analysis of the egg proteome data sets had been performed on software specifically designed to human pathologies and consequently the obtained networks and pathways did not relate to egg functions.

Biological activities of the egg molecules showed antimicrobial activities or are involved in eggshell formation, the physical barrier against bacterial penetration. These natural defences of the egg can be reinforced by selection or by controlling the environmental factors affecting their level of activity. On one hand, some of the novel egg proteins are candidates to be used as biological markers for a marker-assisted selection to reinforce the protective systems of the egg (Dunn *et al.*, 2008). On the other hand, the study of environmental and breeding factors which might affect their activity or levels can be used to optimise the egg defences. Altogether, these approaches will contribute to reduce the risk of foodborne disease outbreaks for egg consumers. Finally, many of the new proteins identified in yolk, white and shell by both transcriptomics and proteomics, may have biological activities of major interest for human and animal health, and for developing non-food use of eggs and egg products.

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8

The eggshell: structure and protective function

M. Hincke, University of Ottawa, Canada, J. Gautron and Y. Nys, Institut National de la Recherche Agronomique, France, A. B. Rodriguez-Navarro, University of Granada, Spain and M. D. McKee, McGill University, Canada

Abstract: Changes in eggshell properties are directly related to increasing risk of egg contamination and foodborne outbreaks for the consumer. This review focuses on the results of recent genomic, transcriptomic and proteomic analyses of the eggshell in order to draw attention to current understanding of eggshell mineralization and structural features that contribute to shell strength. The majority of constituents of the chicken eggshell have been identified. Genes involved in eggshell formation and mineralization are functional candidates for marker-assisted selection to improve egg and eggshell quality. An active field of development is research to identify constituents of eggshell mineral and membranes derived from breaker operations that are suitable for industrial exploitation.

Keywords: eggshell, calcite, microstructure, ovocleidin, ovocalyxin, texture.

8.1 Introduction

The calcified eggshell provides protection to the egg contents and embryo against physical damage and contamination by microorganisms. This complex bioceramic also regulates the exchange of metabolic gases and water, and provides calcium to the developing embryo. A vast number of eggs are produced annually for human consumption worldwide (1140 billion eggs in 2008); an understanding of eggshell formation and its control is of primary importance to avoid egg contamination. Changes in eggshell properties are directly related to increasing risk of foodborne disease for the consumer. During mineralization of the avian eggshell, there is a sequential and orderly

deposition of both matrix and mineral phases. Hence the eggshell is an excellent model for studying matrix–mineral relationships and the regulation of calcitic mineralization. The majority of constituents of the chicken eggshell have been identified. This chapter will focus on the results of recent genomic, transcriptomic, proteomic and structural analyses of the eggshell, in order to draw attention to the impact of the data on current understanding of eggshell mineralization and its ultrastructure/microstructure that contribute to shell strength. Such investigations continue to provide new insights into the function of integrated defense strategies that operate at biomineralized barriers. Genes involved in eggshell formation and mineralization are functional candidates for marker-assisted selection to improve egg and eggshell quality. In addition, the massive scale of egg production throughout the world provides a ready supply of eggshell mineral and membranes that are under active investigation for industrial exploitation.

8.2 Structure of eggshell 1: composition and characterization

Calcified matrices in vertebrate biology are biphasic composites that usually contain collagenous and noncollagenous elements in intimate contact with mineral (Robey, 1996). These basic features are also present in the avian eggshell, where two overlapping yet distinct compartments are present: the eggshell membranes and the compact mineralized layers. The avian eggshell is a complex and highly structured porous calcitic bioceramic with extensive intermingling of both its organic and inorganic phases; it demonstrates a modest overlap between the noncalcified eggshell membrane and the calcified eggshell (Arias *et al*., 1993; Dennis *et al*., 1996; Nys *et al*., 1999, 2004) (Fig. 8.1). The egg is composed of a central yolk surrounded by the albumen, eggshell membranes, calcified eggshell and cuticle (Hincke *et al*., 2008a). During formation of the avian egg, it sequentially acquires all of its layers as it passes through specialized regions of the oviduct. Pores span the eggshell and permit diffusion of metabolic gases and water vapor that are necessary for proper embryonic development (Ar and Rahn, 1985).

8.2.1 Cuticle: composition and function

The majority of shell pigments (two-thirds) and eggshell cuticle are deposited on the surface of the immobilized egg during the last 1.5 hours before oviposition (egg expulsion). The outermost shell layer is the eggshell cuticle, a noncalcified organic layer of variable thickness, which may even be absent. A thickness range of 5–10 μm for the chicken cuticle has been reported (Romanoff and Romanoff, 1949). The cuticle is composed of glycoproteins, polysaccharides, lipids and inorganic phosphorus including

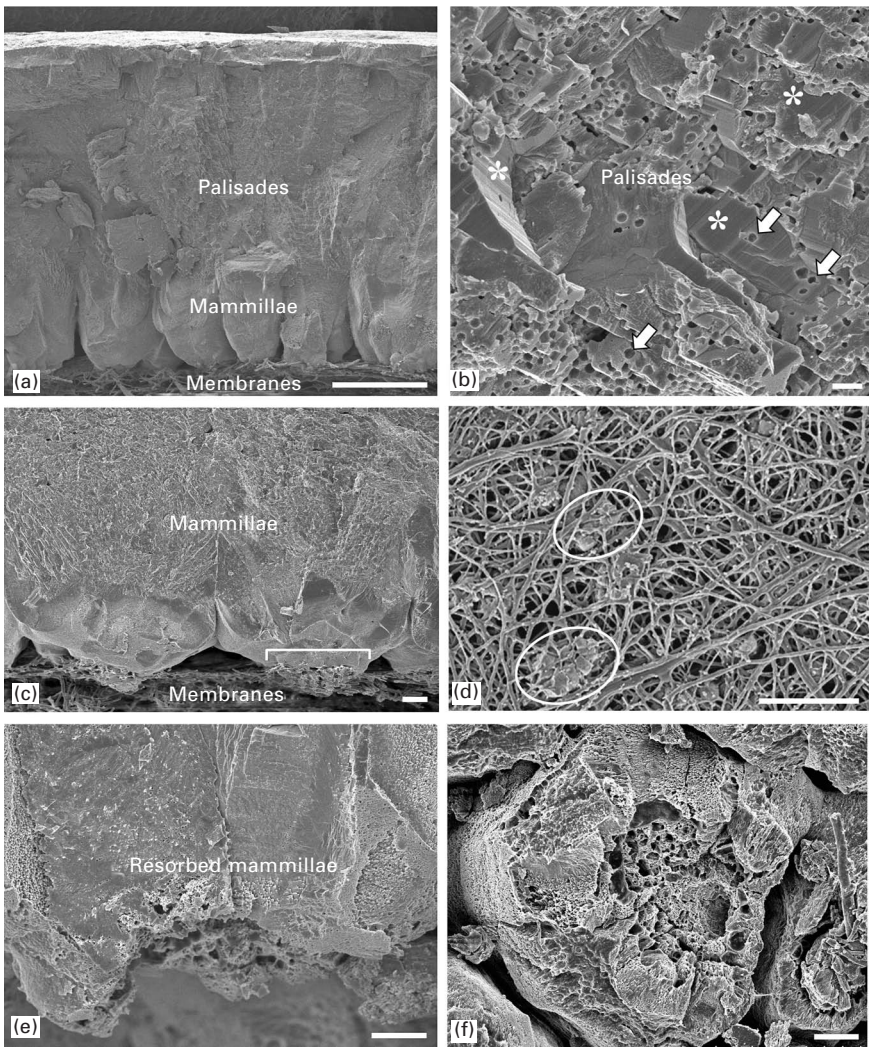

Fig. 8.1 Scanning electron micrographs of avian eggshell after complete formation (a–d), and after partial resorption following egg fertilization (e–f). The palisade layer consists of groups of aligned calcitic columns (palisades) that, overlay an array of mammillae aligned against the fibrous shell membranes. (a, b) Cross-fractured eggshell in the palisade region reveals extensive and irregular cleaved planes of calcite (asterisks) and an abundance of spherical voids (arrows) within the calcite mineral. (c, d) Shell membrane fibers intercalate with the tips of the mammillae (bracket in panel c, and oval outlines as viewed *en face* from the eggshell interior in panel d) to attach to each other at this junction. (e, f) Following egg fertilization and incubation, mineral in the mammillae is dissolved to provide a source of calcium for the growing embryonic chick skeleton leaving a resorbed appearance at the mammillary tips (resorbed mammillae in profile in panel (e) and viewed *en face* from the eggshell interior in panel (f)). Magnification bars equal 100 µm (a), 1 µm (b), 10 µm (c, e, f) and 50 µm (d).

hydroxyapatite crystals (Burley and Vadehra, 1989; Dennis *et al.*, 1996). The cuticle constituents also plug the eggshell pores and thus physically limit bacterial entry at these sites. The cuticle is thought to play a role in controlling water exchange by repelling water or preventing its loss, and may function in limiting microbial colonization of the eggshell surface (Hincke *et al.*, 2008a).

8.2.2 Calcified shell: inorganic constituents

Remarkable in eggshells is the interwoven fabric of organic and inorganic constituents that constitute the palisade and the mammillary layers (Fig. 8.1a). These two layers form the bulk of the avian eggshell (0.3–0.35 mm thick in the chicken eggshell), and their architectures are particularly noteworthy in terms of how proteins interface and intercalate with the calcitic mineral phase. Also remarkable in a fertilized, incubated egg is how the carefully regulated dissolution of eggshell mineral provides an essential source of calcium for the calcium phosphate apatite of bone in the growing embryonic skeleton (Burley and Vadehra, 1989; Dieckert *et al.*, 1989; Arias *et al.*, 1993). It is precisely the nature of the matrix-mineral relationships within the shell that appear to guide its growth, define shell architecture substructure by delineating mineral boundaries (Chien *et al.*, 2008), provide unique mechanical properties to the shell (Nys *et al.*, 2004), and finally establish a structure conducive to partial dissolution to supply calcium for skeletal growth with concomitant weakening of the shell for pipping (hatching) (Dennis *et al.*, 1996; Chien *et al.*, 2009a). Understanding such diverse properties for the shell implicitly requires considerable knowledge of the hierarchies in shell structure ranging from the macro- to the nanoscale, together with an appreciation of molecular organization at the organic–inorganic interface (Chien *et al.*, 2009b). Importantly, recent new information about this hierarchical structure has allowed for a more integrated and better understood view of how an organic matrix and its individual constituents interface with, and function within, the calcitic mineral phase of the shell. Both the palisade and the mammillary layers are unique in terms of their architecture and composition – for both the organic and inorganic phases – and details of these two layers are emerging as described below.

Mammillary layer

The mammillary bodies (Fig. 8.1a, c), representing the narrow tips of the calcitic columns/cones that radiate outwards towards the exterior shell surface, are complex and key structures in both the formative and resorptive 'life' of an eggshell. Indeed, the initial formation of the very tips of the mammillary bodies on the just-assembled shell membranes constitutes the first construction event leading to mineralized cone structure. The simultaneous focal assembly of mammillary tip proteins and mineral at discrete and evenly spaced locations on the shell membranes represents the 'birth' of the

calcified shell (Fig. 8.1d). Little is known of how this process is so precisely temporo-spatially nucleated, patterned and regulated, nor the nature of the discrete focal compositional differences/changes in the membrane fibers that lead to this site-specific nucleation. In this regard, *in vitro* studies reveal that shell membranes isolated with attached remnants of the mammillary layer promote further calcium carbonate deposition from a metastable solution at these specific sites (Wu *et al.*, 1992, 1994). This observation supports the hypothesis of control of crystal nucleation by the organic core of the mammillae and possibly by the matrix deposited concomitantly with calcite to form the calcium reserve assembly (Dieckert *et al.*, 1989; Dennis *et al.*, 1996) (see next section). In particular, a keratan sulfate proteoglycan, termed mammillan, has been implicated; immunohistochemistry reveals that keratan sulfate is prominent in the mammillary knobs as compared to the palisade layer (Arias *et al.*, 1992; Fernandez *et al.*, 1997, 2001). The appearance of keratan sulfate proteoglycan, which is secreted by the isthmus gland cells (Fernandez *et al.*, 1997), coincides with the formation of the mammillae 5.15 h post-oviposition, and its localization within the substructure termed calcium reserve body (Fernandez *et al.*, 2001) corresponds with the site of nucleation of the first crystals and the eventual removal of calcium by the incubated chick embryo. The protein core of this key molecule has not yet been characterized.

Further outward growth of the calcitic cones coincides with a massive accumulation of organic material/matrix within the mammillary bodies, and a remarkable compartmentalization of the mammillae to include a so-called calcium reserve body or assembly (Terepka, 1963a,b; Wyburn *et al.*, 1973; Dieckert *et al.*, 1989; Dennis *et al.*, 1996). Mammillary structure appears more complex than palisade structure, perhaps reflecting its nucleating role and its function as a calcium source for the growing embryonic skeleton in fertilized and incubated eggs. Perhaps best studied are the subcompartments of the calcium reserve body, and how they are thought to contribute calcium for skeletal growth. Selective dissolution of calcium reserve body structures and components appear to release mineral ions, which may then travel along protein-defined conduits to be released at the interior of the shell (Fig. 8.1e,f) (Chien *et al.*, 2009a). To date, only morphological and microanalytical evidence exists to support this possibility.

Palisade layer

The palisades region of the shell (Fig. 8.1b) is defined as the region where the mineralized cone-like substructures originating from the mammillae become tightly apposed to one another to form an approximating polygonal array of compacted cones; however, narrow pores are found at some of the cone junctions that permit gaseous exchange. Clearly, this cone-like compacted architecture where the cones are wider in dimension towards the shell exterior provides a segmental, rounded arch structure to resist exterior compressive

forces, while permitting chick pipping from the inside by pushing outwards on the narrow cone bases at the time of chick hatching. Remarkably, and previously poorly understood, the shell contains a surprisingly diverse organic matrix (large number of organic matrix components) accompanying the calcitic mineral phase (Terepka, 1963a,b; Mann *et al.*, 2006, 2007; Rose and Hincke, 2009). Biochemical, immunochemical and proteomic investigations have identified a vast array of protein components within the shell, likely many of which are in the palisades region. These are discussed in more detail in Section 8.3.3.

It was intriguing to many over decades of study how such an organic matrix could co-exist with the highly organized and oriented, columnar calcitic mineral phase. Reconciling this mineral architecture with an interpretation of how proteins could be either layered or intercalated within seemingly single calcite crystals was at best difficult and, at its worst, incomprehensible. Even today, although we understand that proteins are occluded within the mineral phase, possibly with crystallographic face selectivity (Aizenberg *et al.*, 1996; Chien *et al.*, 2008), we are far from understanding their three-dimensional organization within the calcite. Inherent to most sample preparation protocols used to study mineralized tissues is a significant risk of inducing artifact in either the organic or inorganic phases, or both. The procedures themselves are usually optimized to study one of the shell phases at a time, and rarely do they work for both. Despite this, in recent years, compromise treatments of shell fragments have allowed direct visualization *in situ* of some protein structure and architecture as it relates to the mineral phase. For example, the use of light etching techniques, for both mineral and organics, has allowed partial dissolution of fractured calcite surfaces, and partial degradation of protein constituents at these same surfaces, to render surface topography amenable to ultrastructural and immunochemical labeling methods capable of interrogating surface structure and composition (Chien *et al.* 2008, 2009b). Collectively, from using these approaches, we now know that certain proteins appear to have a preference for certain crystallographic faces, whose selective binding is thought to influence calcite crystal growth (Aizenberg *et al.*, 1994). Certainly, while the eggshell is one of the fastest known biomineralizing systems, where one might intuitively think there would be an overall absence of inhibitors to crystal growth, data from many organismal biosystems, including eggshell, indicate that the mineral-binding proteins function to carefully guide crystal growth rather than to provide an overall inhibition. This hypothesis is supported by modeling of calcite growth on a membrane surface, which shows that eggshell texture and preferred orientation can be explained by competitive and anistropic crystal growth (Fig. 8.2) (Rodriguez-Navarro and Garcia-Ruiz, 2000). Thus, in the eggshell, protein binding at specific sites may selectively assist in shaping and sizing crystal domains and mineral form, and in compartmentalizing and limiting mineralized interfacial boundaries where calcite growth must be terminated. Such activities most surely could lead to defining shell cone structure and

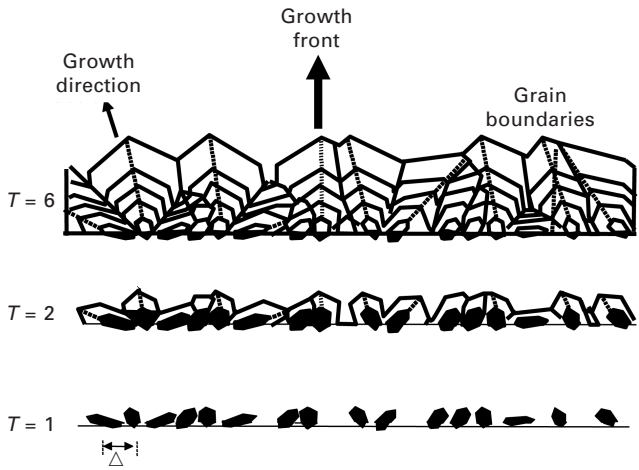

Fig. 8.2 Computer simulation of development of shell texture by competition between adjacent nucleation sites. Only calcite crystals growing perpendicular to the surface are not blocked by adjacent crystals. (After Rodriguez-Navarro and Garcia-Ruiz, 2000.)

its relations with neighbouring cones, pores and mammillary bodies (Chien *et al.*, 2008).

The apparent organization of extensive and abundant planar lamellae of organic material that appear in samples of decalcified eggshell (Chien *et al.*, 2008) likely is partly artifactual, resulting from the collapse of the organic phase after exhaustive decalcification procedures. However, some proteinaceous networks do indeed appear to reside within the mineral phase (both in the palisade and in the mammillary layers, see above), and understanding the precise nature of these interactions in three dimensions is only now beginning to be understood. One possibility is that in addition to modulating crystal growth and mechanical properties of the eggshell, they might also serve as surface area-increasing nano/micro-conduits that facilitate mineral dissolution and transport of calcium towards the interior of the shell for release to the growing embryonic chick skeleton (Chien *et al.*, 2009a). Finally, essentially nothing is known of the formation and function of the spherical voids abundant in the palisade region (Fig. 8.1b) (Simons, 1971; Wyburn *et al.*, 1973). They contain dermatan sulfate proteoglycan (Fernandez *et al.*, 2001) and may be created by circular protein pinning of growth steps during crystal growth (Chien *et al.*, 2008).

8.2.3 Characterization of eggshell microstructure and crystallographic texture

The mechanical properties of the shell are exceptional: the thickness of the chicken eggshell is slightly greater than 0.3 mm, although it is able to resist

static pressures over 3 kg. Eggshell thickness is the main factor contributing to the mechanical strength of the eggshell (Romanoff and Romanoff, 1949; Tyler, 1961). Nevertheless, there is also evidence that the structural organization of the eggshell at different levels significantly influences its mechanical properties (Meller *et al*., 1973; van Toledo *et al*., 1982; Rodriguez-Navarro *et al*., 2002; Lammie *et al*., 2006). Ultrastructure (defined by the extent and disposition of major structural units) and microstructure (defined by the size of crystals, their shape and crystallographic orientation – also known as 'texture') are especially important. The ultrastructure of the chicken eggshell is extremely regular. It is a polycrystalline calcium carbonate ceramic consisting of only one polymorph, calcite. The mammillary cones are composed of calcite crystals that are small in size and are deposited without privileged orientation. The palisade region of the chicken eggshell is about 200 μm thick, and is composed of irregular juxtaposed columns that vary in diameter from 10 to 30 μm. In the palisade region the crystals increase their size progressively and elongate along the calcite *c*-axis towards the eggshell surface.

Quantification of microstructural parameters is important to understand eggshell changes due to multiple factors (hen age, diet, pollution; Rodriguez-Navarro *et al*., 2002; Ahmed *et al*., 2005). Traditionally, microstructure is characterized using optical microscopy (OM), scanning electron microscopy (SEM) or conventional X-ray diffraction to determine crystal orientation (Meller *et al*., 1973; van Toledo *et al*., 1982; Sharp and Silyn-Robert, 1984; Rodriguez-Navarro *et al*., 2002). Although the microscopic techniques provide detailed information about the structural organization of eggshell, they are time-consuming and do not always provide quantitative information about different microstructural parameters. Alternatively, 2D X-ray diffraction techniques provide more accurate and quantitative information (Rodriguez-Navarro *et al*., 2007). For such analyses, an X-ray diffractometer equipped with an area detector is used. From the 2D diffraction pattern of a sample, information regarding both crystal sizes and orientation can be automatically extracted using adequate software (Fig. 8.3). This technique allowed the detection of subtle but significant differences in hen eggshell microstructure associated with molting (Ahmed *et al*., 2005).

The orientation of crystals in a polycrystalline sample is adequately described using pole figures, which display the 3D distribution of specific [*hkl*] crystallographic directions or poles (Fig. 8.4). The orientations of crystallographic directions are displayed as projections in 2D plots using polar coordinates. As crystals become preferentially oriented, poles are concentrated in specific part of the pole figure diagram (Figs 8.3, 8.4). Pole figures can be generated using an X-ray texture diffractometer or a single crystal diffractometer with an area detector. With this type of equipment it is possible to measure the variation of intensity of a given [*hkl*] reflections for any possible orientation of the sample and from there generate the associated *hkl* pole figure (Klug and Alexander, 1974; Rodriguez-Navarro *et al*., 2007).

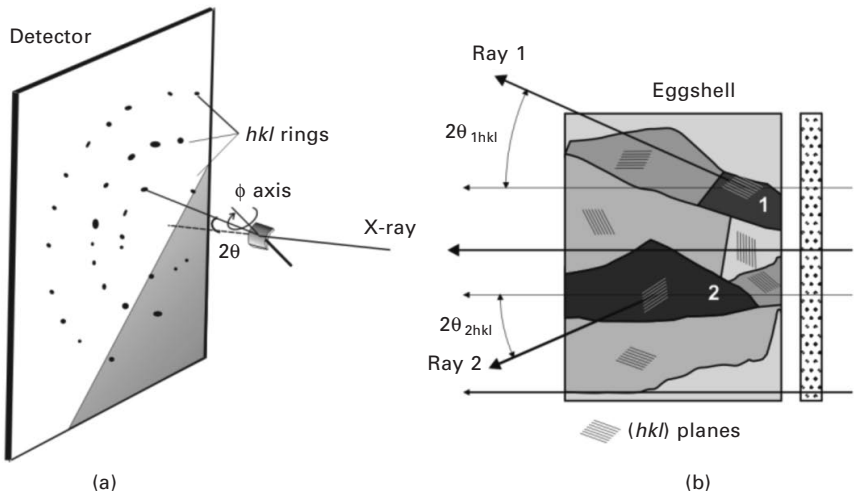

Fig. 8.3 X-ray diffraction of eggshell. (a) The diffraction pattern of a polycrystalline sample such as an eggshell consists of rings formed by spots produced by individual crystals. (b) Schematic drawing of crystals that make up the eggshell. Only those crystals whose (*hkl*) planes are oriented in Bragg condition diffract (e.g. crystals 1 and 2). The intensity of spots recorded in the *hkl* ring is proportional to the size of the diffracting crystals. Thus crystal 2 will contribute a more intense spot than crystal 1. (Provided by A.B. Rodriguez-Navarro.)

Influence of microstructure on mechanical properties

Microstructure characteristics (crystal size and orientation) can vary significantly from one eggshell to another, even within the same species. Weaker eggshells are formed by crystals of abnormal sizes (generally larger) and shapes which negatively affect their mechanical performance (Rodriguez-Navarro *et al.*, 2002; Ahmed *et al.*, 2005), and may also have a larger number of defects or scratches that act as nucleation sites for crack formation. In addition, the preferential orientation of crystals has a strong influence on eggshell mechanical properties. This dependence on crystallographic orientation is probably attributable to the fact that calcite is easily cleaved along specific crystallographic directions. Thus, eggshells composed of smaller calcite crystals, which are less mutually aligned, are stronger than those formed by larger and highly oriented crystals. This crystalline structure is revealed by optical microscopy of transverse sections in polarized light (Fig. 8.4). In most avian species, the majority of the crystals making up the shell are progressively directed in a single privileged direction; the *c*-axis of calcite tends to a perpendicular orientation to the shell surface in its upper third (chicken, quail, pheasant, turkey, ostrich) (Fig. 8.5). Exceptions have been noted for eggs of guinea fowl, duck and goose.

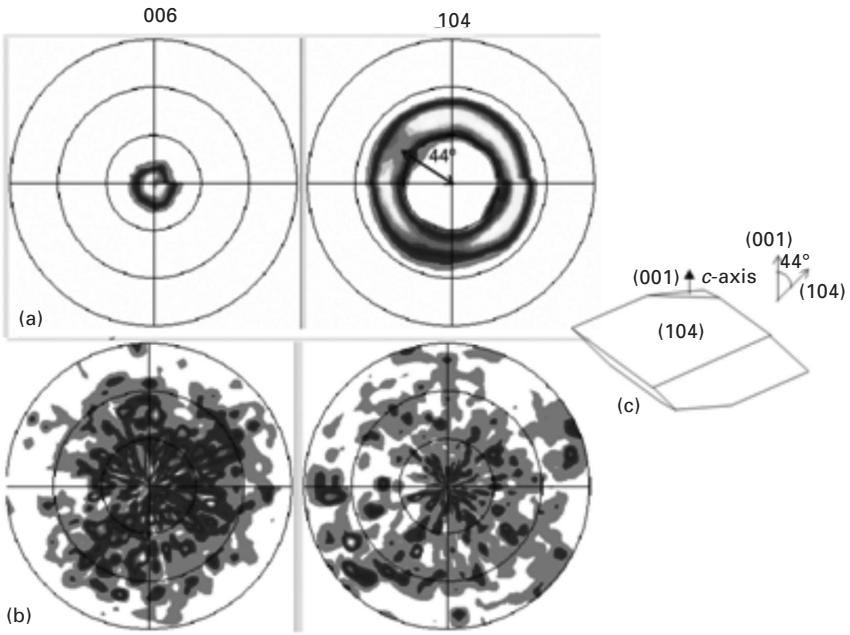

Fig. 8.4 The 006 and 104 pole figures for ostrich and chicken eggshells. (a) Ostrich: The central maximum at the 006 pole figure and ring at the 104 pole figure indicate that calcite crystals are strongly oriented with their *c*-axis perpendicular to the outer surface of eggshell but randomly rotated around it. The angular separation of about 44° between the 006 central maximum and the 104 ring, correspond to the interfacial angle between the (001) and the (104) planes. (b) Chicken: multiple maxima are distributed around the pole figure due to the coarse crystal size of calcite crystals. The scattered distribution of maxima indicates that the there is no preferential orientation of crystals. (c) Sketch of a calcite crystal showing the interfacial angle between (001) and (104) planes. (Provided by A.B. Rodriguez-Navarro.)

8.3 Structure of eggshell 2: biosynthesis and constituents

8.3.1 Biosynthesis/formation of the shell

The mineralized tissues of living organisms differ from each other by their shape, mineral composition, ultrastructure, crystallographic texture and mode of manufacture. The eggshell is a porous ceramic that is formed at low temperature in a cell-free environment, from the uterine secretion of its organic and inorganic precursors (Nys *et al.*, 1999). The mineralization of the shell is characterized by controlled precipitation of calcium carbonate on a membrane, and occurs in the extracellular space between the forming egg surrounded by its soft shell membranes and the tubular wall of the distal oviduct. During this process, the egg expands progressively, due to hydration of its egg white proteins (plumping), while it rotates around its polar axis. It is bathed in a uterine fluid containing 6 to 10 mM of ionized calcium and about 70 mM of bicarbonate ions (Nys *et al.*, 1991). Thus the concentrations

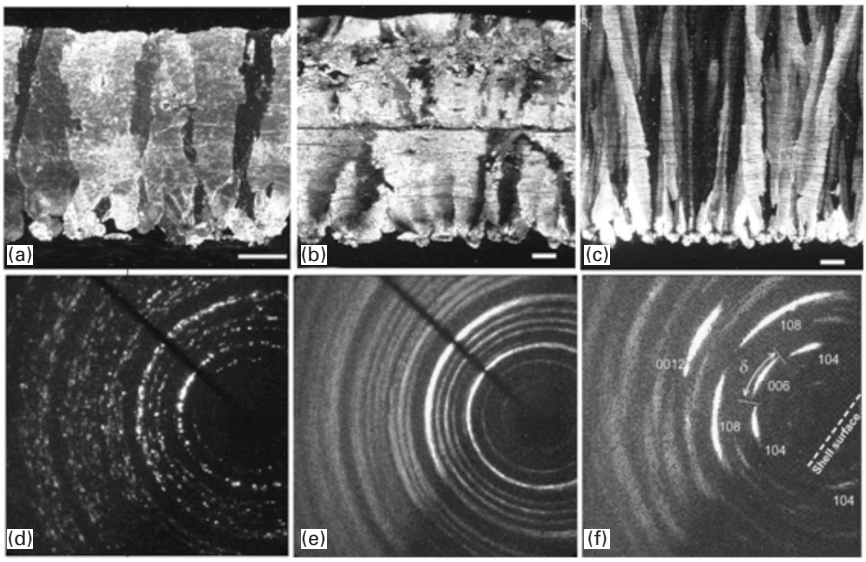

Fig. 8.5 Microphotographic views under cross-polarized light of eggshells from different bird species: (a) chicken, (b) emu and (c) ostrich. Scale bars are equivalent to 100 μm. The mineral part of the eggshells is constituted by columnar calcite crystal units (palisade layer) which show varying degrees of light extinction due to differences in their orientation. 2D X-ray diffraction patterns of the same eggshells: (d) chicken, (e) emu and (f) ostrich. Note that the differences in microstructure of eggshells from different bird species translate into differences in the appearance of rings. Ostrich eggshell displays a strong preferential orientation of crystals which is manifested in the reflection spots merging into short continuous arcs. The δ angle indicates the degree of dispersion of *c*-axes of calcite crystals. In the case of emu eggshell, formed by randomly oriented microcrystals, the diffraction pattern displays continuous rings. In chicken eggshell, which is formed by larger and randomly oriented crystals, the diffraction pattern consists of spotty rings resulting from isolated reflection spots of individual crystals. (Provided by A.B. Rodriguez-Navarro.)

of the precursor ions of calcium carbonate are 80–120 times greater than the solubility product of calcite (K_{sp}), during all phases of shell mineralization. In this supersaturated milieu calcium carbonate precipitates spontaneously in the form of calcite (the most thermodynamically stable polymorph at body temperature and atmospheric pressure). Moreover, it has been demonstrated *in vitro* that the organic constituents of uterine fluid promote formation of calcite, as opposed to other polymorphs of calcium carbonate (aragonite, vaterite) (Hernandez-Hernandez *et al.*, 2008a,b,c).

Mineralization of the shell lasts almost 18 h and is the lengthiest phase of egg formation (Fig. 8.6). It occurs in three stages. The 1st stage is about 4 h in duration and corresponds to the initiation of mineralization in which the first crystals of calcite are formed at the sites of the organic aggregates present on the surface of external shell membranes. The 2nd stage is the rapid growth of polycrystalline calcite, during which there is a

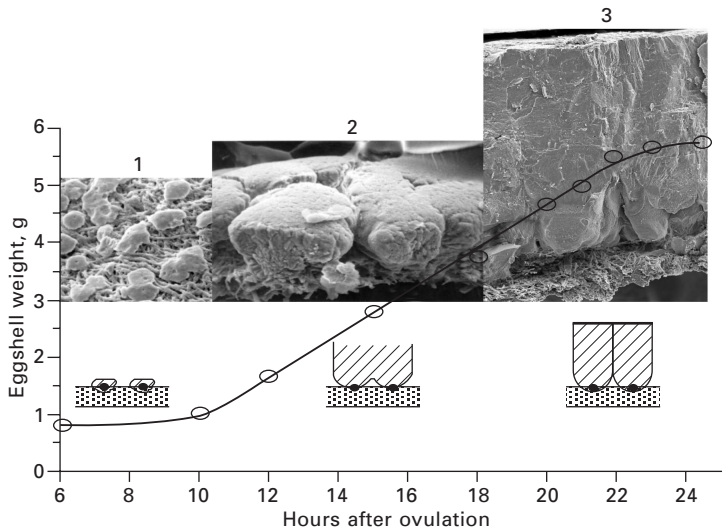

Fig. 8.6 Kinetics of eggshell mineralization. Three periods of eggshell formation can be distinguished, corresponding to: (1) initiation of calcification; (2) growth and active deposition of calcium carbonate; and (3) termination. (Based on original artwork of J. Gautron.)

linear deposition of 0.33 g/h of calcium carbonate for about 12 h. The last stage corresponds to termination of calcification and lasts about 1.5 h (Nys *et al.*, 2004).

Calcification of the shell begins in the red isthmus (stage 1), where small crystals of calcite are deposited at the sites of organic aggregates, thereby nucleating the growth of the mammillary cones. The distribution of nucleation sites on the surface of the external eggshell membrane is under genetic control and varies among species. The size of the mammillary cones, the cylindrical diameter of the palisades in the compact layer of the shell and, ultimately, the strength of the shell are determined by the spacing of these sites. During the first stage of mineralization, crystal growth is radial calcite. The shell membrane fibres prevent mineralization from occurring *into* the egg. The nucleation sites become the origins of the mammillary cones; as they grow outwards they gradually come together to form the bases of the palisade layer, at which point radial crystal growth is inhibited because of competition for space (Fig. 8.2) (Nys *et al.*, 2004). Only the crystals growing perpendicular to the surface will be able to continue to elongate during the second stage of mineralization. Formation of the palisade layer occurs into the available free space, producing crystals growing perpendicular to the surface of the forming shell.

This hypothesis of competitive growth between adjacent nucleation sites explains the observation of a preferred orientation in the upper shell. A prerequisite for this hypothesis is anisotropic growth of calcite crystals,

that is to say unequal growth of crystal faces in different directions. Thus constituents of the organic matrix, which bind to surfaces parallel to the calcite *c*-axis by hydrophilic or hydrophobic bonds and therefore inhibit the growth of these faces, encourage the elongation of the crystal along the *c*-axis (Hernandez-Hernandez *et al*., 2008c). Another example of this phenomenon, which controls mineral texture, is the preferential localization of osteopontin on specific calcite crystal faces in the palisade layer (Chien *et al*., 2008) (page 170). Termination of calcification (stage 3), which occurs 1.5 h prior to oviposition, takes place in a uterine fluid that remains supersaturated in calcium and bicarbonate ions. Although not well understood, it is believed that mineralization is inhibited by phosphorylated proteins, since this process is mimicked by the inhibition of $CaCO_3$ precipitation *in vitro* by high molecular weight components of the terminal phase uterine fluid (Gautron *et al*., 1997). Moreover, phosphorus is detected in the superficial layers of the chicken shell (Cusack *et al*., 2003), and phosphate anion is known to inhibit calcium carbonate precipitation (Lin and Singer, 2005).

Evidence for control of shell construction by its protein organic matrix

A number of elements demonstrate the functional role of organic components in shell fabrication: (1) specific protein profiles of the uterine fluid are unique to each stage of mineralization; (2) demonstration *in vitro* of modification in the crystallization kinetics and morphology of calcite in the presence of these molecules; and (3) there is a relationship between *in vivo* ultrastructural or mechanical properties of eggshell and the eggshell matrix protein content. The last point is reinforced by the results of association studies with specific SNPs of genes of proteins involved in mineralization.

Changes in the composition of uterine fluid

The shell forms in a cell-free environment containing the mineral and organic secretions of the isthmus and uterus. This medium remains supersaturated with respect to the solubility product of calcite during the three phases of mineralization (Nys *et al*., 1991) and therefore the particular process observed respectively at initiation, growth and arrest of calcification is suspected to be controlled by the different protein profiles revealed at each phase of mineralization by electrophoretic analysis (Gautron *et al*., 1997). Following collection of uterine fluid at specific stages, calcium carbonate precipitates spontaneously within a few hours to pull down a subset of the protein constituents. These components are only synthesized in the tissues where mineralization occurs (red isthmus and uterus). The expression of their mRNA is detectable only in sexually mature animals. Finally, some are overexpressed each day in synchrony with the mechanical stimulation induced by the entry of the egg into the distal oviduct during mineralization (Lavelin *et al*., 1998; Gautron *et al*., 2007). Moreover, when the electrophoretic profile of uterine fluid was examined before and after spontaneous precipitation of calcite (Gautron *et al*., 1997; Hernandez-Hernandez *et al*., 2008a), the

disappearance of specific proteins was noted, namely ovocleidin-116 and ovocalyxin-32.

Control of calcium carbonate mineralization in vitro by organic constituents

In vitro precipitation of calcium carbonate is delayed in a dose-dependent manner by the soluble eggshell matrix obtained by decalcification (Gautron *et al.*, 1996). A similar effect is observed with partially purified eggshell proteoglycan (Arias *et al.*, 1992) and with the macromolecules present in uterine fluid (Gautron *et al.*, 1997). Moreover, the morphology of calcite crystals grown *in vitro* is strongly modified in the presence of uterine fluid. Constituents of the uterine fluid control the polymorphic form of calcium carbonate: in the presence of low concentrations of uterine fluid (at whatever its stage of sampling), only calcite is formed *in vitro* in contrast to the control without protein where calcite (50%), aragonite (25%) and vaterite (25%) are obtained (Hernandez-Hernandez *et al.*, 2008b). The introduction of uterine fluid decreased by almost 1000-fold the latency for formation of the first crystals, while their number increases significantly (>10 000 instead of 40); furthermore, crystal size is reduced 5- to 20-fold. In addition, large changes in the kinetics of calcite crystal formation, their size and morphology were observed when a soluble extract of the decalcified organic matrix is introduced into a test solution for crystal growth of calcium carbonate *in vitro* (Gautron *et al.*, 1996; Dominguez-Vera *et al.*, 2000; Hernandez-Hernandez *et al.*, 2008b).

Many attempts have been made to identify the constituents responsible for this interaction with the crystal formation. The morphology of calcite crystals is strongly modified in the presence of chicken lysozyme, ovotransferrin and ovocleidin-17 (Hincke *et al.*, 2000; Gautron *et al.*, 2001a; Reyes-Grajeda *et al.*, 2004) or goose ansocalcin (Lakshminarayanan *et al.*, 2002), likely due to the conformational details of charged amino acids (Ajikumar *et al.*, 2005). For example, ovotransferrin (500 µg/ml) induced characteristic surface changes; the (104) remain flat but their edges converging in the *c*-axis show an irregular appearance with faces (018). The crystal grows and forms a chevron structure reminiscent of observations in the palisades region (Gautron *et al.*, 2001a). In chickens, a likely candidate is the dermatan sulfate proteoglycan, termed ovoglycan, whose core protein is ovocleidin-116, and which possesses polyanionic characteristics (Fernandez *et al.*, 2001, 2004). Also phosphorylated proteins such as osteopontin, ovocleidin-17, ovocleidin-116 and the ovocalyxin-32 are potential candidates (Mann *et al.*, 2007) (Section 8.3.3). Mechanistic details are not yet fully understood; however, these observations support the hypothesis that matrix proteins control the process of eggshell mineralization.

In vitro experiments have been confirmed by *in vivo* observations. Variation in many factors, whether nutritional, genetic or physiological (age) are known to affect the strength of the shell. The well-known reduction in shell

proportion in eggs from hens at the end of production only partially explains the 50% diminution in shell breaking strength. However, this weakness coincides with a change in the relative proportions of matrix proteins in the shell (Panheleux *et al.*, 2000) and alterations in crystallographic texture. Molting restored the strength of the shell and reversed the changes previously observed for matrix composition and crystalline texture of the shell (Ahmed *et al.*, 2005). Finally, association studies between polymorphisms of genes encoding shell proteins and shell quality have revealed osteopontin alleles associated with the hardness of the shell, those of ovocleidin-116 related to elasticity and thickness of the shell and ovocalyxin-32 correlated to the thickness of the mammillary layer (Dunn *et al.*, 2008).

8.3.2 Organic constituents

The eggshell mineral is associated with an organic matrix composed of proteins, glycoproteins and proteoglycans, termed 'eggshell matrix proteins', which are progressively incorporated from the precursor milieu (uterine fluid) during calcification. Their function is thought to influence the fabric of this biomaterial and/or to participate in its antimicrobial defenses (described in Chapter 9). These non-mineral constituents represent about 2% by weight of the calcified eggshell, and can be released for study by demineralization of the eggshell by calcium chelation or acid demineralization, which yields soluble and insoluble constituents. A complex array of distinct protein bands was demonstrated in the soluble intra- and extra-mineral compartments by 1D-electrophoresis (sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SDS-PAGE) (Hincke *et al.*, 1992; Gautron *et al.*, 1996), and in the precursor uterine fluid, showing different patterns between the three stages of the eggshell calcification process (initial, growth and terminal) (Gautron *et al.*, 1997). A varied combination of N-terminal and internal amino acid sequencing, molecular cloning, immunochemistry and bioinformatic database mining have been useful to identify the most abundant components in uterine fluid and decalcified eggshell (reviewed in Rose and Hincke, 2009). Such studies led to the concept that the most abundant eggshell matrix protein components form three characteristic groups:

- '*Egg white' proteins* which are also present in the eggshell – these include ovalbumin, the most abundant egg white protein (Hincke, 1995), lysozyme, an antimicrobial protein with hydrolytic activity against peptidoglycans on cell walls of Gram-positive bacteria (Hincke *et al.*, 2000) and ovotransferrin, which sequesters iron necessary for bacterial growth (Gautron *et al.*, 2001a).
- *Ubiquitous proteins* that are found in many tissues – examples are osteopontin, a phosphorylated glycoprotein present in bone and other hard tissues of birds and mammals (Pines *et al.*, 1994; Hincke and St. Maurice, 2000; Fernandez *et al.*, 2003; Hincke *et al.*, 2008b; Chien *et al.*, 2008, 2009b), and clusterin, a widely distributed secretory glycoprotein

that is also found in chicken egg white (Mann *et al*., 2003), as well as a wealth of low abundance proteins identified by sensitive proteomic methods (see below).

- *Eggshell-specific matrix proteins* unique to the shell calcification process that are secreted by cells in specific regions of the oviduct where eggshell mineralization is initiated (red isthmus) and continues to completion (uterus). These matrix components are termed ovocleidins (*ovo*, Latin – egg; *kleidoun*, Greek – to lock in, implying a functional role) or Ovocalyxins (*ovo*, Latin – egg; *calyx*, Latin – shell, referring to their shell location), with distinction based on apparent molecular weight by SDS-PAGE when initially characterized (reviewed in Rose and Hincke, 2009).

In 2006, the total number of identified eggshell matrix proteins was less than ten. The recent development of high-throughput methods used in combination with chicken EST and genomic databases has generated new approaches for the characterization of new and less abundant egg components and led to the identification of hundreds of eggshell matrix proteins in a very short period of time (described in Chapter 7). A high-throughput tandem-mass spectrometry approach (MS/MS) identified more than 500 eggshell matrix proteins (Mann *et al*., 2006), including the most abundant proteins that had been identified by traditional approaches. It is highly unlikely that all 520 proteins perform eggshell-specific functions or are involved in eggshell assembly. Rather, the eggshell matrix probably contains a vast background of cellular components that are nonspecifically released by breakdown of the cells lining the oviduct during normal turnover and become incorporated into the shell during mineralization (Mann *et al*., 2006). This view is supported by recent reports that mitochondrial and nuclear maternal hen DNA are also present within the eggshell matrix (Egloff *et al*., 2009; Oskam *et al*., 2010), suggesting that non-specific low-level incorporation of a variety of cellular remnants, including cytoplasm and organelles, occurs during eggshell formation.

Eggshell mineralization is essentially a uterine event; proteins specifically expressed and secreted by uterine cells, and not in other oviduct segments, are therefore likely to play an eggshell-specific role. In this context, the use of transcriptomics to identify specifically expressed uterine genes is a complementary approach to identify genes coding for eggshell matrix proteins. A total of 605 genes were identified as the uterine-specific transcriptome (Jonchère *et al*., 2010) (described in Chapter 7). A list of 54 proteins potentially secreted by uterine cells that could be deposited in the eggshell was inferred from this approach. In addition to proteins involved in uterine ion transport for providing the calcium, bicarbonate and other ions, proteins potentially involved in shell mineralization are those that have been described in mineralized tissues, or bind calcium or could play a role in the proper folding of other eggshell matrix proteins (detailed in Chapter 7).

The 'eggshell-specific' proteins that are selectively described in detail in the next section of this chapter are abundant components of the eggshell matrix and are considered to be highly relevant to eggshell function. Supportive evidence for an *eggshell-specific* role would be: restricted high level expression in a uterine-limited oviduct segment, up-regulation of expression in synchrony with movement of the forming egg through the oviduct, demonstration of a secretory process (i.e. signal peptide, colloidal gold immunocytochemistry to demonstrate secretion granule localization) and secretion during eggshell formation, and finally, evidence for a role in eggshell function. Many of these criteria have been met by the ovocleidins and ovocalyxins.

Two possible roles for eggshell-specific matrix proteins have been proposed; both reflect the protective function of the eggshell in avian reproduction: regulation of eggshell mineralization and antimicrobial defense. Egg calcification occurs in three distinct phases (initiation, active calcification and termination of shell calcification); each phase of shell mineralization is associated with a specific protein electrophoretic profile for the uterine fluid, suggesting that these molecules play specific roles during the calcification process (Gautron *et al.*, 1997). The matrix proteins described in the next section are abundant components of the eggshell matrix and exhibit characteristics that are relevant to eggshell mineralization. Antimicrobial proteins are described in detail in Chapter 9.

Ovocleidin-17 (OC-17)

Ovocleidin-17 (OC-17) was the first eggshell-specific matrix protein to be isolated and characterized following its chromatographic purification after eggshell decalcification (Hincke *et al.*, 1995). OC-17 is an abundant eggshell-matrix specific protein (40 μg/g shell) (Mann *et al.*, 2002). It is secreted by the tubular gland cells in the shell gland; within the shell it is distributed throughout the shell matrix, but concentrated in the mammillary bodies (Hincke *et al.*, 1995). OC-17 exists in differentially phosphorylated and glycosylated forms (Mann, 1999; Mann and Siedler, 1999; Mann *et al.*, 2007). The phosphorylation sites are preserved in closely related eggshell proteins isolated from other avian species (see below), suggesting their importance. Detailed studies have identified eggshell matrix proteins that are homologous to OC-17 in shell from goose (ansocalcin), ostrich, emu and rhea (Lakshminarayanan *et al.*, 2003; Mann and Siedler, 2004, 2006). It is likely that related proteins are found in the shells of all other bird species. Database searches with these eggshell protein sequences reveal that they belong to a heterogeneous group of proteins consisting of a single C-type lectin domain (CTL). The X-ray structure of OC-17 has been determined; it reveals a mixed alpha helix/beta sheet structure and verifies the C-type lectin-like domain (Reyes-Grajeda *et al.*, 2002, 2004).

The properties of purified OC-17 and its goose homolog (ansocalcin), and their influence upon calcite crystallization patterns have been investigated

and compared (Lakshminarayanan *et al.*, 2002, 2003, 2005; Reyes-Grajeda *et al.*, 2004). Functionally, OC-17 and ansocalcin do not appear to be completely equivalent in their effect on calcite crystal growth *in vitro* (Lakshminarayanan *et al.*, 2002; Reyes-Grajeda *et al.*, 2004). Ansocalcin showed reversible concentration-dependent aggregation in solution, and was reported to induce pits on growing calcite rhombohedral faces at lower concentrations (<50 μg/ml) and to nucleate polycrystalline aggregates of calcite crystals at higher concentrations (Lakshminarayanan *et al.*, 2003). Aggregated ansocalcin may act as a template for the nucleation of calcite crystal aggregates (Lakshminarayanan *et al.*, 2002). However, under the same conditions, OC-17 was not observed to aggregate in solution nor induce the nucleation of calcite aggregates. Nevertheless, under different experimental conditions, Reyes-Grajeda and coworkers (2004) reported that OC-17 could modify the crystalline habit of calcium carbonate and the pattern of crystal growth at concentrations of 5–200 μg/ml. Ovocleidin-17 and ansocalcin may also have an antimicrobial role (Wellman-Labadie *et al.*, 2008).

Ovocleidin-116 (OC-116)

Ovocleidin-116 (OC-116) was the first eggshell matrix protein to be cloned, by expression screening a uterine library using an antibody raised to the abundant 116 kDa protein observed in hen uterine fluid during the active calcification phase of shell formation (Hincke *et al.*, 1999). OC-116 is the most abundant eggshell matrix protein, estimated at 80 μg/g eggshell powder (Mann *et al.*, 2002). It is relatively eggshell specific; however, it is also present in young chick cortical bone, laying hen medullary bone and growth plate hypertrophic chondrocytes, suggesting that it also plays a role in bone mineralization (Horvat-Gordon *et al.*, 2008). Genomic analyses indicate that OC-116 is the avian ortholog of MEPE, a mammalian mineralization-specific protein which also plays a role in phosphate metabolism (Kawasaki *et al.*, 2004; Kawasaki and Weiss, 2006; Bardet *et al.*, 2010). However, OC-116 is uniquely specialized to function during calcitic mineralization. OC-116 is the core protein of the dermatan sulfate proteoglycan (ovoglycan) which exists in two forms (116 and 180 kDa) (Carrino *et al.*, 1997; Fernandez *et al.*, 2001). Moreover, it is phosphorylated to a variable and partial extent on at least 22 serine and threonine residues and has two sites of glycosylation (Mann *et al.*, 2007).

Ultrastructural immunocytochemistry indicates that OC-116 is synthesized and secreted from the granular cells of the uterine epithelium, and is incorporated into, and widely distributed throughout, the palisade region of the calcified eggshell (Hincke *et al.*, 1999). Transmission electron microscopy (TEM) of the organic matrix of the avian eggshell reveals two structural features within the palisade layer; vesicular structures with electron-lucent cores intermingle between flocculent sheets of organic material. OC-116 is predominately associated with the periphery of the vesicular structures that probably correspond to the walls of microvesicular holes (voids) in the calcitic

eggshell (Hincke *et al*., 1999). Such localization studies do not distinguish between the differentially phosphorylated, N-glycosylated or glycanated forms of OC-116, nor would possible differences in eggshell distribution between the 116 and 180 kDa forms be detected by this technique. Single nucleotide polymorphisms (SNPs) in the OC-116 gene are significantly associated with eggshell elastic modulus and thickness and egg shape (Dunn *et al*., 2008).

Ovocalyxin-32 (OCX-32)

Ovocalyxin-32 (OCX-32) was originally identified as a 32 kDa uterine fluid protein that is abundant in the terminal phase of shell formation (Gautron *et al*., 2001b; Hincke *et al*., 2003). Ovocalyxin-32 is expressed at high levels in the uterine and isthmus regions of the oviduct and is secreted by the surface epithelial cells that line the lumen. In the eggshell, OCX-32 localizes to the outer palisade layer, the vertical crystal layer, and the cuticle of the eggshell, in agreement with its demonstration by western blotting at high levels in the uterine fluid during the termination phase of eggshell formation (Gautron *et al*., 2001b; Hincke *et al*., 2003). OCX-32 is one of the major phosphoproteins of the eggshell matrix (Mann *et al*., 2007). The timing of OCX-32 secretion into the uterine fluid has been interpreted to suggest that it plays a role in the termination of eggshell calcification (Gautron *et al*., 1997). This hypothesis originated from the observations of morphological changes in calcite crystals by uterine fluid collected during the terminal phase of calcification and the location of OCX-32 in the mineral pellet after its precipitation with calcium carbonate *in vitro* from fresh uterine fluid (Dominguez-Vera *et al*., 2000; Hernandez-Hernandez *et al*., 2008c). However, there is also evidence that it plays a role in antimicrobial defense of the cuticle (Xing *et al*., 2007) (reviewed in Chapter 9).

Ovocalyxin-36 (OCX-36)

Ovocalyxin-36 (OCX-36) is a prominent 36 kDa protein present in the uterine fluid collected during the active calcification stage of shell mineralization. The protein is detected only in the regions of the oviduct where eggshell formation takes place (isthmus and uterus). Moreover, the uterine OCX-36 message, quantified by real time reverse transcription polymerase chain reaction (RT-PCR), is strongly up-regulated during eggshell calcification (Gautron *et al*., 2007). OCX-36 localizes to the inner calcified eggshell and is particularly abundant in the shell membranes. The OCX-36 protein sequence displays significant identity with mammalian proteins that are associated with the innate immune response, such as lipopolysaccharide-binding proteins (LBP), bactericidal permeability-increasing (BPI) proteins and palate, lung and nasal epithelium clone (Plunc) family proteins which act as the first line of host defense (Bingle and Craven, 2004). OCX-36 is reviewed in more detail in Chapter 9.

Osteopontin (OPN)

Osteopontin (OPN) is a phosphoglycoprotein associated with many types of calcium biominerals in birds and mammals (McKee and Nanci, 1996). In the chicken, osteopontin is found in both bone (calcium phosphate hydroxyapatite) and eggshell (calcium carbonate) (Pines *et al*., 1994; Hincke *et al*., 2008b). The oviduct expression of osteopontin is entirely uterine-specific and is temporally associated with eggshell calcification through coupling of physical distension of the uterus to osteopontin gene expression (Lavelin *et al*., 1998). Localization studies show that OPN is concentrated in the palisade region of the eggshell (Fernandez *et al*., 2003, Hincke *et al*., 2008b; Chien *et al*., 2009b). Osteopontin exists as two to three predominant forms in both eggshell and bone, indicating that bone and eggshell OPN differ in their posttranslational modifications (Hincke *et al*., 2008b). Dephosphorylation of eggshell OPN greatly diminishes its ability to inhibit precipitation of calcium carbonate from a supersaturated solution (Hincke and St. Maurice, 2000). Osteopontin and ovocalyxin-116 are synthesized and secreted by the granular epithelial cells of the shell gland (Pines *et al*., 1994; Hincke *et al*., 1999, 2008b; Fernandez *et al*. 2003), suggesting that this epithelial cell type plays a major role in secretion of the eggshell matrix.

After decalcification and processing of the eggshell for TEM and SEM, an extensive organic matrix network is observed throughout all regions. OPN is associated with protein sheets in the highly mineralized palisade region, but not with the vesicular structures where OC-116 is localized (Hincke *et al*., 2008b; Chien *et al*., 2008, 2009b). The elongated calcite crystals in the palisades region tend to be preferentially orientated with the (001) planes parallel (*c*-axis perpendicular) to the shell surface, which orients the {104} plane at 44° tangential to the surface (Silyn-Roberts and Sharp, 1986; Rodriguez-Navarro *et al*., 2002). Since osteopontin specifically interacts with the {104} eggshell calcite faces (Chien *et al*., 2008), which is the natural cleavage plane, it could modify the resistance of the shell to fracture along this plane.

Unusual patterns of uterine OPN expression may underlie certain defects in eggshell mineralization that are observed in egg production. In birds laying eggs with normal eggshells, OPN is expressed uniformly by all the epithelial cells facing the uterine lumen; however, reduced or absent OPN expression in specific regions of the uterine luminal epithelium has been associated with eggshell defects such as corrugations, pimples and cracks (Arazi *et al*., 2009). A candidate gene association analysis with eggshell matrix genes recently revealed that OPN SNP's were associated with eggshell fracture toughness (Dunn *et al*., 2008).

8.3.3 Non-calcified shell: eggshell membranes

The albumen is surrounded by the inner and outer shell membranes, which are considered to be the innermost layers of the eggshell (Fig. 8.1). They

are deposited as the egg traverses the proximal (white) isthmus. The inner membranes remain uncalcified, while the fibers of the outer shell membrane penetrate the tips of the mammillary cones of the calcified shell since they initially provided the nucleation sites for mammillary cone mineralization (Fig. 8.1c) (Arias *et al.*, 1993; Nys *et al.*, 2004). Both inner and outer membranes are constructed of parallel meshworks composed of interconnecting networks of fibres (Fig. 8.1d). The fibers consist of 10% collagens (types I, V and X) and 70–75% of other proteins and glycoproteins containing lysine-derived cross-links (Wong *et al.*, 1984; Arias *et al.*, 1991; Fernandez *et al.*, 2001). The ratio of collagen types I:V is approximately 100:1. Within the membranes, a major disulfide-rich structural protein termed CREMP (**c**ysteine-**r**ich **e**ggshell **m**embrane **p**rotein) was recently identified (Kodali *et al.*, 2011). Transcriptome analysis reveals both collagen X and CREMP as highly expressed genes of the white isthmus transcriptome (Du, Hincke, Gautron and Nys, personal communication). Lysyl oxidase activity has been identified in the white isthmus, which catalyses intra- and interchain collagen cross-linking (Harris *et al.*, 1980). Inhibition of collagen cross-linking by treatment of laying hens with dietary osteolathyrogens leads to large disruptions in shell membrane structure and arrangement of fibres (Chowdhury and Davis, 1995; Arias *et al.*, 1997).

The eggshell membranes envelop the albumen, but are semipermeable and allow the exchange of gases and water while retaining the albumen proteins. In addition, they act as a physical and chemical barrier to bacteria. In the distal portion of the isthmus, or red isthmus/tubular shell gland, organic aggregates are deposited on the surface of the outer eggshell membranes in a quasi-periodic array, where calcium carbonate begins to aggregate and are the origin of the mammillary knobs (Figs 8.1d, 8.6) (Nys *et al.*, 2004).

8.4 Applications: eggshell as an industrial raw material

The eggshell represents about 10% of the egg weight. It is composed mostly of calcium carbonate (95%) with its associated organic matrix (3.5%). As outlined in the previous sections, the shell is lined by the shell membranes (about 3% of the shell weight), which consists of cross-linked structural proteins, egg white proteins and eggshell matrix proteins. In the egg-producing industry a large percentage of eggs are diverted to breaker operations to produce liquid egg products and are not consumed as shell eggs (i.e. 32% in United States, close to 25% in Europe). Such operations generate eggshell residue for which a commercial application remains elusive. Eggshell waste has been used as animal feed or as a fertilizer or lime substitute. In many countries, it is accepted practice for eggshells to be dried at high temperature to be decontaminated and used as a source of calcium in animal feeds. A polypropylene composite with crushed eggshell as filler has been recently fabricated and characterized for industrial applications (Toro *et al.*, 2007a,

b). Shells have been dried, crushed, acid-treated, abraded and tumbled, but the membrane remains tenaciously attached to the shell by the outer membrane fibers that penetrate the mammillary cones (Figs 8.1a, c). A variety of processes to separate the membranes from ground shell have been proposed; for example, based on differential flotation of shell fragments and membrane particles in water (Yoo *et al.*, 2009). Separation of eggshell into its mineral and membrane constituents allows different applications for each material to be developed. For example, the membrane free shell powder can be used in the paper industry as coating pigments for ink-jet printing paper (Yoo *et al.*, 2009), or in agriculture as a lime substitute or calcium supplement.

8.4.1 Nutraceutical and clinical applications

Eggshell powder as a dietary calcium supplement

Bioavailability of calcium is very high in eggshell mineral, which also contains useful amounts of a large range of microelements such as strontium (Sr), fluorine (F) and selenium (Se). Several studies have investigated the impact of consuming chicken eggshell powder enriched dairy-based products on bone mineral density in healthy late post-menopausal women and in persons with osteoporosis or osteopenia, with some benefits noted (Schaafsma and Pakan, 1999; Schaafsma *et al.*, 2002). Eggshell powder has also been compared to purified calcium carbonate as a calcium source in animal feed for piglets (Schaafsma and Beelen, 1999).

Eggshell membrane

Consumption of eggshell membrane supplement (Natural Eggshell Membrane (NEM®) as a dietary supplement was reported to significantly reduce both joint pain and stiffness compared to placebo in human clinical trials for osteoarthritis of the knee (Ruff *et al.*, 2009a, b). Anecdotal reports suggest that fresh eggshell membrane can be utilized as an antimicrobial tissue adhesive to aid healing of certain skin lacerations (Zadik, 2007).

Clinical applications to promote bone healing

Eggshell membrane has been evaluated as a biodegradable bone regeneration inhibitor (Arias *et al.*, 2008). Eggshell as a bone substitute suitable for grafting to promote bone healing has been explored in animal models using rat calvaria and cranial defects in rabbit (Durmus *et al.*, 2003; Park *et al.*, 2007a).

8.4.2 Bioremediation

A number of studies have demonstrated that eggshell membranes bind metal ions and other positively charged molecules (perhaps due to immobilized ovotransferrin content). Ground eggshell waste may be utilized as an

adsorbent for the removal of anionic dyes or heavy metals from aqueous solution (Chojnacka, 2005; Vijayaraghavan *et al.*, 2005; Park *et al.*, 2007b; Tsai *et al.*, 2008). Carbonate hydroxylapatite derived from eggshell waste shows potential for adsorption of cadmium, copper and lead ions from aqueous solution (Zheng *et al.*, 2007; Liao *et al.*, 2010). Eggshell membranes alone, after manual stripping from eggshell, are capable of uptake and recovery of gold ions from electroplating wastes, as well as biosorption of actinides (uranium and thorium ions) from dilute waste solution (Suyama *et al.*, 1994; Ishikawa *et al.*, 1999, 2002a, 2002b; Goto and Suyama, 2000).

8.4.3 Chemical processing support

Eggshell membranes can be used in a variety of nanotechnology applications as a solid phase support for biomimetic chemical processing. Eggshell membrane can serve as a reactive surface for synthesis of novel functional nanomaterials; for example, biomimetic synthesis of $BaSO_4$ nanotubes using eggshell membrane as a template (Liu *et al.*, 2004a). *In situ* synthesis of lead sulfide nanoclusters for optoelectronics applications has been achieved on eggshell membrane fibers (Huilan *et al.*, 2008). In another approach, barium chromate nano-superstructures were synthesized with the bioactive eggshell membrane serving as a template for self-assembly of nanoparticles (Liu *et al.*, 2004b). Hierarchically ordered thin films with a macroporous network structure made up of crystalline ZrO_2 tubes were obtained through sol–gel mineralization of an eggshell membrane template (Yang *et al.*, 2003). In another application, electrodialysis of racemic mixtures of amino acids through eggshell membranes resulted in selective chiral separation (Kondo and Yoshikawa, 2001).

8.4.4 Solid phase enzyme immobilization

Eggshell membranes are a durable solid-phase support that are suitable for immobilization of enzymes, and have been adapted to a number of biosensor applications: oxalate oxidase for determination of urinary oxalate (Pundir *et al.*, 2009); hydrogen peroxide biosensor using immobilized catalase (Choi and Yiu, 2004); chemiluminescence flow-through biosensor for glucose using immobilized glucose oxidase and horseradish peroxidase (Li *et al.*, 2008), optical glucose biosensor using immobilized glucose oxidase with subsequent covering of the surface with an oxygen-sensitive optode membrane (Choi *et al.*, 2001), bienzyme system for detection of aspartame, composed of chymotrypsin and alcohol oxidase immobilized onto an eggshell membrane, with an oxygen-sensitive optode membrane as the transducer (Xiao and Choi, 2002). Eggshell membrane has also been adapted to serve as an immobilization platform for determination of human IgG in a sandwich immunoassay (Tang *et al.*, 2009).

8.4.5 New composite biomaterials

Eggshell membranes have been a starting point for preparing a novel ingredient for composite biomaterials. A soluble extract of eggshell membranes, termed soluble eggshell membrane protein (SEP), can be produced from eggshell membrane by aggressive methods that target the disulfide-reinforced crosslinkages. A combination of performic acid oxidation/pepsin digestion yields an SEP which can be conjugated to pepsin-solubilized collagen to form well-developed collagen networks for cell adhesion (Takahashi *et al.*, 1996; Ino *et al.*, 2006). Reductive cleavage of eggshell membrane with 3-mercaptopropionic acid in acetic acid yields SEP which is soluble in 10–100% aqueous acetic acid and is a starting point for fabrication of new composite biomaterials with potential application in tissue engineering (Yi *et al.*, 2003, 2004). SEP-modified poly (D, L-lactic acid) membranes have enhanced cytocompatibility compared to poly (D, L-lactic acid) membranes alone (Lu *et al.*, 2009). SEP/chitosan blended films have superior material properties and enhanced biocompatibility for cell culture of NIH3T3 cells compared to pure chitosan (Qi *et al.*, 2009). Nonwoven poly(S-caprolactone) (PCL) nanofibers can be prepared by electrospinning, followed by SEP immobilization on the nanofibers after surface modification. SEP immobilization enhanced the attachment, spreading, and proliferation of human dermal fibroblasts (HDFs) compared with the unmodified PCL nanofibers (Jia *et al.*, 2008). SEP can also be electrospun directly with PCL to form an SEP/PCL web fiber web (Kim *et al.*, 2008). Poly(vinyl alcohol) (PVA) has been blended with soluble eggshell membrane protein (SEP) to improve the mechanical properties of the brittle SEP film (Yi *et al.*, 2006a). Polyethylene (PE) film surface is modified by combining plasma treatment and soluble SEP immobilization, with resulting improvement of the PE hydrophilicity (Yi *et al.*, 2006b). Further improvements in the properties of nanofibers prepared from SEP/PVA and SEP/poly(ethylene oxide) (PEO) mixtures can be obtained by adding catechin (Kang *et al.*, 2010).

8.5 Conclusions

A large number of eggs are produced worldwide for human consumption; changes in eggshell properties are directly related to increasing risk of foodborne disease for the consumer. Therefore, an understanding of eggshell formation and its control is of primary importance to minimize egg contamination. This chapter summarizes recent genomic, transcriptomic and proteomic analyses of the eggshell and impact of this information on our understanding of eggshell structural and textural features that are important for shell strength. Genes involved in eggshell formation and mineralization are functional candidates for marker-assisted selection to improve egg and eggshell quality, and therefore food safety, of this nutritious foodstuff.

Research for value-added uses for eggshell mineral and membranes is a field under active development.

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8.7 References

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9

Molecules involved in chemical defence of the chicken egg

S. Réhault-Godbert, V. Hervé-Grépinet, J. Gautron, C. Cabau and Y. Nys, Institut National de la Recherche Agronomique, France and M. Hincke, University of Ottawa, Canada

Abstract: Molecular egg defence is ensured by all the proteins displaying antimicrobial activity or contributing to the overall homeostasis of the egg. With the development of transcriptomic and proteomic approaches, many proteins have been newly identified in chicken egg, the functions of which have not yet been explored. To identify candidates potentially involved in egg defensive mechanisms, we examined protein sequences for specific domains and in some cases searched for homology with their mammalian counterparts. This chapter gives an overview of the peptides and proteins potentially participating in the chemical defence of the egg.

Key words: chicken egg, antimicrobial defence, stress response, homeostasis.

9.1 Introduction

Egg defence relies on two major mechanisms: one is based on physical defence essentially ensured by the calcified eggshell and its membranes (Chapter 8) and the other results from the molecules involved in chemical defence. Until lately, only a few proteins were considered to participate in the antimicrobial resistance of the egg: lysozyme, ovotransferrin, avidin, riboflavin-binding protein, cystatin, ovostatin, ovomucin, phosvitin, Tenp and ovocalyxin-36. The recent development of high throughput methods (proteomic and transcriptomic approaches; see Chapter 7) allowed the identification of hundreds of egg proteins potentially involved in various physiological processes. The recent analysis of egg white and egg yolk proteins, identified by combinatorial peptide ligand libraries and mass

spectrometry-based approaches, allowed elaboration of network and pathway analyses in order to convey a unified view of these proteomes. It revealed a role for proteins involved in cell development, proliferation and migration, cell-to-cell interaction and hematological system development (D'Alessandro *et al*., 2010). Using Ingenuity Pathway Analysis software only 15 proteins were ascribed to antimicrobial components (D'Alessandro *et al*., 2010).

In fact, the complete characterization of the activities and physiological functions of the egg white and yolk proteins depends fundamentally on the availability of a pure and active protein (either purified or produced as recombinant protein), which can be difficult and time-consuming. Nevertheless, some functions can be predicted by identifying homologous proteins in other species and/or by investigating the presence of conserved domains in protein sequences. Using these bioinformatic analyses, new candidate proteins that could have a major role in egg defence were identified. In this chapter, we pay particular attention to molecules that could play a role against pathogens but also molecules that might help to face oxidative and heat stresses and therefore contribute to overall egg homeostasis.

9.2 Molecules degrading microbial components

9.2.1 Hydrolases

Lysozyme (P00698) has been first identified as a major egg white protein. However, its presence in the other egg compartments, eggshell, eggshell membranes, egg yolk and vitelline membrane has been disclosed by the various proteomic and biochemical analyses published in the past few years (D'Ambrosio *et al*., 2008; Farinazzo *et al*., 2009; Hincke *et al*, 2000; Mann, 2007, 2008, Mann and Mann, 2008; Mann *et al*., 2006). Lysozyme is an N-acetyl-muramidase that is well known for its potent antibacterial activity. This 14 kDa protein is widely distributed in mucus, body fluids and tissues, in animals, plants and insects. It possesses a broad-range spectrum of activity. Its muramidase activity exhibits bactericidal effects against Gram-positive strains by hydrolysing the bacterial peptidoglycan. However, this activity cannot account for the protective effect of lysozyme against Gram-negative bacteria, the peptidoglycan of which is partly protected by bacterial lipopolysaccharide. Several articles have reported that lysozyme would also exert antimicrobial activity through a mechanism that is independent of its catalytic activity (Nash *et al*., 2006) and that would involve the cationicity of the molecule.

Similar to **acyloxyacylhydrolase** (IPI_00589382) is an egg white protein that shares 62% sequence identity with the murine counterpart. This lipase removes secondary fatty acyl chains from bacterial lipopolysaccharides and thereby can limit their toxic effect (Feulner *et al*., 2004).

Cathepsin L1 (P09648) has been shown to be a component of eggshell (Mann *et al*., 2006) and might participate in antigen presentation by assisting the overall degradation of proteins and pathogens in lysosomes (Hsieh *et al*.,

2002). The expression of cathepsin L has been shown to be differentially regulated following infection by an avian pathogenic *Escherichia coli* strain (Lavric *et al.*, 2008). Its role as an antimicrobial agent in egg and more precisely in eggshell has never been explored.

Other proteases have been identified through the various approaches used recently. Proteases are involved in many different biological processes and there is no evidence in the literature that the other egg proteases would play a significant role in egg defence, although such activities cannot be excluded.

Lysosomal protective protein (P10619) is a protective protein with a cathepsin-A like activity (Galjart *et al.*, 1991). It associates with lysosomal beta-galactosidase and neuraminidase, toward which it exerts a protective function necessary for their stability and activity. Recent gene expression profiling of the chicken uterus identified an overexpressed gene transcript corresponding to lysosomal protective protein (Jonchere *et al.*, 2010). This protein was predicted to be secreted in the uterine fluid and could be deposited in the eggshell.

9.2.2 Antimicrobial peptides

Many natural antimicrobial peptides of innate immunity, such as avian beta-defensins and histones, have been identified in egg. These peptides are expected to be involved in the protection of the embryo during its development and to contribute to production of pathogen-free eggs. The **avian β-defensins** (AvBDs) are small cationic non-glycosylated peptides (1–10 kDa), with a three-stranded β-sheet structure connected with a loop of β-hairpin turn (Evans *et al.*, 1994; Sugiarto and Yu, 2004). Their structures are very similar to those of mammalian β-defensins (Landon *et al.*, 2004; Pazgier *et al.*, 2006). β-defensin molecules possess six highly conserved cysteines with the following consensus sequence motif: X_n-C-$X_{2\text{-}4}$-G-$X_{1\text{-}2}$-C-$X_{3\text{-}5}$-C-$X_{9\text{-}10}$-C-$X_{5\text{-}6}$-CC-X_n where C is a cysteine, G a glycine and X any amino acid. The six cysteines in β-defensins are linked to form disulphide bridges in a 1–5, 2–4 and 3–6 pairing pattern. The mechanism of action of AvBDs is not completely known. AvBDs have a specific amphipathic tridimensional structure, with three intramolecular disulphide bridges, and possess opposite domains of clustered hydrophobic and cationic amino-acid chains. Exposed cationic sites are thought to interact electrostatically with negatively charged membrane components, such as lipopolysaccharide of Gram-negative bacteria, or acidic polysaccharides in Gram-positive bacteria (Hancock, 1997). Following peptide accumulation, parallel to the membrane surface, dimers and multimers could be formed, resulting in the creation of a pore (Wellman-Labadie *et al.*, 2007). Subsequently, inside the bacteria, AvBDs could interact with DNA or RNA, altering DNA and RNA functions including protein synthesis (Lehrer *et al.*, 1989). In spite of mRNA expression of AvBD-1, -2, -3, -4, -5, -8, -9, -10, -11 and -12, in the different segments of

the oviduct (Mageed *et al*., 2008), only three AvBDs have been identified, in the hen egg, by proteomic approaches (Table 9.1): AvBD10 in the eggshell; AvBD11 in the eggshell, the egg white and the vitelline membranes; and gallin (similar to meleagrin or cygnin) in the egg white (Mann, 2007, 2008; Mann *et al*., 2006). AvBD9 was found to be overexpressed in the uterus and might also be incorporated in the eggshell (Jonchere *et al*., 2010).

Synthetic and recombinant versions of AvBD9 (previously named gallinacin 6) have been shown to exhibit antimicrobial activities towards Gram-negative and Gram-positive bacteria, and fungi (van Dijk *et al*., 2007). Similarly, recombinant AvBD10 peptide displays antibacterial activities against Gram-negative and Gram-positive bacteria (Liao *et al*., 2008). AvBD11 is composed of 82 residues and is the only member of the beta-defensin family that contains two beta-defensin motifs with 12 cysteines and 6 disulphide bonds. This peptide was found to possess a broad antimicrobial activity towards both Gram-positive and Gram-negative bacteria (Herve-Grepinet *et al*., 2010). Gallin is a cationic peptide of 41 residues, which is related to the AvBD family. The production of a recombinant gallin peptide revealed antimicrobial activity against *E. coli* (Gong *et al*., 2010). Its gene was localized on chromosome 3, close to the genes of the other AvBDs. Moreover, the presence of three copies of this gene in the genome was confirmed in

Table 9.1 Antimicrobial peptides in egg

Protein name	Accession numbers (Swissprot, REFSEQ or IPI_CHICK)	Localization*
Beta-defensins		
AvBD-11	Q6IV20	ES, EW,VM
AvBD-10	Q6QLQ9	ES
Gallin	XP_429907	EW
AvBD-9	Q6QLR1	Ut
Histone proteins		
Histone H2A.Z	Q5ZMD6	ES, EW, EY
Similar to histone protein	IPI_00576977	EW
Histone H1	P09987	EW
Histone H2A-III	P35062	ES, EW, EY
Histone H2A	Q788R3	EY
Histone H4	P62801	ES, EY
Histone H2A-VIII	Q92069	ES
Histone H2A-IV	P02263	ES, EW, EY
Histone H2A.J	P70082	ES, EY
Histone H2A.V	P02272	ES, EY
Histone H2B 1/2/3/4/6	P0C1H3	EY
Histone H2B 5	P0C1H4	EY
Histone H2B 8	Q9PSW9	EY
Histone H4 type VIII	P70081	ES, EY

* Data compiled from D'Ambrosio *et al*. (2008), Farinazzo *et al*. (2009), Jonchere *et al*. (2010), Mann (2007, 2008), Mann and Mann (2008), Mann *et al*. (2006); ES, eggshell, EY, egg yolk, EW, egg white, Ut, uterus, VM, vitelline membrane.

three chicken lines. These results suggest that gallin could be duplicated in order to increase its production, in particular in the egg white, because each of the three copies was expressed in the magnum (Gong *et al.,* 2010).
Histones are principal structural proteins of eukaryotic chromosomes. They are polypeptides rich in lysine and/or arginine. Histones H2A, H2B, H3 and H4 are core proteins responsible for the basic structure of chromatin, the nucleosome core. The linker histone H1 interacts with linker DNA between nucleosomes and functions in the compaction of chromatin into higher-order structures (Kasinsky *et al*., 2001). There is increasing evidence that histones, in addition to their role in nucleosome formation, might play an important role in innate host defence against intracellular or extracellular microbe invasion. Different histone H2A-derived antimicrobial peptides were studied to understand the structural requirements necessary for their antimicrobial activity and their mechanism of action. Four features were highlighted: (1) the carboxy-terminal α-helical region providing an amphipathic stable α-helical, (2) the proline hinge for the cell-penetrating property, (3) basic residue for the membrane-binding activity and (4) lysine residue for the 'barrel-stave' formation (Koo *et al*., 2008; Park *et al*., 2000).

Many histones were identified in the hen egg. In *Gallus gallus*, histone H2A and histone H2B carboxy-terminal fragments exhibited antimicrobial activities against both Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli* D31) bacteria. Moreover, these activities were thermostable and salt-resistant. Histones H1 and H2B purified from the hen reproductive system (ovary and oviduct) were shown to display antimicrobial activity against Gram-positive and Gram-negative strains (Silphaduang *et al*., 2006). These data combined to the ubiquitous distribution of histones in egg (Table 9.1) suggest that these molecules are major components of the egg immune system.

9.2.3 Lipopolysaccharide binding – bactericidal/permeability increasing (LBP-BPI) proteins

Lipopolysaccharide binding (LBP) and bactericidal/permeability increasing (BPI) proteins are well known in mammals for their involvement in defence against bacteria. They belong to the superfamily of proteins known to be key components of the innate immune system which act as the first line of host defence (Bingle and Craven, 2004). LBP proteins initiate the inflammatory host response upon the detection of a pathogen (Schumann *et al*., 1990). LBP binds the lipid A component of the lipopolysaccharide (LPS) layer of Gram-negative bacteria and transfers them to CD14, an LPS receptor (Hailman *et al*., 1994). BPI also binds LPS, followed by permeabilization of the cytoplasmic membrane and a decrease in the electrochemical gradient of the bacterial cell leading to death (Dann and Eckmann, 2007). The genes coding for BPI-like proteins are organized in two loci that are syntenous across animal genomes. Most members are encoded within a contiguous region which in humans is

located on chromosome 20 (Wheeler *et al.*, 2007). Avian BPI-like genes are located on the corresponding chicken chromosome 20, where the OCX-36 gene (LOC419289) is nested in the tandemly arranged BPI/LBP/PLUNC gene cluster; it is next to Bpil3, Lplunc-3 and 4 (Tian *et al.*, 2010). TENP is also localized in the same locus in the chicken genome; both phylogeny and genomic comparison suggest that the Tenp gene is orthologous to the mammalian BPI-Like-1 but that OCX-36 is uniquely avian (Tian *et al.*, 2010). Two other BPI-like proteins are encoded by a locus on chromosome 1 in chickens; BPI-Like-2 (LOC771461) and similar-to-BPI (LOC427911). The colocalization of these genes is conserved in mammalian genomes (shared synteny). Examination of the expressed sequence tag (EST) database for tissue-specific expression of BPI-like genes indicates that OCX-36, TENP, BPI-like-2 (BPIL2) and similar-to-BPI are expressed in chicken reproductive tissues; moreover, sensitive proteomic scans of egg compartment proteins reveal that these members of the BPI-like family are present at detectable levels.

Ovocalyxin-36 (OCX-36) (IPI_00573506.2) was the first member of this family of antimicrobial proteins to be identified in the egg. It is a prominent 36 kDa protein present in the uterine fluid collected during the active calcification stage of shell mineralization (Gautron *et al.*, 1997). Antibodies raised to the uterine protein were used to expression-screen a hen uterine library, and a novel clone was identified which was the basis for additional rounds of hybridization-screening (Pilon *et al.*, 2000). The resulting consensus sequence was subsequently assembled with public ESTs to obtain complete full-length cDNA (Gautron *et al.*, 2007). The protein is only detected in the regions of the oviduct where eggshell formation takes place (isthmus and uterus). Transcriptomic profiling of hen oviduct expression reveals that OCX-36 is upregulated in uterus during shell mineralization (Jonchere *et al.*, 2010), as previously revealed by qRT-PCR (Gautron *et al.*, 2007). Uterine OCX-36 expression is strongly upregulated in sexually mature hens compared to juveniles (Dunn *et al.*, 2009). OCX-36 protein localizes to the inner calcified eggshell, and is particularly concentrated in the shell membranes (Gautron *et al.*, 2007; Mann *et al.*, 2006); it is also detected in vitelline membrane (Mann, 2008). The OCX-36 protein sequence is 20–25% similar to mammalian proteins associated with the innate immune response, such as LBP, BPI proteins and palate, lung and nasal epithelium clone (Plunc) family proteins (Gautron *et al.*, 2007). OCX-36 may therefore participate in natural defence mechanisms that keep the egg and oviduct free of pathogens. Chicken and zebra finch OCX-36 exhibit a large degree of similarity throughout the protein sequence (56% identity), which implies that there is conservation of function between evolutionarily distant bird species (Rose and Hincke, 2009).

Three additional LBP-BPI like proteins have been identified in egg. However, their functional characterization has not yet been explored. Although the chicken **TENP** transcript (XM_429272) was originally cloned as a gene transiently expressed during neurogenesis (Yan and Wang, 1998), the protein (XP_429272) has only been detected in various egg compartments: egg white

(D'Ambrosio *et al*., 2008; Guerin-Dubiard *et al*., 2006; Mann, 2007), yolk (Farinazzo *et al*., 2009; Mann and Mann, 2008) and vitelline membrane (Mann, 2008). The protein **BPIL2** (XP_001234743, IPI_00585627.1) was detected in egg white and vitelline membrane (Mann, 2007, 2008). Uterine BPIL2 expression was found to be strongly upregulated in sexually mature hens compared to juveniles (Dunn *et al*., 2009). **Similar-to-BPI** (XP_425484, LOC427911) protein is detected in egg white (D'Ambrosio *et al*., 2008) and its transcript was identified in a chicken reproductive tract library. The function of these BPI-like molecules is the subject of intense research to characterize their putative LPS-binding properties and antimicrobial activity.

9.2.4 C-type lectin-like proteins

C-type lectin-like proteins are major components of the calcified eggshell of multiple avian species. In mammals, C-type lectin proteins specifically capture carbohydrate antigens present on the surface of pathogens and are crucial in tailoring adaptative response to pathogens (Den Dunnen *et al*., 2010). **Ovocleidin 17** (OC-17, Q9PRS8) was the first eggshell matrix protein purified to homogeneity (Hincke *et al*., 1995). OC-17 is a 142 amino acid phosphorylated protein with a C-type lectin domain (Mann and Siedler, 1999; Reyes-Grajeda *et al*., 2004) that has also been identified in egg white, vitelline membrane and eggshell in the recent proteome surveys (D'Ambrosio *et al*., 2008; Mann 2008; Mann *et al*., 2006). Related proteins with a similar C-type lectin domain in other avian species have been reported. Ansocalcin is a goose protein of 40% identity with OC-17 (Lakshminarayanan *et al*., 2002, 2003, 2005). In ostrich, struthiocalcin I and II showed 65% identity with goose ansocalcin and 41% with OC-17 (Mann and Siedler, 2004). Emu and rhea also exhibit C-type lectin-like proteins related to OC-17; they have been named dromaiocalcin-1 and 2 and rheacalcin-1 and 2 (Mann and Siedler, 2006). Purified ovocleidin 17 has shown bactericidal activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Wellman-Labadie *et al*., 2008). Proteomic analyses of the various egg compartments pointed out the presence of three additional proteins with C-type like lectin domains: **Tetranectin** (Q9DDD4) in egg yolk (Farinazzo *et al*., 2009; Mann and Mann, 2008), **Collagen XVIII** (O93419) and **DEC-205 protein** (Q4LDF5) in the eggshell (Mann *et al*., 2006). Their potential activity as antimicrobial proteins remains to be explored.

9.3 Molecules decreasing bioavailability of iron and vitamins

By binding vitamins and iron, chelators of vitamins and metal ions diminish their bioavailability for microorganisms and can thus affect growth and survival of bacteria.

9.3.1 Iron chelators

The antimicrobial activity of **ovotransferrin** (P02789) is mainly due to its ability to chelate iron that is essential for the growth of some bacterial strains such as *Pseudomonas* spp, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* or *Streptococcus mutans* (Valenti *et al*., 1983). Ovotransferrin is thought to be the major anti-*Salmonella enterica* Enteritidis agent that is present in egg (Baron *et al*., 1999), and is widely distributed in all compartments (D'Ambrosio *et al*., 2008; Farinazzo *et al*., 2009; Mann, 2007, 2008; Mann and Mann, 2008; Mann *et al*., 2006). In addition, ovotransferrin and some derived peptides have a direct action on pathogens by permeabilizing the bacterial membrane (Aguilera *et al*., 2003; Ibrahim *et al*., 2000).

Vitellogenin 1 (P87498) and **vitellogenin 2** (P02845) are produced by the chicken liver, secreted into the blood and then sequestered by receptor-mediated endocytosis into growing oocytes, as component of egg yolk (Deeley *et al*., 1975; Farinazzo *et al*., 2009; Mann and Mann, 2008). They are generally cleaved, giving rise to the respective yolk components lipovitellin and phosvitin as sources of nutrients for the embryo. **Phosvitin** is believed to be of importance in sequestering calcium, iron and other cations for the developing embryo. Its ability to bind iron suggests that phosvitin could play a similar role as ovotransferrin in the protection of egg against the previously mentioned pathogens, as has been demonstrated for *E. coli* (Choi *et al*., 2004).

9.3.2 Vitamin chelators

Avidin (P02701) possesses a high affinity for biotin (Green, 1975). It is a major egg white protein which is also present in the vitelline membrane and eggshell (Mann, 2007, 2008; Mann *et al*., 2006). A homologous protein '**similar to avidin**' (XP_429212) has also been identified in egg yolk (Farinazzo *et al*., 2009; Mann and Mann, 2008). Similarly, the **riboflavin-binding protein** (P02752), **retinol binding protein 4** (P41263) and **vitamin D binding protein** (Q9W6F5) bind riboflavin, retinol and vitamin D with high affinity, respectively. Riboflavin-binding protein has been identified in all compartments (Farinazzo *et al*., 2009; Mann, 2007, 2008; Mann and Mann, 2008; Mann *et al*., 2006), whereas the plasma retinol-binding protein and the vitamin D-binding protein have been found in egg yolk and/or eggshell (D'Ambrosio *et al*., 2008; Farinazzo *et al*., 2009; Mann *et al*., 2006). All three proteins were previously found to be incorporated into the growing oocyte from egg yolk (Maclachlan *et al*., 1994; Ono and Tuan, 1991; Vieira and Schneider, 1993). To date, vitamin chelators in egg yolk have never been considered as potential antimicrobials, but rather assumed to supply nutrients and vitamins for the embryo.

9.4 Molecules inhibiting the activity of microbial proteases

Protease inhibitors are widely used in several therapeutic strategies to overcome bacterial and viral infections (Supuran *et al*., 2002). Most microorganisms secrete proteases, which play a major role in various processes associated with proliferation and colonization by pathogens. These microbial proteases can hydrolyse host proteins to inactivate them or to facilitate their assimilation by microorganisms as nutrients. They can also limit the immune response, and induce tissue damage that favours dissemination of pathogens. Bacterial proteases are considered to be major virulence factors. Fortunately, host organisms possess an arsenal of antiproteases that regulate and limit the deleterious activity of exogenous proteases (Armstrong, 2006). Antiproteases are highly represented in egg. Their biological role remains undefined; however, with regards to homologous proteins in other species, all of them could be considered as potential antimicrobial proteins. We can distinguish four main classes of antiproteases based upon their specificity of action and targeted proteases.

9.4.1 Inhibitors of serine proteases

This group of antiproteases is largely represented in all egg compartments and is listed in Table 9.2. We distinguish inhibitors with kazal-like domains, serpins (serine proteases inhibitors) and inhibitor containing kunitz domains.

Kazal-type inhibitors are canonical serine proteinase inhibitors that interact with their cognate enzymes through their reactive site. These inhibitors are widely distributed in all kingdoms of life. The reactive site of kazal-type inhibitors is extremely variable but is structurally conserved. The kazal-type domain contains six cysteine residues engaged in disulphide bonds according to a specific pattern (Fig. 9.1).

Many kazal-like inhibitors have been identified in egg (Table 9.2). Among them there are two well-known inhibitors, **ovoinhibitor** (P10184) and **ovomucoid** (P01005) that are major egg white proteins, the functions of which are still under investigation. Additionally, one to nine kazal-like domains have been identified in **flik protein** (Q9W600), **SPARC** (P36377), **trypsin inhibitor ClTI-1** (P85000), **complement component C6** (Q811M5), **agrin** (P31696), **follistatin** (Q90844) and **follistatin-related protein 1** (Q12841) (Table 9.2). There are some data in the literature reporting the antibacterial activity of kazal-like inhibitors. Such inhibitors, that display potent *in vitro* bactericidal activity against *Staphylococcus aureus,* have been identified in the metazoan *Hydra* (Augustin *et al*., 2009). Moreover, in crustaceans, kazal-type serine proteinase inhibitors in hemolymph are believed to function as regulators of the host-defence reactions but also as inhibitors of proteinases from microorganisms (Li *et al*., 2009).

Serpins (serine protease inhibitors) are a group of structurally related proteins, which contain a C-terminal reactive site-loop that interacts with targeted serine proteases. Upon interaction, the loop is cleaved by the

Table 9.2 Serine protease inhibitors in egg

Protein name	Accession number (Swissprot, REFSEQ)	Localization*	Domains, activity
Inhibitor with kazal-like domains			
Ovoinhibitor	P10184	ES, EW, EY, VM	7, inhibitory
Ovomucoid	P01005	ES, EW, EY, VM	3, inhibitory
Flik protein	Q9W600	ES	2, unknown
SPARC	P36377	ES	1, unknown
Trypsin inhibitor ClTI-1	P85000	ES	1, unknown
Complement component C6	Q811M5	EW	2, unknown
Agrin	P31696	ES, VM	9, unknown
Follistatin	Q90844	ES, EW, VM	3, unknown
Follistatin-related protein 1	Q12841	Ut	1, unknown
ov-serpins and serpins			
Ovalbumin-related protein Y	P01014	ES, EW, EY, VM	Unknown
Ovalbumin-related protein X	P01013	ES, EW, EY, VM	Unknown
Heparin cofactor II	O73840	ES, EW, EY	Unknown
Similar to plasma protease C1 inhibitor	XP_421063	EY	Unknown
Antithrombin	Q91422	EY	Unknown
Neuroserpin	Q90935	ES	Unknown
Similar to alpha1-antitrypsin	XP_421343	EY	Unknown
Similar to serpina1d-prov	XP_421342	EY	Unknown
Alpha-2-antiplasmin	P08697	Ut	Unknown
Similar to alpha2-plasmin inhibitor	XP_415807	EY	Unknown
Inhibitors with kunitz-like domains			
Beta-amyloid protein 751 isoform	Q9DGJ7	ES, Ut	Unknown
Amyloid beta A4 protein	P79307	Ut	Unknown
Tissue factor pathway inhibitor 2	Q7YRQ8	Ut	Unknown
Similar to pancreatic secretory trypsin inhibitor	XP_001233307	EW	Unknown
Similar to alpha1 microglobulin/ bikunin	XP_001234121	EY	Unknown
Similar to inter-alpha-trypsin inhibitor	XP_414253	EY	Unknown
Similar to kunitz-like protease inhibitor	XP_001235178	EW	Unknown
Similar to serine peptidase inhibitor, kunitz type 1	XP_421130	EW	Unknown

Table 9.2 Continued

Protein name	Accession number (Swissprot, REFSEQ)	Localization*	Domains, activity
Similar to putative porin	XP_417473	EY	Unknown
Others			
BMP-binding endothelial regulator protein	Q8N8U9	Ut	Unknown
Ovomucin-alpha subunit	Q98UI9	ES, EW, VM	Unknown
OCX-32	Q90YI1	ES, EW, VM	Inhibitor of carboxypeptidase A

* Data compiled from D'Ambrosio *et al*. (2008), Farinazzo *et al*. (2009), Jonchere *et al*. (2010), Mann (2007, 2008), Mann and Mann (2008), Mann *et al*. (2006); ES, eggshell, EY, egg yolk, EW, egg white, Ut, uterus, VM, vitelline membrane.

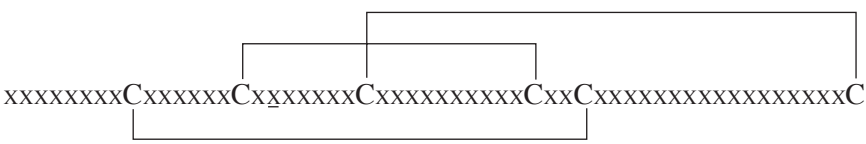

Fig. 9.1 Schematic representation of kazal-type domain. C, cysteine residue; X, amino acid residue, X, active site residue; — disulphide bonds.

protease and the serpin undergoes a conformational rearrangement in which the protease is covalently complexed and inhibited (Silverman *et al*., 2001). Serpins share common structural features, along with a specific signature consisting of a stretch of well-defined residues. This family of proteins also contains serpins with no inhibitory activity (ov-serpin). Ovalbumin, the major egg white protein is the archetype of the ovalbumin-related serpin (ov-serpin) subgroup. Ov-serpins are characterized by short N and C-termini compared with the prototype α1-antitrypsin and lack a conventional secretory signal peptide (Remold-O'Donnell, 1993). Ovalbumin has no inhibitory activity and is therefore not listed in this chapter. Several serpins have been identified in egg. **Ovalbumin-related protein X** (P01013) and **ovalbumin-related protein Y** (P01014) are closely related to ovalbumin. Some have observed that their sequences diverge less than that of ovalbumin from the consensus sequence of inhibitory serpins, and suggested that they might have inhibitory activity, in contrast to ovalbumin (Benarafa and Remold-O'Donnell, 2005). **Heparin cofactor II** (O73840) is present in all compartments except the vitelline membrane (D'Ambrosio *et al*., 2008; Farinazzo *et al*., 2009; Mann and Mann, 2008; Mann *et al*., 2006) and has been identified by similarity with homologous proteins in mammals. **Antithrombin** (Q91422) has been identified in egg yolk (Farinazzo *et al*., 2009; Mann and Mann, 2008), and **α2-antiplasmin** (P08697) is potentially a constituent of the eggshell since

it is overexpressed in uterus where eggshell formation takes place (Jonchere *et al*., 2010). The antibacterial activities of the chicken heparin cofactor II, chicken antithrombin and chicken α2-antiplasmin have never been explored. **Neuroserpin** (Q90935) is described in the proteomic analysis of the eggshell (Mann *et al*., 2006) and has been shown elsewhere to inhibit plasmin and plasminogen activators. It is involved in the reorganization of the synaptic connectivity during development and synapse plasticity (Yepes and Lawrence, 2004) and would have neuroprotective effect during embryonic development (Lebeurrier *et al*., 2005). Its role in egg as an antimicrobial protein has not yet been investigated. Additional serpins have been identified as proteins similar to homologous serpins and have no correspondence in the Swiss-Prot database. Information on their activity is therefore not available. These include **similar to α2-plasmin inhibitor** (XP_415807), **similar to plasma protease C1 inhibitor** (XP_421063), **similar to α1-antitrypsin** (XP_421343), **similar to serpina1d-prov** (XP_421342), all identified in egg yolk.

The **pancreatic trypsin inhibitor (kunitz)** family is one of the numerous families of serine proteinase inhibitors. They are short proteins, the fold of which is constrained by three disulphide bonds as shown in Fig. 9.2.

These inhibitors have been identified in egg white, eggshell and egg yolk (Table 9.2). The sequence of these inhibitors have been either predicted (**similar to kunitz-like protease inhibitor**, **similar to serine peptidase inhibitor kunitz type 1**, **similar to putative porin**, **similar to pancreatic secretory trypsin inhibitor, similar to α-microglobulin/bikunin**) or have homologues in mammals (**amyloid beta A4 protein**, **tissue factor pathway inhibitor 2**). **Beta-amyloid protein 751 isoform** (Q9DGJ7) has been shown to be expressed during chick embryogenesis. It might function as a cell surface receptor and performs physiological functions on the surface of neurons relevant to neurite growth, neuronal adhesion and axonogenesis (Carrodeguas *et al*., 2005), as does its human counterpart. The participation of all these newly identified inhibitors in egg defence is not known.

Other types of potential peptidase/protease inhibitors have been identified in egg, although outside of the main families described above. **Ovocalyxin 32** possesses a latexin-like domain (Gautron *et al*., 2001), which confers an inhibitory activity against carboxypeptidase A. This protein has been shown to affect *Bacillus subtilis* growth (Xing *et al*., 2007). **Ovomucin** is one of the major proteins of egg white and vitelline membrane and contains a cysteine-rich trypsin inhibitor-like domain. It contributes to the gel-like properties

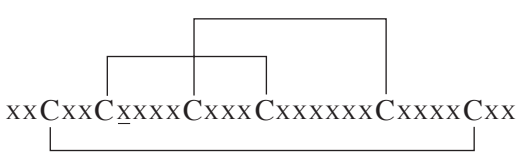

Fig. 9.2 Schematic representation of kunitz-type domain. C, cysteine residue; X, amino acid residue, X, active site residue; — disulphide bonds.

of thick egg white. There is some evidence that ovomucin can act as an antibacterial agent (Kobayashi *et al*., 2004) but it is not known whether its cysteine-rich trypsin inhibitor-like domain is responsible for this property. BMP-binding endothelial regulator protein (Q8N8U9) harbours a trypsin-inhibitor like domain, which typically contains ten cysteine residues that form five disulphide bonds. Its antimicrobial activity has not been studied.

9.4.2 Inhibitors of cysteine proteases

The major egg cysteine protease inhibitor is **cystatin** (P01038), which is ubiquitously distributed in egg (D'Ambrosio *et al*., 2008; Farinazzo *et al*., 2009; Mann, 2007; 2008; Mann and Mann, 2008; Mann *et al*., 2006). Cystatin is active against bacteria, viruses, yeasts and parasites. Chicken cystatin has been described to reduce the production of polio-virus after infection and to be effective against rotavirus (Korant *et al*., 1985; Ebina and Tsukada, 1991). Cystatin displays antibacterial activity against several bacteria including *Porphyromonas gingivalis* (Blankenvoorde *et al*., 1998; Wesierska *et al*., 2005) and is also a potent inhibitor of cysteine proteases expressed by *Trypanosoma cruzi* parasite (Serveau *et al*., 1996). In addition, it has been shown to have immunomodulatory effects (Verdot *et al*., 1999). **Similar to prekininogen** (XP_422766) contains three cystatin-like domains and has been identified in egg yolk (Farinazzo *et al*., 2009). Its antimicrobial effect has not been explored.

9.4.3 Metalloprotease inhibitors

Two metalloprotease inhibitors have been found in egg, **tissue inhibitors of metalloprotease type 2 and 3**, TIMP-2 (O42146) in egg white and eggshell (D'Ambrosio *et al*., 2008; Mann *et al*., 2006) and TIMP-3 (P26652) in egg white, vitelline membrane and eggshell (D'Ambrosio *et al*., 2008; Mann, 2007, 2008; Mann *et al*., 2006). There is no evidence in the literature of the involvement of these inhibitors in host defence. However, it is noteworthy that many pathogens express metalloproteases (Guillemet *et al*., 2010; Karim *et al*., 2010; Kulkarni *et al*., 2006) and the presence of such inhibitors in egg might interfere with the deleterious activity of some bacterial metalloproteases.

9.4.4 Broad spectrum inhibitors: macroglobulin-type proteins

Macroglobulin-type antiproteases are able to inhibit all four classes of proteinases by a unique 'trapping' mechanism. This protein has a peptide stretch, called the 'bait region' which contains specific cleavage sites for different proteases. The cleavage of the bait region by a protease induces the conformational change of the macroglobulins. The resulting entrapped enzyme remains active against low molecular weight substrates whereas activity against high molecular weight substrates is greatly reduced due to

Table 9.3 α2-macroglobulins in egg

Protein name	Accession number (Swissprot, NCBI or IPI_CHICK)	Localization*	Activity
Ovostatin	P20740	ES, EW, EY, VM	Inhibitory
Similar to MGC82112 protein	XP_416480	EW	Unknown
Complement C3	Q90633	ES	Unknown
Complement component 3d	Q2MV09	ES, EY, VM	Unknown
Similar to MGC68875	IPI_00589043	EY	Unknown
Similar to α2-macroglobulin	XP_425514	EY	Unknown
Similar to α2-macroglobulin-1	IPI_00595847	EW	Unknown

* Data compiled from D'Ambrosio *et al*. (2008), Farinazzo *et al*. (2009), Jonchere *et al*. (2010), Mann (2007, 2008), Mann and Mann (2008), Mann *et al*. (2006); ES, eggshell, EY, egg yolk, EW, egg white, VM, vitelline membrane.

steric hindrance. There is some evidence in the literature that macroglobulin-like proteins play a major role in innate immunity (Armstrong and Quigley, 1999).

Ovostatin, the main alpha2 macroglobulin of egg has been identified in all compartments (Table 9.3). Ovostatin is able to inhibit many pathogenic proteases, such as 64K and 56K metalloproteases and cysteine protease 70K produced by *Serratia marcescens*, elastase expressed by *Pseudomonas aeruginosa* or alkaline protease from *Bacillus stearothermophilus* (Miyagawa *et al*., 1991; Molla *et al*., 1987). Other macroglobulin-like proteins have been found in egg (Table 9.3). Their predicted inhibitory activity suggests that they would have a similar antimicrobial effect as ovostatin: **complement C3** (Q90633), **complement component 3d** (Q2MV09), **similar to MGC68875** (IPI00589043.1), **similar to α2-macroglobulin** (XP_425514), **similar to α2-macroglobulin-1** (IPI_00595847) and **similar to MGC82112 protein** (XP_416480).

9.5 Immunoglobulin superfamily

9.5.1 Immunoglobulins

Immunoglobulins (Igs) or antibodies (Abs) are the principal operators of the adaptive humoral immune response. Immunoglobulins are glycoproteins that mediate antigen recognition and presentation resulting in their neutralization and elimination (pathogens, toxins, etc.). In chickens, only three immunoglobulin classes have been fully identified, IgM, IgA and IgY. However, some evidence of the other IgD and IgE immunoglobulins has also been reported (Burns and Maxwell, 1981; Chen *et al*., 1982). All three immunoglobulins M, A and Y, have been identified in egg yolk and

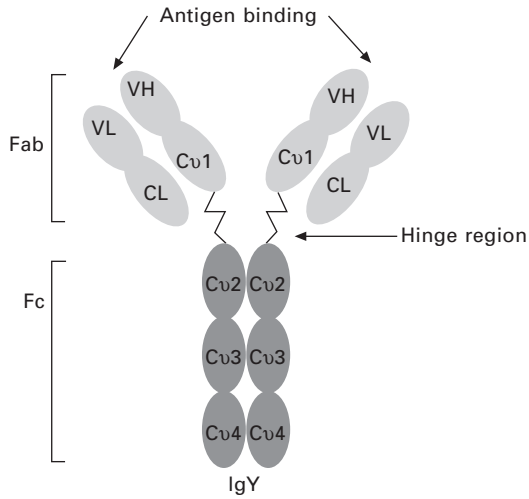

Fig. 9.3 Schematic representation of Ig Y. VL, variable region of the light chain; VH variable region of the heavy chain; CL, constant region of the light chain; Cυ, constant region of the heavy chain.

egg white. Chicken IgY concentration in egg yolk varies significantly with breed lines (Hamal *et al*., 2006); nevertheless, IgY are the most predominant immunoglobulins present in egg with concentration over 1 mg/mL of yolk as opposed to concentrations rounding 20 μg/mL for IgM and IgA (Hamal *et al*., 2006). In contrast, concentrations of all three immunoglobulins in egg white are quite similar (about 10 μg per mL of egg white) (Hamal *et al*., 2006). Chicken IgY was initially associated with the gamma-livetin fraction of the egg yolk. The basic structure of IgY molecules is comparable to that of mammalian IgG: IgY is a tetramer of two light chains and two heavy chains linked by disulphide bonds (Fig. 9.3). IgY molecules are exported to the egg yolk by the hen and contribute to protection of offspring from early infections. This passive immunity is of major importance for the developing embryo since its immune system matures later after hatching. The transfer of immunoglobulins from the bloodstream of the hen to the yolk is mediated by oocyte membrane receptors and involves both the Fc part of the antibody and hinge region (Morrison *et al*., 2002) (Fig. 9.3). During incubation, IgY molecules are exported from the embryonic yolk sac into the bloodstream of the embryo after binding to the chicken yolk sac IgY receptor (FcRY), through a pH-dependent mechanism (West *et al*., 2004).

9.5.2 Proteins with Ig-like domains

The Ig-like domain is probably the most widespread domain, at least in animals. This domain can be considered as a heterogeneous group built on a common fold (Bork *et al*., 1994). Proteins containing an Ig-like domain

differ in their tissue distribution, amino acid composition and biological role. All Ig-like domains appear to be involved in binding functions and many of them might participate in host defence as antibiotic proteins, enzymes, cytokine receptors or components of immune system (Halaby and Mornon, 1998). In the chicken egg, many molecules containing Ig-like domains were initially noted to possess cell adhesion activity and might therefore be involved in morphogenesis and tissue differentiation (Table 9.4). However, their role as antimicrobial proteins cannot be excluded. We also found three proteins involved in the immune response that might therefore play a role in its activation against pathogens: **ICOS ligand** (Hutloff *et al*., 1999), **β-2**

Table 9.4 Proteins with Ig-like domains in egg

Protein name	Accession number (Swissprot)	Localization*	Activity
CEPU-Se alpha 2 isoform	O57596	ES, EW, VM	Cell adhesion
CEPU-1	O93242	ES, VM	Cell adhesion
Protein CEPU-1	Q90773	ES, EW, VM	Cell adhesion
Neogenin	Q90610	ES	Cell adhesion
Basement membrane-specific heparan sulphate proteoglycan core protein	Q6KDZ1	ES	Cell adhesion, tissue development and differentiation
Neuroplastin	P97300	Ut	Cell adhesion, synaptic plasticity
Pro-neuregulin-1, membrane-bound isoform	Q05199	EW	Growth factor activity, embryonic development
Semaphorin-3C	O42236	ES, VM	Receptor activity, nervous system development
Muscle, skeletal receptor tyrosine protein kinase	Q8AXY6	EW	Receptor tyrosine kinase activity
Butyrophilin subfamily 1 member A1	Q62556	Ut	Unknown
Basigin	P17790	ES	Unknown
VH1 protein	A2N887	ES	Unknown
ICOS ligand	O75144	Ut	Receptor binding, defence and immune response
Beta-2-microglobulin (IR)	P21611	ES, Ut	Antigen presentation, immune response
T-cell surface glycoprotein CD1b4	Q9QZY9	EW	Antigen presentation, immune response

* Data compiled from D'Ambrosio *et al*. (2008), Farinazzo *et al*. (2009), Jonchere *et al*. (2010), Mann (2007, 2008), Mann and Mann (2008), Mann *et al*. (2006); ES, eggshell, EY, egg yolk, EW, egg white, Ut, uterus, VM, vitelline membrane.

microglobulin as a component of the class I major histocompatibility complex and **T-cell surface glycoprotein CD1b4** involved in antigen presentation (Androlewicz *et al*., 1994; De Libero *et al*., 2009). It is noteworthy that these proteins have been identified as secreted proteins in the transcriptomic survey of the uterus (Jonchere *et al*., 2010). These molecules could be secreted into the uterine milieu to protect the egg against microbial invasion prior to shell formation and then incorporated into the calcified shell as organic matrix proteins. During its development, the embryo mobilizes the eggshell calcium. The most internal eggshell matrix proteins would be solubilized and consequently could contribute to natural egg defence by providing chemical protection to the embryo.

9.6 Cytokines and other mediators of immune response

Four additional molecules in egg have been identified as potential mediators of immune system. These proteins might only reflect the stimulation of immune system to locally protect the epithelium of uterus. The biological role of these molecules in egg after oviposition is unknown and it would be interesting to study whether their presence in egg contributes to the protection of the embryo. These molecules consist of lymphocyte antigen 86 which has been identified in all compartments of the egg except vitelline membrane (D'Ambrosio *et al*., 2008; Farinazzo *et al*., 2009; Mann, 2007; Mann and Mann, 2008; Mann *et al*., 2006) and three eggshell cytokines, osteopontin, MIP 1 α, Interleukin-like protein (Mann *et al*., 2006). Cytokines encompass a large and diverse family of polypeptide regulators with immunomodulatory activity.

By similarity with mouse lymphocyte antigen 86 (also named MD-1), chicken **lymphocyte antigen 86** (Q90890) is thought to mediate the innate immune response to bacterial lipopolysaccharide (LPS) and cytokine production. It might be involved in LPS recognition and signalling by cooperation with cell surface molecules such as the B-cell CD180 (Nagai *et al*., 2002).

Osteopontin (P23498) is believed to play an important role in regulating eggshell mineralization. Its expression in uterus is stimulated by entry of the egg into the uterus due to distension of the uterine wall (Lavelin *et al*., 1998). Consequently, osteopontin is released in the uterine fluid and accumulates in the eggshell to be a component of the eggshell matrix where it regulates shell mineralization (Chapter 8). The mammalian homologue of osteopontin has been shown to regulate the production of diverse cytokines and is essential in natural killer T cells functionality (Diao *et al*., 2008).

Chicken **macrophage inflammatory protein 1-alpha** (B5TME2) would have chemokine activity (Wolpe and Cerami, 1989) and potential antiviral activity (Amella *et al*., 2005) by similarity with its mammalian homologue. As a member of the interleukin family, chicken **interleukin-like protein**

(Q6VXX8) could also be involved in immune response (Koskela *et al.*, 2004). The potential effect of these four molecules in egg is not clear. All these molecules are mediators of cell responses and therefore are not likely to participate in defence of unfertilized table eggs. In contrast, they might play a role in defence during embryonic development. As suggested previously, cytokines identified in the eggshell might also result from activation of inflammation and immune system in uterus and might not selectively accumulate in the eggshell but rather, would be present in the eggshell due to passive incorporation.

9.7 Molecules involved in protection against stress and oxidative injury

Most organisms possess many cellular and molecular mechanisms to respond to a variety of stresses and maintain cellular homeostasis. These include the rapid synthesis of various proteins that assist in the proper folding of molecules. Some of them appear to be critical to protect the cells from damages associated with stresses such as ischaemia, inflammation, and energy depletion. These various stresses also trigger accumulation of toxic molecules such as oxygen radicals that can contribute to numerous functional dysregulations. In normal situations, the generation of such molecules is usually regulated by a potent antioxidant system allowing for cell detoxification. Careful analysis of egg proteomes and the uterus transcriptome allowed identification of many actors participating in the stress response.

9.7.1 Molecules assisting protein folding

We distinguish chaperone proteins, including heat shock proteins, as proteins transiently involved in the non-covalent folding, assembly and/or disassembly of other polypeptides, as well as proteins that catalyse formation of disulphide bonds in proteins (Ma and Hendershot, 2004). Chaperone proteins help to maintain cellular homeostasis and protein conformation by functioning to reactivate denatured or malformed proteins. Hyperthermia, ischaemia, oxidative, cytokine and muscular stress, glucose deprivation, and alterations in calcium and pH are potent inducers of chaperone expression in different types of cells and tissues (Diller, 2006).

Most of the chaperone proteins that have been identified were essentially related to the eggshell proteome or the uterus transcriptome (Table 9.5). Interestingly, analyses revealed the presence of numerous chaperone-related proteins in the eggshell as compared with the other tissues (Jonchere *et al.*, 2010; Mann *et al.*, 2006). This raises the question of the presence of a very specific environment temporally regulated during eggshell formation. In fact, uterine fluid contains all the protein precursors of the eggshell matrix.

Table 9.5 Proteins assisting protein folding

Protein name	Accession number (Swissprot, REFSEQ)	Localization*
Heat shock proteins		
Heat shock protein HSP 90-alpha	P11501	ES
Heat shock 70 kDa protein	P08106	ES, EY
Heat shock cognate 71 kDa protein	O73885	ES, EY, VM
Heat shock cognate protein HSP 90-beta	Q04619	ES
Heat shock protein 10	O42283	ES
Putative uncharacterized protein HSP70	Q5F497	ES
78 kDa glucose-regulated protein	Q90593	ES, EW, Ut
Endoplasmin	P08110	Ut
Other chaperone proteins		
LDLR chaperone MESD	Q5ZKK4	ES
DnaJ homologue subfamily C member 3 (SR)	Q5ZI13	ES
Lysosomal alpha-mannosidase	O46432	Ut
Calnexin	Q5R440	Ut
FK506-binding protein 9	Q2KJC8	Ut
Clusterin	Q9YGP0	ES, EW, VM
Ovocalyxin-21	XP_417666	ES, Ut
Disulphide bond catalysts		
Protein disulphide-isomerase	P09102	ES
Peptidyl-prolyl *cis-trans* isomerase B	P24367	ES, EW, VM
Sulphydryl oxidase 1	Q8JGM4	ES, EW, VM

* Data compiled from D'Ambrosio *et al*. (2008), Farinazzo *et al*. (2009), Jonchere *et al*. (2010), Mann (2007, 2008), Mann and Mann (2008), Mann *et al*. (2006); ES, eggshell, EY, egg yolk, EW, egg white, Ut, uterus, VM, vitelline membrane.

These precursors need to support the transition from a soluble status to an insoluble matrix, without denaturation of their functional properties. In this context, chaperone proteins in uterine fluid could play an important role in proper folding of the eggshell matrix, which generates the crucial template for eggshell calcification.

However, although eggshell proteins are likely to be gradually mobilized during embryonic development (Chien *et al*., 2009), there is no evidence to date that these chaperone proteins would have a specific role in the egg after laying, such as the protection of the embryo from various stresses.

9.7.2 Antioxidant proteins

Cells are protected against oxidative stress by an interacting network of antioxidant enzymes. These molecules are capable of slowing or preventing oxidation of other molecules and of detoxifying the organism from oxygen radicals, which initiate chain reactions leading to cellular damage.

This detoxification pathway is the result of multiple enzymes, some of which have been identified in egg: superoxide dismutases (**similar to extracellular superoxide dismutase**, XP_420760, eggshell), several peroxidases catalysing the reduction of various peroxides (**glutathione peroxidase** 3, P22352, egg white; **similar to glutathione peroxidase**, NP_001156704, egg yolk; **peroxiredoxin-6**, Q5ZJF4, eggshell; **glutathione *S* transferase 3**, P26697, eggshell; **glutathione *S* transferase 2**, P20136, eggshell**; thioredoxin domain-containing protein 16**, Q9P2K2, uterus; **UDP-glucuronosyltransferase 1**, Q63886, uterus) (D'Alessandro *et al*., 2010; D'Ambrosio *et al*., 2008; Farinazzo *et al*., 2009; Jonchere *et al*., 2010; Mann, 2007, 2008; Mann and Mann, 2008; Mann *et al*., 2006). Moreover, many data have reported the antioxidant activity of other components of egg including peptides resulting from hydrolysis of egg white or egg yolk (Davalos *et al*., 2004; Sakanaka *et al*., 2004) but also ovotransferrin and phosvitin, two major egg proteins, which have high affinity for iron and thereby can limit free radicals generated by this metal ion (Ibrahim *et al*., 2007; Ishikawa *et al*., 2004; Lu and Baker, 1986).

To conclude, chicken egg contains many molecules with potent defensive activity, either confirmed or predicted. This chapter sheds light on new elements to better understand the mechanisms and molecular actors that can be mobilized to protect the egg and the developing embryo from physical, chemical and microbial aggression. Many questions arise from this study. Are these molecules specifically secreted to protect the reproductive system from various aggressions or are they actively incorporated in the egg to intimately participate in the protection of the embryo and fortuitously contribute to the safety of table eggs? How are these molecules regulated/activated during embryonic development? It is now crucial to better define the relative involvement of all these molecules in the various systems of egg defence to better appreciate their biological significance.

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10

Advances in egg defect detection, quality assessment and automated sorting and grading

K. Mertens, B. Kemps, C. Perianu, J. De Baerdemaeker, E. Decuypere and B. De Ketelaere, Katholieke Universiteit Leuven, Belgium and M. Bain, University of Glasgow Veterinary School, UK

Abstract: Efficient control of the quality of eggs for human consumption facilitates the production of enough eggs that meet consumer requirements. The development of techniques for assessing the different quality aspects of eggs is an essential prerequisite for achieving this goal of quality. Novel technologies which are fast, automated and reliable offer the possibility of assessing quality in all individual units instead of relying on the evaluation of a subsample. Factors affecting the choice of techniques include consistency within a single product, measurement speed, instrumentation cost and the sorting efficiency required. This chapter reviews available techniques, focusing on advances, for the assessment of egg quality.

Key words: egg grading, egg quality, non-destructive measurement techniques.

10.1 Introduction

While avian eggs are a vehicle for reproduction, they are also a staple food within the human diet and have a natural balance of essential nutrients. However, eggs have to compete for sales with an increasing number of other products in the modern food industry: to succeed they have to overcome certain disadvantages. For example, eggs are fragile commodities and are subject to quality loss with age. Furthermore, each of the main components of the egg (the shell, the albumen and the yolk) has a natural variability which is not in line with the modern consumer's requirement for consistency. Planned

production methods and efficient quality control procedures help to reduce this variation and are fundamental to the successful marketing of eggs.

Egg quality measurements should not be limited to packing houses or to providing information for the consumer. Non-destructive (and also destructive) egg quality measurements can indeed have applications in grading of eggs for enhanced safety and quality assurance towards consumers, but they can also be of benefit to producers since such measurements give information on the outcome of the egg production process and hence on the health condition of the flock or on the climatic conditions in the poultry house.

The development of sensors that are able to ensure the quality of the products is the first step towards achieving consistent egg quality and ensuring the quality is high. The advantages of an automated quality evaluation over a human one are higher reliability, higher productivity and the ability to perceive more than human beings (for instance to 'see through' the products). Fast, reliable and completely automated technologies offer the possibility of assessing quality of all individual eggs instead of taking samples. The choice of techniques depends on the consistency within a single product, the measurement speed, the instrumentation cost and the required sorting efficiency. The availability of fast computers and new sensor technologies has directed the research towards such fast, objective and accurate technologies. This chapter reviews available techniques, focusing on advances, for the assessment of egg quality.

10.2 Assessment of eggshell quality

From a biological point of view, the eggshell is meant to protect the developing bird embryo and to allow gas exchange between the embryo and the environment. From a food point of view, the eggshell acts as the natural packing material of the egg contents, allowing easy transport of these nutritious fluids and preventing the ingress of potentially harmful bacteria which would otherwise cause spoilage of the egg contents. From a consumer's point of view, the visual aspect of the eggshell quality is a major issue, since dirty, pale or unevenly coloured eggs will be rejected. Furthermore, dirty eggs might increase the risk of bacterial contamination of the eggs.

10.2.1 Shell colour

The eggshell colour has no influence on the nutritional value of the egg for humans, but it has an important influence on consumer preferences, thereby making the colour of the eggshell an important economic quality parameter (Wei and Bitgood, 1989). Shells of commercial table eggs can be brown, white or tinted (in between brown and white). The brown colour of eggs is mainly caused by the pigments protoporphyrin-IX, biliverdin-IX and zinc

chelate. Most of these pigments are located in the cuticle of the egg, yet they can also be found in the shell. That is why, even from white-shelled eggs, protoporphyrin can be extracted (Kennedy and Vevers, 1973; Lang and Wells, 1987).

Shell colour, particularly of brown eggs, diminishes when hens age (Mills *et al*., 1991; Nys *et al*., 1991; Odabasi *et al*., 2007). Odabasi *et al*. (2007) suggested that this phenomenon is the result of the increased egg size as they found no proportionate change in the quantity of pigment deposited over the shell surface.

Furthermore, Mills *et al*. (1991) and Nys *et al*. (1991) reported shell colour variation (see Fig. 10.1) between hens, but also between subsequent eggs of the same hen, albeit that the between hen variation is larger. In addition, these authors report a within-egg variation of pigmentation with the blunt end of the eggs being darker than the sharp end.

The shell colour can also be influenced by disease, management and stress. Mills *et al*. (1991) and Nys *et al*. (1991) stated that stress results in egg whitening as a consequence of premature termination of shell pigmentation. It is indeed known that stress or disease can influence the deposition of eggshell pigmentation during egg formation (Whittow, 1999). In commercial laying hens, for instance, viral diseases infecting the reproductive tract may cause pale eggs. Different kinds of stress (environmental or social) might also induce a higher degree of spottiness (Butcher and Miles, 2003). In normal conditions each bird could be identified by the patterns of the pigment spots. More information on eggshell pigmentation in both commercial laying hens and wild birds can be found in Solomon (1987).

Traditionally, eggshell colour is measured using reflectometry in which reflected light is recorded at three specific wavelength bands, being the characteristic wavelengths of red, green and blue light (Wei and Bitgood, 1989). These three values are combined to calculate a specific colour value which defines the shell colour as a percentage between the black and the white reference, with the former expressed as 0% and the latter as 100%

Fig. 10.1 The variability in brownness of eggs. Picture property of Moba BV.

reflectance. Similarly, shell colour can also be quantified in other colour systems such as the *L*a*b** colour space.

Visible/near infrared (VIS/NIR) spectroscopy: the transmission colour value

Recently, Mertens *et al*. (2010) introduced a novel measure, called the transmission colour value (TCV), for defining the colour of brown eggs based on visual/new infrared (VIS/NIR) spectroscopy. Results of Kemps (2006), who tried to predict internal egg quality (Haugh unit and albumen pH) using VIS/NIR spectroscopy (see page 222), indicated that the observed changes in the VIS/NIR spectra of consumption eggs were more related to changes in shell pigments than to internal quality aspects and this author therefore stated that the use of VIS/NIR transmission spectroscopy could prove useful in defining and monitoring shell colour.

The setup used for measurement of the TCV is the same as described on page 223 (Fig. 10.8). The TCV is calculated from the smoothed (Savitzky-Golay filter, bandwidth 10 nm) VIS/NIR transmission spectra. The TCV was calculated in analogy with the blood value parameter (see Section 10.3.3). The most important absorbance peak of the molecule mainly responsible for the shell colour, protoporphyrin-IX, is situated at 643 nm (Kadish *et al*., 1999). The variability in egg characteristics, such as shell thickness and yolk colour, prevents the absolute value of the intensity of the light transmitted at 643 nm from being used directly. A thick eggshell will absorb more light than a thin one, resulting in a lower transmitted intensity at 643 nm in a thick-shelled light coloured egg. Therefore, a relative measurement is required, such as the ratio between the transmission intensity at two wavelengths namely 643 nm and a reference band not influenced by protoporphyrin-IX. The TCV is therefore defined by the following ratio:

$$\mathrm{TCV} = \frac{T_{643}}{T_{610}} \qquad 10.1$$

with T_{643} the relative transmission at 643 nm and T_{610} the relative transmission through the egg at the reference wavelength 610 nm. Higher TCV values correspond to a higher transmission rate of light at the protoporphyrin-IX wavelength and hence less pigment is present in the shell.

The results of Mertens *et al*. (2010) indicated that the assessment of shell colour through the amount of pigmentation is especially of interest for detecting changes in shell colour as a result of stress (e.g. heat stress) or diseases (e.g. infectious bronchitis). Mills *et al*. (1987) already suggested the use of reflectometry as a measure of stress in commercial layers. Work by Siefferman *et al*. (2006), Moreno *et al*. (2006) and Martinez de la Puente *et al*. (2007) indicated that eggshell colour and spottiness are indicators of stress and the general health of birds. As already mentioned in the introduction to shell colour, stress and disease often result in egg whitening as a consequence of premature termination of shell pigmentation (Mills *et al*., 1991; Nys

et al., 1991; Whittow, 1999), or might induce a higher degree of spottiness (Butcher and Miles, 2003).

Comparing this novel method with the more traditional reflectometry measurement by means of a colorimeter, in, e.g., the *L*a*b** colour space, it is considered a more complete measure of colour because it contains more information on actual pigment deposition in and on the eggshell. Although colour results of reflectometry contain information on only the outer pigment component, namely PPIX, deposited in the cuticle, the TCV value quantifies the total presence of PPIX pigmentation in the egg including the eggshell (Lang and Wells, 1987). Furthermore, reflectometry measures are influenced by the measurement site (Mertens *et al*., 2010).

Front-face fluorescence

The basics of front-face fluorescence is that the absorption of light by a molecule causes the excitation of an electron moving from a ground state to an excited state. After the electron has been excited, it rapidly relaxes from the higher vibrational state to the lowest vibrational state of the excited electronic state. After reaching the lowest vibrational state of the excited electronic state, the excited state may decay to the ground state by the emission of a photon which can be observed as the phenomenon of fluorescence (Genot *et al*., 1992a,b).

Related to shell colour, eggs show maximum emissions at 635 and 672 nm after ultraviolet excitation. These excitation wavelengths are mainly related to the amount of protoporphyrin. The autofluorescence of a fresh egg is stronger than that of an old one because the intensity of autofluorescence depends on the amount of porphyrin on the shell surface (Karoui *et al*., 2006a).

10.2.2 Cuticle quality

The quality of the cuticle of a consumption egg is an important factor for protection against bacterial penetration. Amongst others, Messens *et al*. (2005) and DeReu *et al*. (2006a,b) stated that the cuticle is the critical factor in protection of the egg against bacterial penetration: the poorer the quality of the cuticle, the higher eggshell penetration.

A method for assessment of the cuticle quality was developed by Board and Halls (1973). In this method an egg is first immersed for 1 minute in an aqueous solution containing Tartrazine and Green S, rinsed for excess dye and dried. Quantification of cuticle deposition is done by making the difference between a colour measurement (e.g. with a colorimeter) of the egg before staining and a measurement after staining (De Reu *et al*., 2006a,b; Messens *et al*., 2005, 2009).

10.2.3 Shell strength

It is of great importance that consumption eggs have high shell strength in order to resist all impacts an egg is subjected to during the production chain

(Bain, 1990). Broken eggs cause economic damage in two ways: they cannot be sold as first quality eggs and the occurrence of hair cracks raises the risk for bacterial contamination of the broken egg itself and of other eggs when leaking, affecting external quality, internal quality and even food safety (Mertens *et al.*, 2005).

Shell damage is directly related to shell strength. Shell strength is directly influenced by shell thickness and shell matrix organization. A number of techniques and instruments have been developed to measure eggshell strength. Shell strength diminishes with age in commercial laying flocks. This may be related to the increasing size and changes in availability and metabolism of calcium to build the calcium carbonate crystals. Hence more eggs are broken towards the end of lay and handling of eggs from older flocks is more critical than for younger flocks (Mertens *et al.*, 2006; Solomon, 1997; Wolford and Tanaka, 1970).

Traditional techniques

Different mechanical techniques have been used over many years to evaluate the mechanical properties of the shell – mainly determined by its strength and the presence of cracks (De Ketelaere *et al.*, 2004). In general, eggshell strength methods can be subdivided into direct methods and indirect methods (Hamilton, 1982). Different direct methods are described in the literature. Most widely used is the compression fracture force measured during quasi-static compression (Abdallah *et al.*, 1993; Hamilton, 1982; Voisey and Hunt, 1967), providing a measure of eggshell's material strength. Other methods include puncture tests and impact tests. All of these direct methods are destructive. Another parameter related to eggshell strength is Young's modulus, the ratio of stress to strain. Determination of Young's modulus is not straightforward in the case of an eggshell due to its particular natural curvature. Furthermore, measurements are destructive and very time-consuming due to eggshell sample preparation and standardization (Kemps *et al.*, 2004).

Indirect methods can be destructive or non-destructive, and usually measure a parameter that is related to the eggshell strength. Measuring the thickness of the eggshell for example is frequently used as an indicator of eggshell strength. Another indirect measure for the strength of the egg is provided by the calculation of the weight percentage eggshell of an egg measured, for example defined by means of the specific gravity a method developed by Olsson (1934). A third widely used indirect method is to measure the deformation of the egg using a fixed non-destructive load during a quasi-static compression test. The static stiffness of the egg (K_{stat}) can be determined from the slope of the force–deformation curve. The non-destructive deformation, defined as the amount the eggshell bends or deflects under the applied fixed load, can also be used to estimate the force required to fracture the eggshell (Hamilton, 1982) by extrapolation. This test measures a structural property of the egg. Indirect methods are used to measure eggshell strength on the assumption that the indirect values are correlated with the direct values.

However, values found in the literature indicate that there are only moderate correlations among those parameters. This observation points out that there are several mechanisms involved in shell fracture in addition to the strength of the eggshell material.

Novel methods of assessing eggshell strength

The acoustic response technique provides an opportunity to obtain information relating to eggshell strength in a fast, objective and non-destructive way (Coucke, 1998; Coucke *et al*., 1999; De Ketelaere and De Baerdemaeker, 2000; De Ketelaere *et al*., 2002; Lin *et al*., 2010; Wang *et al*., 2004), which is in contrast to the aforementioned techniques.

Coucke (1998), Coucke *et al*. (1999), De Ketelaere and De Baerdemaeker (2000) and De Ketelaere *et al*. (2002) used vibration analysis of the egg after an impact to define its resonant frequency. This resonant frequency was used to estimate the eggshell strength of intact eggs. For this purpose, Coucke (1998) modelled the egg as a mass–spring system and defined a novel eggshell strength parameter which relates to the egg's dynamic stiffness (K_{dyn}). The equipment used for this (Fig. 10.2) is of laboratory scale and consists of two rolls to support the egg and to rotate it around its longitudinal axis, a hammer made of a light rod to tap the eggs, and a microphone to record the resulting egg vibration (De Ketelaere *et al*., 2004).

While the egg is turning around the hammer taps on the egg four times. Figure 10.3 gives an example of a typical registered time signal of the vibrational response of the egg after impact with the light hammer. Based on these time signals, frequency spectra are calculated, and from these the dominant resonant frequency of the intact egg is extracted. Figure 10.4 shows an example of extracted frequency spectra of an intact egg.

The dynamical stiffness, K_{dyn}, is then calculated as

$$K_{dyn} = A \cdot EW \cdot RF^2 \qquad 10.2$$

with EW the mass of the egg in kg, A a constant (set to 1) and RF the first resonant frequency of the vibration in Hz. The dynamic stiffness is calculated on four equidistant places on the equator of the egg and the average value defines the final K_{dyn} value.

Coucke (1998) and Coucke *et al*. (1999) reported a correlation of 0.71 between the static and dynamic stiffness of eggs. The Pearson correlation between the eggshell thickness and the dynamic stiffness was 0.60. Later, De Ketelaere and De Baerdemaeker (2000) and De Ketelaere (2002) extended the initial mass–spring model towards a mass–spring–damper model and showed that the damping of the vibration provides extra information. This was obtained by linking the dynamic measurements to static measurements, although interpretation remains a matter of debate. Furthermore, they showed that the shape of the egg is needed to link dynamic to static measurements. A multiple linear regression model was constructed using the dynamic stiffness, the damping and the shape index of the eggs as explanatory variables in order

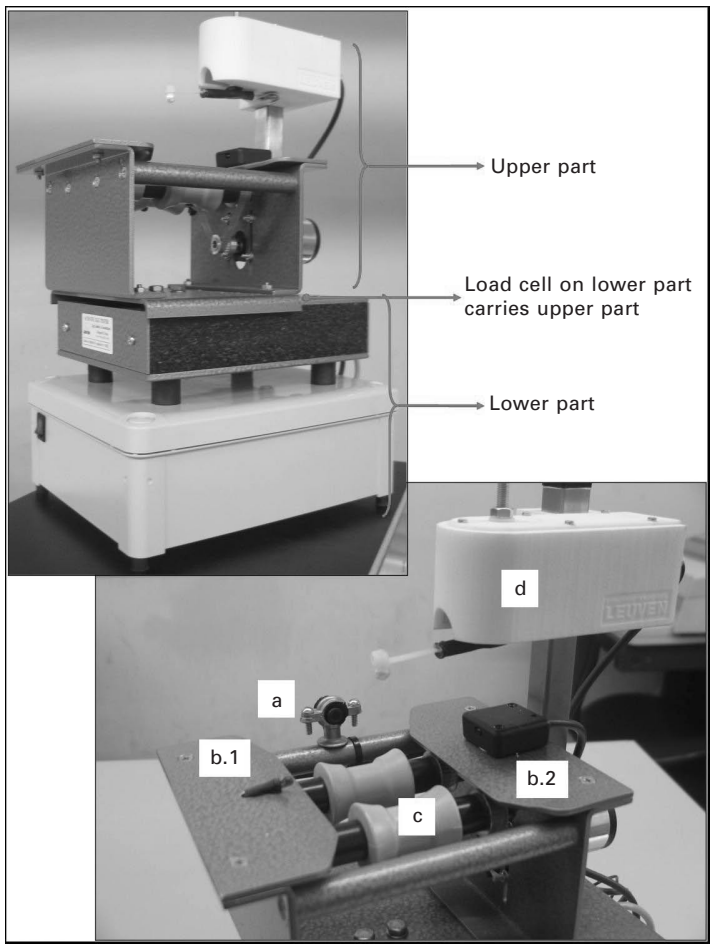

Fig. 10.2 The acoustic egg tester: lab-scale setup for analysis of the vibrational behaviour of the egg after a slight impact. This device can be used to detect cracks in eggshells and for intact eggs it is used to define the dynamical stiffness (K_{dyn}). Furthermore an electronic load cell is included to automatically weigh the egg: a microphone; b egg presence detector, b.1. IR beam sender b.2 receiver; c supporting rolls for egg rotation; d hammer mechanism with the light hammer and a magnetic coil (not visible). Picture property of Katholieke Universiteit Leuven.

to predict the static stiffness with a correlation of 0.90. The same explanatory variables were used to estimate shell thickness and breaking strength, but correlations were lower (0.78 and 0.64, respectively).

It should be questioned, however, whether one has to compare new quality indicators, such as the dynamic stiffness, to older and imperfect indicators such as static stiffness, or whether one should use the new indicators. Recent results from different researchers have proved K_{dyn} to be an interesting and practical relevant measure for eggshell strength. Eggs with higher K_{dyn}

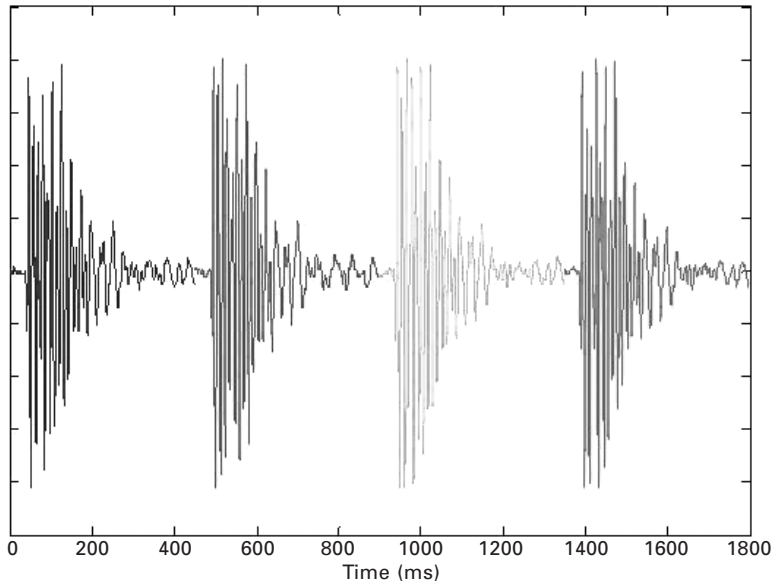

Fig. 10.3 Vibrational responses (four) (time signal) of the egg after impact of the light hammer. Figure property of Katholieke Universiteit Leuven.

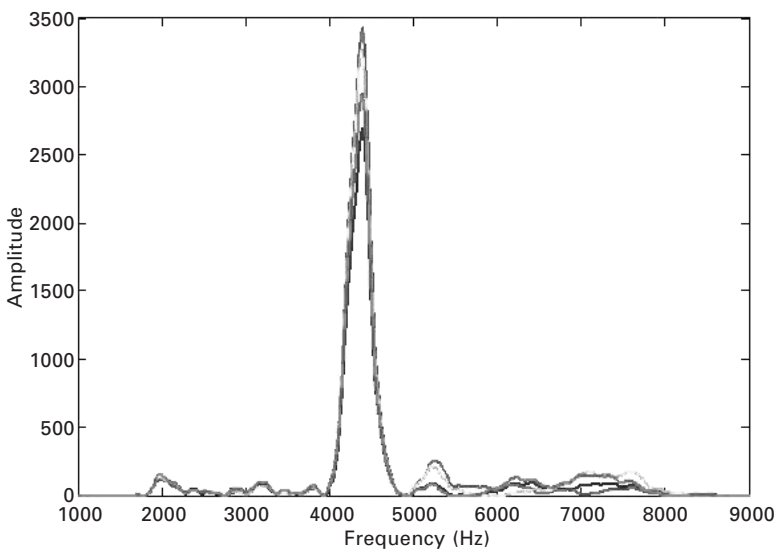

Fig. 10.4 Resulting frequency spectra of an intact egg. This egg shows a typical resonant frequency of about 4300 Hz. Figure property of Katholieke Universtiteit Leuven.

for example have a significantly lower incidence of breakage during their journey along the production chain of table eggs (Bain *et al.*, 2006; Mertens *et al.*, 2006). Furthermore, K_{dyn} can be used as a constant quality parameter

for monitoring egg quality in practice indicating when egg shell strength decreases as a result of stress or disease (Lin *et al*., 2004; Mertens, 2009; Mertens *et al*., 2007). Bacteriological studies investigating penetration of *Salmonella* Enteritidis into table eggs showed that eggs with higher K_{dyn} were penetrated less by *Salmonella* Enteritidis (Messens *et al*., 2007). These results contribute to the fact that K_{dyn} might be interesting for use in selection programmes for shell quality. This idea was proved when Bain *et al*. (2003) and Dunn *et al*. (2005) found the heritability for K_{dyn} to be significantly higher than traditional measures of shell strength (mentioned before).

More recently, Lin *et al*. (2010), using a similar setup as Coucke (1998), built partial least squares (PLS) models based on the complete frequency spectrum to predict shell strength in terms of the maximum breaking force (F_{max}). They obtained a maximum R^2 of 0.59.

10.2.4 Eggshell crack detection

Candling has been used since the 1920s to identify eggs containing cracks and other poor quality aspects such as translucent shells, loose or mobile air cells and egg with inclusions. During the candling operation, a bright light is shone through the egg and the egg is visually inspected, preferably in a dark room. Poor quality shells and eggs with cracks appear more translucent due to the presence of water within the shell.

Vibration analysis

The acoustic response test described on page 215 also provides a more reliable method to detect cracks and defects in eggshells. Several authors have presented a method to detect eggshell cracks based on the acoustic response, amongst others Coucke (1998), De Ketelaere *et al*. (2000), Cho *et al*. (2000), Jindal and Sritham (2003), Wang and Jiang (2005), Deng *et al*. (2010), Lin *et al*. (2009, 2010) and Y. Zhao *et al*. (2010).

The method which is currently most widely used, mainly in quality control and genetic selection, is the one proposed by Coucke (1998) and De Ketelaere *et al*. (2000) which uses the same setup as on page 215. Owing to the axial symmetry of an egg with an intact shell, the impulse response in the frequency domain is very similar and independent of the place of excitation along the equator ring of the egg. In this case the Pearson correlation coefficients are used to quantify the similarity between two independently obtained frequency spectra from the same egg. A value close to 1 will be obtained for two identical spectra. Lower values will be observed for spectra with heterogeneous patterns. As described above, each egg is usually tapped four times (every 90° around the equator ring). These multiple measurements are used to calculate Pearson correlation coefficients for six different combinations of data arrays, namely: (1,2); (1,3); (1,4); (2,3); (2,4) and (3,4). If the lowest correlation value of these six features is lower than a certain threshold value, usually 0.9 or 0.95, the egg is classified as

cracked (De Ketelaere *et al.*, 2000). Based on the time signal (Fig. 10.3), frequency spectra are calculated and used to decide whether the egg is 'intact' or 'cracked'. Figure 10.4 is obtained from an intact egg and Fig. 10.5 shows the frequency spectra obtained from a cracked egg.

It was shown that in this way up to 90% of the cracks can be removed, while the false rejects remain below 1%. The technique is also extremely fast. An egg vibration typically lasts for about 10 ms. The low number of measurements needed makes this technique a very appealing alternative to the existing crack detection systems.

Similar setups and analysis of the acoustic response in the frequency domain have been described by several other authors. Cho *et al.* (2000) used multiple regression and discriminant analysis on extracted features of the frequency spectra to classify eggs. These authors report 94% crack detection, but allowed for false rejects of 4%. Jindal and Sritham (2003) trained artificial neural networks to classify the eggs. They used the complete frequency spectra from eight measurements for each egg and reported a crack detection level of almost 99%, but allowed for more than 10% false rejects.

Wang and Jiang (2005) presented a setup where the impactor consists of a small pendulum and where the acoustic response is registered with a piezoelectric sensor. They extracted different features of the frequency spectrum to discriminate between intact and cracked eggs. Y. Zhao *et al.* (2010) used this setup and performed pattern recognition (principal component analysis and linear discriminant analysis) on the acquired features. They reported an

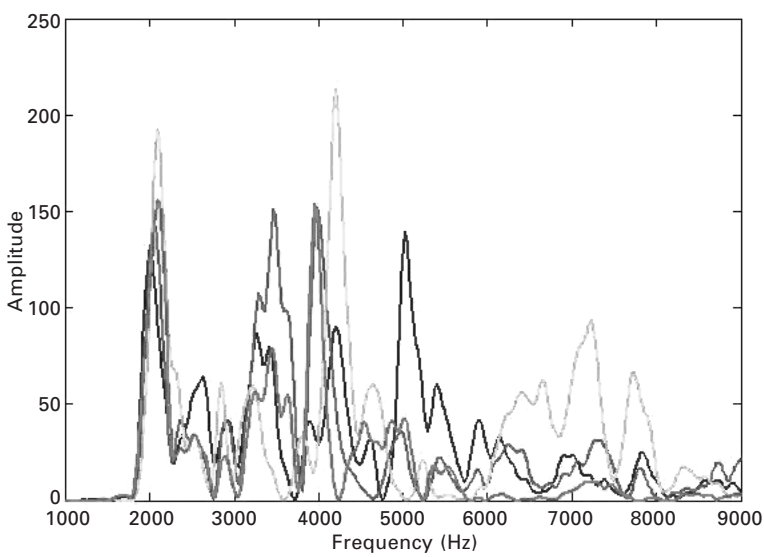

Fig. 10.5 Resulting frequency spectra of a cracked egg. The minimal correlation between these four spectra, –0.04 in this case, is lower than the threshold value of 0.90 and so this egg is evaluated as 'cracked'. Figure property of Katholieke Universtiteit Leuven.

overall performance with 98.7% crack detection and 10.2% false rejects. Lin *et al*. (2009) used a support vector machine to analyse some frequency spectra features for discrimination between intact and cracked eggs. They achieved 90% detection and a false rejection level of 10%.

Rather than transforming the spectrum in the time domain to the frequency spectrum by means of a fast Fourier transformation (FFT), Deng *et al*. (2010) directly used the time domain registrations to detect cracks. They made a difference between intact and cracked eggs by using several time domain signal features and applying a support vector machine for pattern recognition and discrimination. For their egg sample they report a detection rate of 98.9% and 0.8% false rejects.

For crack detection, the percentage of false rejects (intact eggs that are falsely classified as cracked) and the percentage of cracked eggs that are correctly classified as cracked are two competing forces: when allowing for more false rejects, the crack detection level will rise. In order to judge the real potential of a crack detection system, it is good practice to set the false reject percentage to a level that is acceptable for practical purposes (lower than 0.5%), and to report the crack detection percentage achieved at that specific false reject level. This, however, is rarely done which makes it impossible to compare results among researchers working in this field.

Microcracks in eggs

It has been established recently that microcracks initiate in hens' eggs at loads less than that necessary to cause total structural failure (Macleod *et al*., 2006). Using a combination of computational modelling and numerical analysis Macleod *et al*. (2006) showed that very high stress levels developed on the inner surface of the eggshell during mechanical insult. This resulted in a series of microcracks being initiated at the inner surface of the shell which radiated out from the load site for varying distances (Fig. 10.6). A series of concentric circumferential microcracks also developed beneath the cuticle on the outer surface of the shell. Calculations relating to the eggshell's dynamic response indicated that these microcracks have a little effect on the structural stiffness and resonant frequencies of the egg. As a result these microcracks cannot be detected by on-line crack detection systems which rely on mechanical excitation. Eggs in retail outlets have also been shown to contain microcracks as a result of insults experienced during routine handling. Since the eggshell forms the first line of defence against potentially pathogenic microorganisms entering the egg contents, microcracks could potentially compromise egg safety if eggs are then inappropriately handled and/or stored in conditions that favour bacterial contamination and growth.

10.2.5 Detection of dirt and shell anomalies

Dirt on the surface of the eggshell is recognized as being linked to the presence of potentially harmful bacteria which can lead to spoilage of the

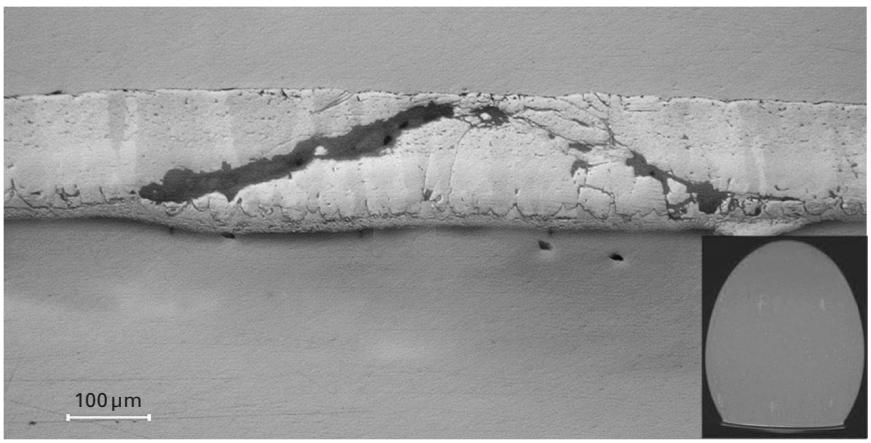

Fig. 10.6 Transverse section of eggshell embedded in resin and polished to reveal extent of damage at microcrack site (see insert). Picture property of the University of Glasgow.

egg contents. Dirt and stains on eggs are mainly caused by contaminants such as faeces (black to light brown stains), uric acid (white stains), yolk, albumen and blood. In most of the grading plants the detection of dirty eggs is still mainly performed by human graders and as egg processing speed runs up to 180 000 eggs per hour, a grader must inspect a dozen eggs per second, resulting in false rejects and false approvals. While false rejects generate economic losses, false approvals can be hazardous to health.

Automatic dirt detection systems are mainly based on camera vision. Patel *et al*. (1996, 1998) carried out a study to differentiate blood spots, dirt stains and cracks by training neural networks with colour histograms obtained from colour images. Mertens *et al*. (2005) recently developed a new system to detect and quantify stains of blood, faeces, uric acid and yolk on brown eggs. In Fig. 10.7 the sequence of images resulting from the detection algorithm of Mertens *et al*. (2005) is given for the detection of the uric acid present on a dirty egg.

10.3 Assessment of the internal egg quality

The internal quality of table eggs is a difficult aspect to measure without breaking the shell. Detection and assessment of internal quality aspects are important since the consumer is sensitive to internal quality (Gerhardy and Ness, 1995; Coutts *et al*., 2007). In the following sections different measuring methods for internal egg quality assessment are discussed.

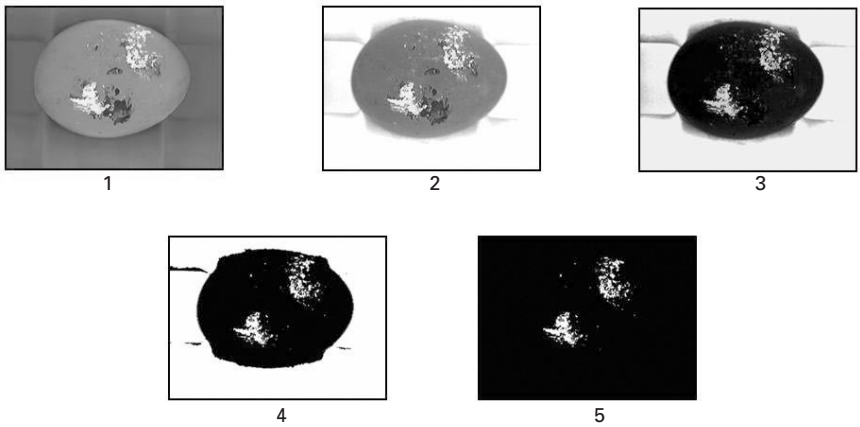

Fig. 10.7 Sequence of images for the detection of white stains on brown eggs caused by uric acid. Pictures property of Katholieke Universtiteit Leuven.

10.3.1 Albumen quality

Consistency (i.e not watery) and clarity (i.e. not mottled or discoloured) are cited as being attributes important to the quality of the albumen (Coutts *et al.*, 2007). The relative importance of these terms varies in different European countries.

The quality of the albumen is usually expressed in terms of height of the air cell, height of the thick albumen or in terms of Haugh units (Haugh, 1937). The Haugh unit is widely used as the reference for albumen freshness, yet doubts have been put on its correctness and objectivity (Silversides, 1994; Williams, 1992). Therefore it might be interesting to link optical or other non-destructive physical measurements to a physical albumen quality parameter such as viscosity or acidity (pH), rather than to the integrative Haugh unit (De Ketelaere *et al.*, 2004). As will be clear from the following, many techniques have been tested for their suitability on predicting egg freshness or albumen quality, yet for use in in-line sorting machines, further research is needed. For further reading, please refer to Romanoff and Romanoff (1949), Solomon (1997) and Karoui *et al.* (2006b).

VIS/NIR spectroscopy

VIS and NIR spectroscopy (wavelengths 350–2500 nm) is a technique based on the measurement of the vibrations caused by the stretching and bending of hydrogen bonds with carbon, oxygen and nitrogen. It has been widely used for determination of the internal quality of agricultural products (Sun, 2009; Williams and Norris, 1987). The analysis of NIR absorption spectra results in quantitative information about constituents such as water and proteins (Giangiacomo and Dull, 1986; Sun, 2009). NIR measurements have several advantages: they are quick, non-destructive, accurate, reliable, contactless and economical. The contactless nature of the technique makes

it very appealing to score egg quality parameters because contact can be minimized for hygienic reasons and a large number of eggs can be graded in a short time. Often, no sample preparation is required (De Ketelaere *et al.*, 2004).

The measurement setup for defining the VIS/NIR transmission spectra of the eggs assesses the transmission spectrum of an egg by means of a light source and a collimating lens receiving the transmitted light (Fig. 10.8). Similarly, reflection spectra can be acquired by capturing the light reflected by the eggshell surface.

In order to avoid the influence of spectral changes of the light source, the transmission measurement is performed relatively. This means that all measurements are compared with a reference measurement through a white Teflon sample – Teflon displays an almost flat transmission spectrum in the VIS and the NIR range (400–2000 nm) – and to the electrical noise of the spectrophotometer. This electrical noise is defined by recording the signal of the spectrum that the spectrophotometer produces when absolutely no light is introduced into the spectrophotometer. As a result, transmission values of light passing through an egg are expressed as a ratio which is the amount of light passing through the egg compared with the amount of light passing through the reference at the same wavelength (Bamelis, 2003). The relative transmission is calculated from:

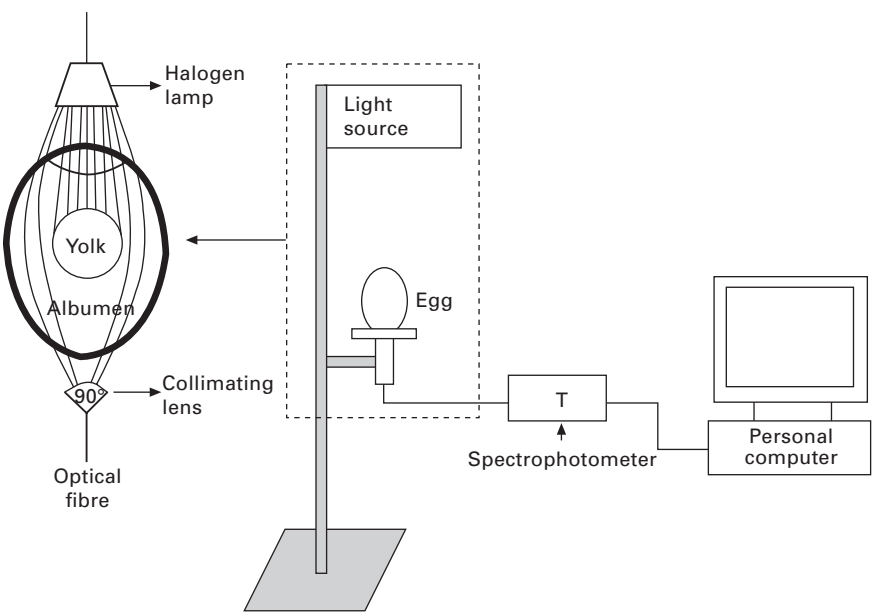

Fig. 10.8 Schematic representation of the setup to measure the transmission spectra of eggs. Picture property of Katholieke Universiteit Leuven.

$$T_\lambda = \frac{T_{\lambda,e} - T_{\lambda,b}}{T_{\lambda,w} - T_{\lambda,b}} \qquad 10.3$$

with T_λ the relative transmission at wavelength λ, $T_{\lambda,e}$ the transmission through the egg at wavelength λ, $T_{\lambda,b}$ the electrical noise at wavelength λ and $T_{\lambda,w}$ the transmission through the Teflon reference at wavelength λ. In the work of Bamelis (2003), Kemps (2006) and Kemps *et al*. (2006, 2007) it was shown that the information captured by the transmission spectra of eggs is located between about 500 and 900 nm. Below 500 nm the light is blocked by the $CaCO_3$ crystals. The spectral information above 900 nm mainly (± 90%) originates from the water in the egg albumen. Figure 10.9 shows an example of typical transmission spectra through brown eggs.

The literature about the link between optical measurements, and more specific VIS/NIR spectroscopy, and physical albumen quality parameters is rather limited. Schmilovitch *et al*. (2002) show that pH could be determined using a PLS regression model on the first derivative of the NIR spectral data. Kemps (2006) tried to estimate the Haugh unit using VIS/NIR spectral data and a PLS regression model. He found that changes in the measured spectra rather reflect changes in the eggshell then changes in albumen quality. Berardinelli *et al*. (2005) and Giunchi *et al*. (2008) used Fourier transformed (FT) NIR reflectance spectroscopy to predict the air cell height, thick albumen height and Haugh unit. The latter authors obtained R^2 values of 0.72, 0.79 and 0.68, respectively. J. Zhao *et al*. (2010) measured NIR reflectance spectra of eggs and used a support vector machine algorithm to discriminate between

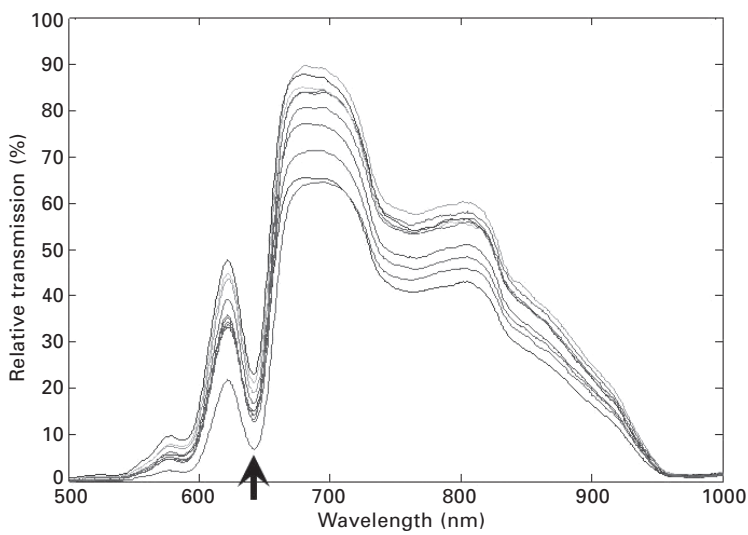

Fig. 10.9 Typical transmission spectra of brown eggs. The spectral information is found between about 500 and 900 nm. The arrow indicates the main peak of PPIX (643 nm).

the spectra of fresh (Haugh unit > 60) and unfresh (Haugh unit < 60) eggs. With this method they correctly classified 93.3% of a small sample of eggs.

No reference was found linking albumen viscosity, as another important physical quality attribute, to spectral data. Moreover, the literature about viscosity measurements of albumen as such is limited, probably due to the Haugh units alternative, and the complex nature of albumen viscosity measurements using a rheological approach (Lucisano *et al*., 1996; Pitsilis *et al*., 1984; Tung *et al*., 1970).

H-NMR spectroscopy

Nuclear magnetic resonance (NMR) is a phenomenon which occurs when the nuclei of certain atoms are immersed in a static magnetic field and exposed to a second oscillating magnetic field. Some nuclei experience this phenomenon, called spin, and others do not.

Low resolution H-NMR spectroscopy was used by Capozzi *et al*. (1999), Schwägele *et al*. (2001) and Kemps *et al*. (2007) as a possible non-destructive method to determine the quality of intact eggs. The longitudinal and transverse relaxation times give information on the chemical and physical changes of the specimen under study and, hence, could be used to assess egg freshness. Changes in the transversal relaxation times during storage were indeed reported by these researchers. These changes were found to be due to the increasing liquefaction of albumen during storage.

Dielectric properties

Dielectric properties of materials vary with moisture content, density, composition and structure, water activity, temperature and frequency of the applied field. They can be measured with several techniques which range from direct current (DC) to microwaves. The kind of dielectric material, frequency, accuracy, availability of instrumentation, cost and suitability for on-line application influence the choice of measurement equipment and the sample holder design. Some of the most commonly used devices and instruments to measure dielectric properties of agri-food materials include the parallel plate capacitor, coaxial probe, waveguide, resonant structure, inductance, capacitance, resistance (LCR) meter, impedance analyser, and scalar and vector network analyser (Ragni *et al*., 2006, 2007).

Data on the first investigations on dielectric properties of eggs were reported by Romanoff and Romanoff (1949). Williams *et al*. (1997) suggested measurements of the total body electric conductivity (TOBEC) to determine the composition of intact eggs. Particularly, this study showed that TOBEC index was useful and accurate to predict the egg lean mass and the mass of some egg components (albumen and water content) (Ragni *et al*., 2006). Budickov (1965, 1968) measured conductivity of eggs by using an electrode chamber filled with electrolyte. This technique allowed to establish correlations between electrical resistance and pH of the yolk and albumen and yolk weight.

Dielectric properties were also determined for egg constituents and used to detect their denaturation (Bircan and Barringer, 2002; Lu *et al*., 1998).

Recently, Ragni *et al*. (2006, 2007, 2008, 2010) tried to predict several egg freshness related quality parameters using the dielectric properties of the egg and its contents. In the work of Ragni *et al*. (2006, 2007, 2008) a frequency range of 50–500 MHz was used. Different statistical models were used to predict egg freshness parameters. Ragni *et al*. (2006) performed non-linear multiple regression on electric parameters to make a prediction for the air cell, yolk index, thick albumen height and Haugh unit, obtaining R^2 values of respectively 0.55, 0.36, 0.34 and 0.32. Ragni *et al*. (2007) predicted egg quality indices from PLS regression of the dielectric constant and loss factor spectra. Their results indicated R^2 values up to 0.92, 0.60, 0.53 and 0.73 for the air cell height, thick albumen height, Haugh unit and yolk index, respectively. Ragni *et al*. (2008) developed PLS models based on the dielectric spectra and got R^2 values up to 0.876 and 0.678 for the prediction of the air cell height and thick albumen height, respectively. In the work of Ragni *et al*. (2010) higher frequency ranges (3 to 20 GHz) were investigated. Their results indicated that in particular the range from 10.5 to 11.5 GHz was interesting for egg quality indices prediction by means of an artificial neural network. The R^2 values obtained were 0.92, 0.85 and 0.91 for the air cell, the thick albumen height and the yolk index, respectively.

Front-face fluorescence spectroscopy

Karoui *et al*. (2006b) presented a study to assess egg freshness using front-face fluorescence (see page 213). They showed that fluorescent spectra recorded on thick and thin egg albumen and targeted on Maillard reaction products such as furosine, have the potential to differentiate fresh from aged eggs.

Electronic nose

Wang *et al*. (2009) were the first to investigate the possibility of using an electronic nose for monitoring internal egg quality (Haugh unit and yolk index; section see 10.3.2). An electronic nose is basically a sensor capable of recording chemical volatiles. Based on the whole set of volatiles of the sample, the electronic nose generates a unique digital pattern which can be considered as the signature of a particular set of aromatic compounds. Based on a database of such patterns, future samples can be discriminated.

Wang *et al*. (2009) used different multivariate statistical techniques to analyse the patterns of the electronic nose to develop their models. Their model for Haugh unit had an R^2 of 0.91 and a standard error of prediction (SEP) of 3.74, while the model for yolk index had an R^2 of 0.93 and an SEP of 0.02.

10.3.2 Yolk quality

There are two quality aspects related to the egg yolk. The yolk colour is mainly important for the consumer's perception, while the physical quality

of the egg yolk can be used as a measure for egg freshness (see Section 10.3.1).

Yolk colour

For consumers yolk colour is considered one of the main attributes of good quality of consumption eggs. Yolk colour is influenced by the hen's diet: the more carotenoids in the diet, the more red and darker the yolk. More detailed information on the effect of diet components on yolk composition can be found in Huyghebaert *et al*. (2002) and Huyghebaert (1995).

The yolk colour is traditionally defined manually using the Roche yolk colour scale (Fig. 10.10) or automatically by means of a reflectance measurement (Fig. 10.11). The instrument measures the ratio of red, green and blue light reflected from the yolk when illuminated by a flashed white light and compares these values with known percentages of the 15 colours in the Roche scale.

This yolk colour as a quality aspect is subjective and related to the perception of the consumer for the yolk colour since the yolk colour has no influence on the nutritional value of the egg. In different consumer surveys performed during the last 10 years in a number of European countries (France, Germany, Italy, UK, Spain, Poland and Greece), when offered samples of eggs with different yolk colours (measuring 8, 10, 12 and 14 on the Roche Yolk Colour Scale), the majority of the people questioned in all countries expressed a preference for the egg yolks with the darkest colour hue (colour score 14) (Coutts *et al*., 2007). Any discoloration of the yolk is perceived as bad quality yolk. The colour may be uneven or patchy (= mottled yolks), or vary from the desired colour range.

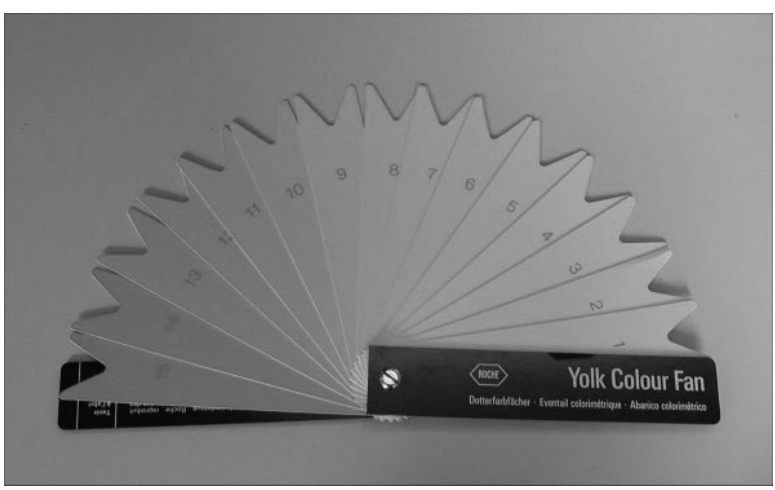

Fig. 10.10 Roche colour scale for yolk colour. Picture property of the University of Glasgow.

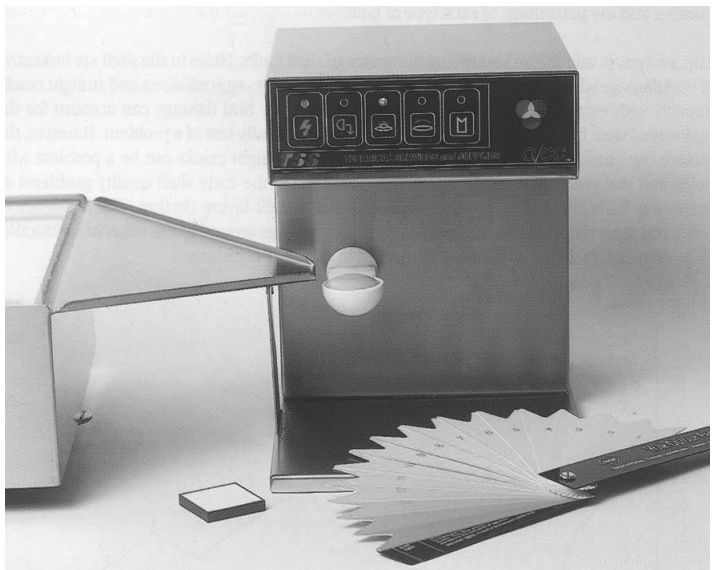

Fig. 10.11 Reflectometer to measure the yolk colour. After measuring the albumen height, the albumen and yolk can be separated and the yolk can be slid into the white cup. Picture property of the University of Glasgow.

Yolk index

The physical quality of the egg yolk can be quantified by the yolk index defined by the ratio of the yolk height to the yolk width. The yolk index depends on the quality of the vitelline membrane surrounding the yolk (see below). A fresh good quality egg typically shows a yolk index of around 0.45. As the egg ages, the yolk absorbs water from the egg white, increasing its size and weakening the vitelline membrane (Funk, 1948). In practice the yolk height and width are usually measured without separating yolk and albumen (Stadelman and Cotterill, 1995).

Vitelline membrane strength

The mechanical properties of the vitelline membrane can be considered a measure for the egg freshness. The strength of the vitelline membrane is an important parameter during egg breaking operations: it has to remain intact for separation of egg white and yolk. Early methods for assessing the vitelline membrane strength made use of a rapid capillary vacuum technique. With this method, a 2 mm capillary tube was placed on the surface of the vitelline membrane, and a vacuum was created in the capillary tube. The vitelline membrane strength was determined by the vacuum (Munro and Robertson, 1935) or the vacuum time (Fromm and Matrone, 1962) required to rupture the membrane. More modern methods measure the rupture energy and the maximum force, obtained by driving a probe of 2 mm diameter into

the highest point of the yolk, by means of a compression (texture) device (Kirunda and McKee, 2000; Berardinelli *et al.*, 2008).

10.3.3 Detection of inclusions by VIS/NIR spectroscopy

Undesired inclusions in eggs, such as blood spots and meat spots, can be present in eggs. While blood spots are the result of haemorrhage of small blood vessels in the ovary or oviduct, meat spots are degenerated blood spots, loose oviducal surface epithelial cells which come off due to, for example, stress (Jacob *et al.*, 2000). These inclusions were classically detected by candling the eggs in a candling booth or with a candling lamp. Development of automatic blood and meat spot detection started in 1953 with Brant *et al.* (1953) who were the first to use the spectrum of the transmitted light through an egg for detecting blood and meat spots in the albumen. Haemoglobin is the detected pigment in blood and has three absorption bands (at 415, 539 and 577 nm). Protoporphyrin IX, the pigment responsible for the brown colour of the eggshell (see page 212), also has three absorption bands (at 539, 589 and 643 nm) (Fig. 10.12).

Below 500 nm, the eggshell itself absorbs most of the light, thus only the 577 nm band is useful for blood detection (Brant *et al.*, 1953). Even at this wavelength there may be some influence of protoporphyrin because the absorption band at 589 nm is very close to 577 nm (Gielen *et al.*, 1979). Similar to the transmission colour value (see page 212), the absolute value of the transmitted light at 577 nm cannot be used for detecting haemoglobin, yet the ratio with an independent wavelength is necessary. Depending on the author, a wavelength between 585 and 610 nm (Gielen *et al.*, 1979) is chosen as a reference because transmission of light at this wavelength is

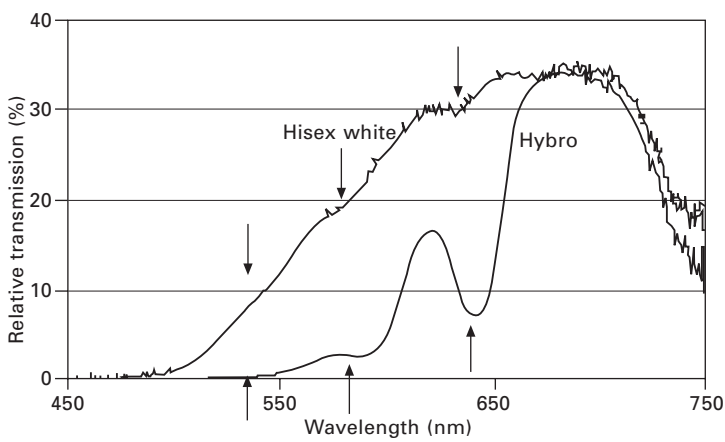

Fig. 10.12 Examples of transmission spectra for a white Hisex™ white egg and a brown Hybro™ egg. Arrows indicate reductions in % transmission due to protoporphyrin IX in the eggshell. Source: Bamelis *et al.* (2002).

influenced by neither the shell pigment protoporphyrin, nor by haemoglobin. The ratio between the two transmission values is called the 'blood value' and is used as a classification criterion in most commercial graders. Research at the Katholieke Universiteit Leuven (Belgium) (unpublished results) pointed out that small amounts of blood in the albumen can be detected only when part of it is diffused in the albumen. Very small blood spots are often not accompanied by dispersed blood and, hence, are very hard to detect. On the other hand, a small amount of dispersed blood without the presence of a blood spot cannot be seen with the human eye, but is recognized by the detecting mechanism and these eggs will be rejected. This last issue is of great importance for consumers who should not eat blood for religious reasons.

The detection success of blood spots in eggs is highly dependent on shell colour: on white eggs (Fig. 10.13) high detection accuracy can be achieved, this is not the case for brown coloured shells. Indeed, one brown pigment of the eggshell, protoporphyrin, has optical properties that are closely related to haemoglobin. It has an adsorption peak at 589 nm, very close to the absorption peak of haemoglobin (577 nm) and this makes the detection of blood in brown shelled eggs particularly difficult. Even the use of a reference wavelength cannot solve this problem (Gielen *et al.*, 1979). Some detectors can adapt the selection threshold based on a colour measurement of the eggshell, but the detection still remains difficult and needs further research (De Ketelaere *et al.*, 2004).

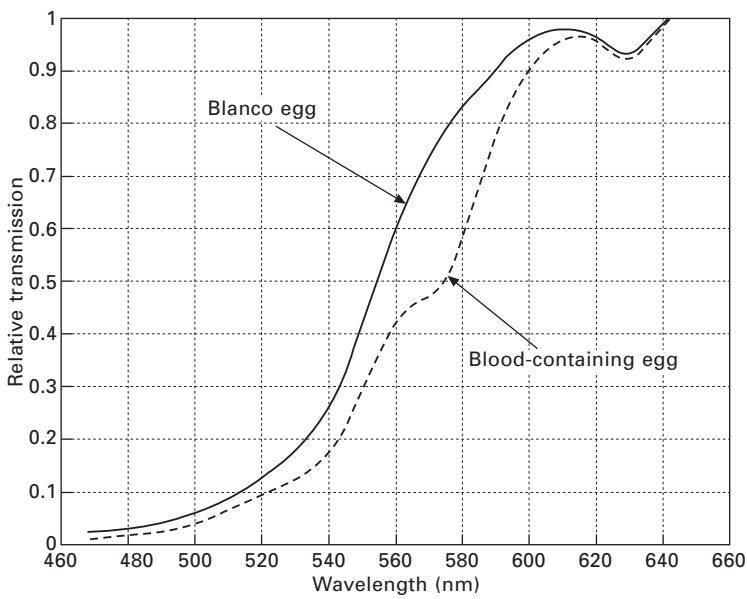

Fig. 10.13 Graph demonstrating the difference in relative transmission between a white egg without blood and a white egg with blood. Picture property of Katholieke Universiteit Leuven.

10.4 Automated industrial egg sorting and grading

The major companies that cover more or less the complete world market in egg sorting, grading and packing machines are Moba (Barneveld, The Netherlands), Diamond Automations Inc. (Michigan, USA), Staalkat International BV (Aalten, The Netherlands) and Nabel Co. Ltd (Kyoto, Japan). The techniques currently applied by these companies in their online machinery are described and discussed in following sections. Most of these technologies have been around for some time and it is only recently that some of the newer technologies are being considered for application in an industrial scale. Figure 10.14 provides a schematic overview of the possible steps in the egg grading process.

10.4.1 Crack detection

It is proven that under commercial circumstances fast, non-destructive mechanical sensors can be used for eggshell crack detection. In two commercial applications a small impactor excites the eggshell. A combination of the amplitude of the rebounds and/or the number of rebounds of the impactor is used as an indication for the local mechanical eggshell integrity. A locally intact eggshell surface allows the impactor to perform several elastic rebounds at high amplitude. In the neighbourhood of a crack, the elasticity of the adjacent shell area is seriously impaired and hence the rebound will be heavily damped. By repeating this action on different places around the eggshell surface, a spatial map of the mechanical state of the eggshell can be built. The role of the impactor can be performed either by a small hammer (Fig. 10.15), a bearing ball built into an electromagnetic probe (Moayeri, 1996 – Fig. 10.16) or by the egg itself. In the latter case the egg rolls over a series of small metal objects under which a piezo-sensor is mounted (Bliss, 1973).

This measuring principle reveals only local shell quality information and the crack detectors have to test several locations for each egg (16 to 32 points per egg) in order to obtain satisfactory results. The crack detection rate ranges from 70% to 85% while the percentage falsely rejected intact eggs is between 0.3% and 1% according to the data offered by the manufacturers of the eggshell crack detectors. These values are obtained under practical, industrial conditions on a high volume of eggs.

10.4.2 Dirt detection

Tests have shown that human candling is not a very effective way of identifying dirty eggs when eggs are not washed. This is especially true for brown eggs which show a large variability in shell colour, making the contrasts created by dirt spots hardly visible. Egg handling companies have therefore developed and invested in new technologies to automate the visual inspection of eggs.

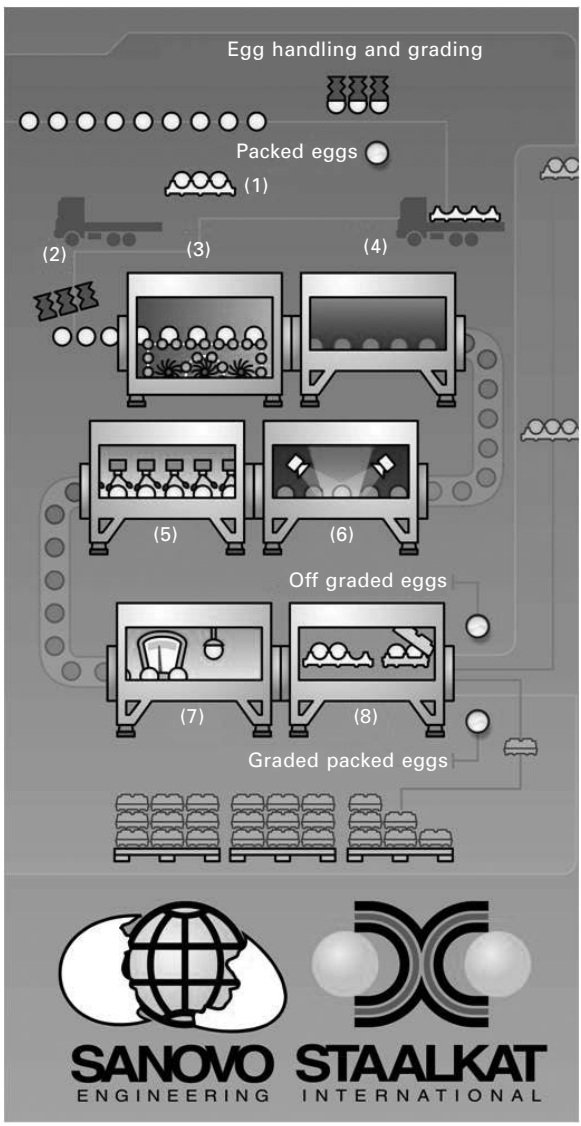

Fig. 10.14 Schematic representation of the egg grading process. (1) Transport of eggs from the farm to the egg processing plant. The eggs are packed in cardboard trays of 30 eggs. (2) Transfer of the eggs from the cardboard trays into the processing line. (3) Washing of the eggs is currently prohibited in Europe, but may be allowed in the near future. (4) UV-disinfection to prevent cross-contamination. (5) Crack detection. (6) Dirt detection and blood spot detection. (7) Egg weighing. (8) Packing the eggs according to egg weight. Picture property of Sanovo Staalkat Group.

For dirt detection, typically a series of digital still cameras are used (Goodrum and Elster, 1992). By using a uniform illumination and an appropriate digital image processing system these systems are capable of not only detecting dirt

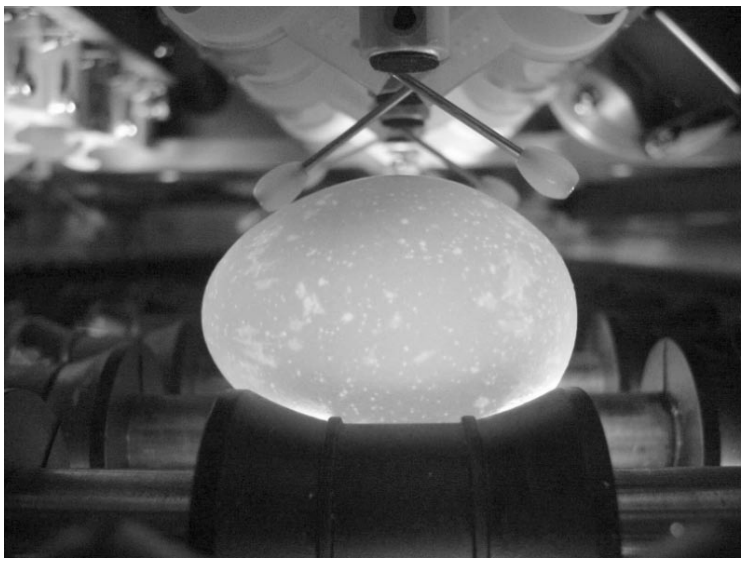

Fig. 10.15 Crack detection method used by Staalkat International BV and Nabel Co. Ltd. Picture property of Sanovo Staalkat Group.

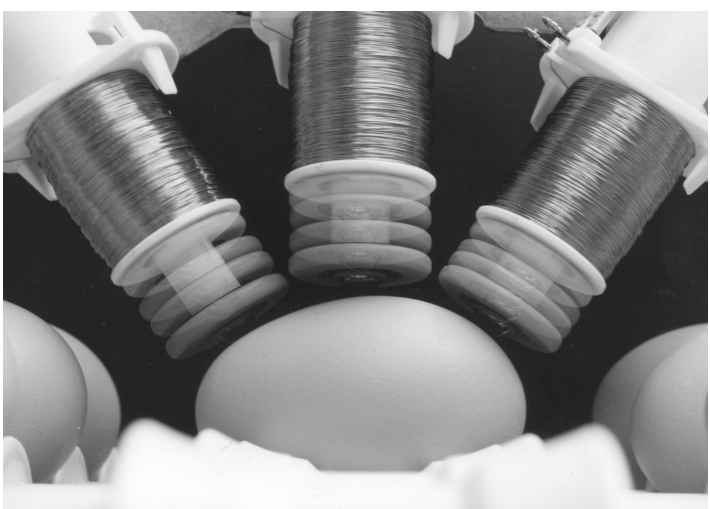

Fig. 10.16 Crack detection method of Moayeri (1996). Picture property of Moba BV.

on eggs, but also discriminating between different sources of dirt (faeces, yolk, blood etc.) and the severity. MOBA (Fig. 10.17), Diamond and Staalkat all offer these systems for on-line application.

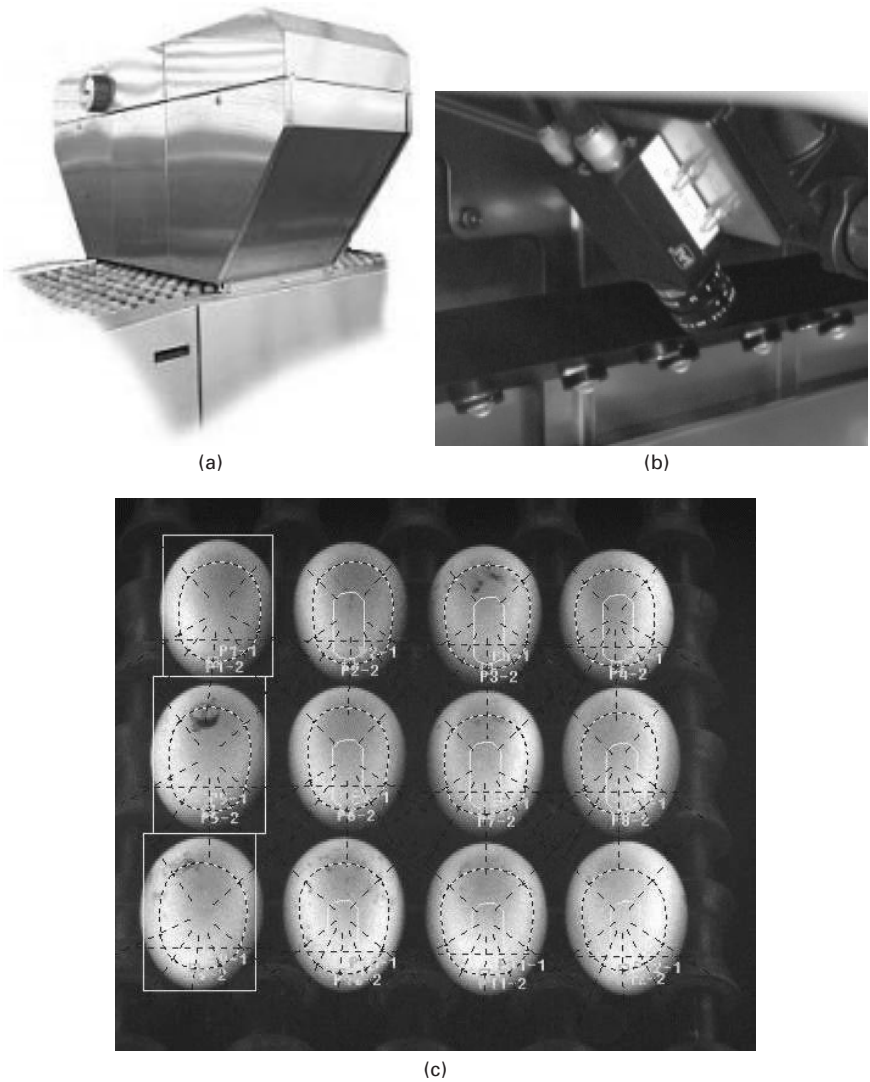

Fig. 10.17 Images of the dirt detection system of Moba BV. (a) The egg inspector system on the sorting line. (b) All eggs are illuminated by single light-emitting diode (LED) illumination and multiple cameras take images of all eggs. (c) A software algorithm detects the eggs and defines whether there is dirt on the eggs. Pictures property of Moba BV.

10.4.3 Detection of internal anomalies

Commercial egg graders offer the opportunity to detect anomalies inside the egg (Fig. 10.18). To detect the presence of blood spots inside the egg, the blood value is calculated according to the method described by Gielen *et al.* (1979) (see Section 10.3.3), using the specific wavelength for haemoglobin

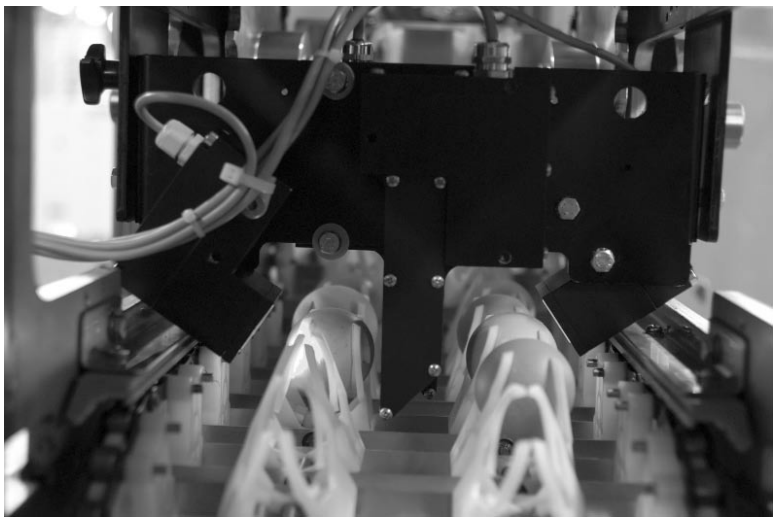

Fig. 10.18 Blood detection in a Staalkat International BV grader. Picture property of SanovoStaalkat Group.

(577 nm) and a reference wavelength (610 nm). Nevertheless, detection efficiency rates in brown eggs suffer from the issue of shell colour mentioned in Section 10.3.3. Grader builders do acknowledge this issue, but still use it due to the lack of alternatives.

10.4.4 Shell colour

As previously mentioned, the reflected light at certain wavelengths when illuminating eggs can be used for measuring shell colour in on-line systems. Eggshell colour is then measured by the reflected light at three specific wavelength bands, being the characteristic wavelengths of red, green and blue light. These three values are combined to calculate a specific colour value depending on the type of the application, which provides information about the colour of the eggshell (Wei and Bitgood, 1989). Also the ratio of the amount of reflected light at a specific wavelength (influenced by protoporphyrin for instance) relative to a reference wavelength not influenced by shell colour can be used. This shell colour measurement is used to discriminate between white and brown eggs (to avoid mixing) and to scale the different kinds of brownness in brown eggs (Fig. 10.1).

10.5 Conclusions and future trends

As a result of consumers' high demands towards food quality and safety, there is a need to evaluate the quality of every single egg. This chapter has

reviewed the available investigated techniques for the assessment of egg quality, with a focus on recent advances in the field. The nature of these techniques is clearly evolving towards speed, automation and reliability to ensure high accuracy and objectivity.

Many proposed techniques and methods have the potential to comply with these demands. Although the results of most papers can be described as promising, rather few of the techniques (except indirect acoustic response for crack detection, transmission spectroscopy for blood detection, computer vision for dirt and leaker detection) are integrated in modern graders.

An important reason that many proposed techniques are not picked up by industry is that many proposed techniques have only been demonstrated on a small (lab) scale. A second reason is that the raw data acquired by many of the proposed techniques require complex data analysis methods. As a result, many researchers start from a certain technique (e.g. acoustic response) and then modify the concept by applying another advanced statistical (modelling) technique (partial least squares, neural network, support vector machine, etc.) for predicting a certain egg quality trait. Usually such complex methods require large numbers of eggs for validation. Furthermore, the market of constructors of industrial egg graders is dominated by a few players which cannot follow every lead on novel technologies presented by research.

Nevertheless, ongoing research is needed for development of novel techniques for egg quality to fill the gaps in current industrial egg quality assessment. Important quality aspects which are not yet covered are the eggshell strength, egg freshness (albumen quality) and the detection of bacterial contamination. Moreover, quality information from each egg is not only relevant to egg grading for enhanced safety and quality assurance towards the consumers, it can also be used as feedback to producers, providing them with valuable information on the quality of the output of the egg production process and hence on the health status of the layer flocks.

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11

Poultry breeding for egg quality: traditional and modern genetic approaches

I. Dunn, University of Edinburgh, UK

Abstract: Egg quality remains one of the key aims for breeders of egg laying poultry. Its deterioration over the life of the hen is probably the major limitation to increases in overall efficiency of the whole industry. Traditional methods of selection combined with new methods of measurement of egg properties, both external and internal, that may lead to real improvements in quality and improvements in the keeping qualities of eggs are discussed. This includes progress on the cuticle, shell, egg white, and vitelline membrane and yolk. Recent data on heritabilities of selected traits and of the correlation between these traits and other measurements are summarised. This review also presents the results of molecular genetic and marker methodologies applied to egg quality and the promise of new techniques is summarised.

Key words: measurement of egg quality, genetic selection, marker-assisted selection, heritability, chicken.

11.1 Introduction

The quality of the egg and its content has focused the minds of farmers for millennia (Columella, 1955) and more recently those of scientists. Eggs have always been prized as a form of food which could be stored for moderate to long periods because of the unique package that has evolved to protect the embryo while it develops: so the shell and the nutritional and keeping qualities of the contents of the egg are important. In the modern world egg quality remains important and its improvement has a benefit in reducing cost, improving efficiency of production, improving the acceptability of the

product and reducing the risk to the consumer from potential pathogens. Selection for egg quality has therefore been an important component of the breeding strategy of companies marketing egg laying-type hens. Almost certainly this will continue to be the case and traditional and novel genetic techniques of selection will continue to be developed. The future offers the possibility of widening the parameters used in selection; however, it must be remembered that new traits can weaken selection pressure on the existing traits. However, if the new traits better measure an existing trait their inclusion may increase selection response for the existing trait because of their positive genetic correlation (Falconer, 1954). New methods of assessing quality must therefore have clear commercial, safety or welfare benefits if they are to be adopted.

11.2 Selection for egg quality

The majority of selection for egg quality has focused on the egg white and the shell's physical properties as well as yolk percentage. However, considerable work has been undertaken to look at other aspects of the egg even if those have not featured in any attempts at commercial selection. In this chapter I will look at both the methods and traits already used and those that might offer potential in the future, progressing from the outer cuticle of the egg into the ovum or yolk.

11.3 The structures of the egg, its formation and the potential for genetic improvement

11.3.1 Cuticle

The cuticle is a proteinaceous covering of the egg which acts to prevent water entering the pores of the egg shell and so prevents bacterial contamination of the egg contents (Board, 1980; Vadehra *et al*., 1970) including by *Salmonella enterica* serovar Enteritidis (Messens *et al*., 2007). Almost 40 years ago it was conjectured that genetics was one of the principal variables which influenced the amount of cuticle present on eggs (Ball *et al*., 1975). The measurement utilised staining with a dye Edicol Supra Pea Green H, but many histological dyes have been shown to effectively stain the cuticle, including malachite green, crystal violet, eosin, gentian violet, methyl violet, fuchsin, toluidine blue and Janus green (Sharp, 1932). However, none of the studies tried to quantify the staining to determine the contribution of genetics to this trait. This assessment is important if progress is to be made in selecting hens with good cuticle coverage. In a recent study using reflectance spectrometry the preliminary results suggest that the cuticle staining is a moderately heritable trait, with a heritability of 0.27 (Bain *et al*., 2009). There is still work to be

done determining to what extent the variation observed in pedigree commercial populations correlates with differences in the propensity for bacteria to enter the egg, but the approach looks promising as a selection tool.

11.3.2 Pigmentation

Pigmentation or colour (Blow *et al.*, 1950) is of concern in the brown egg market, not because it is a marker of quality but rather of preference. Heritabilities are relatively high for this trait (Table 11.1). However, in some cases it would appear that what is desired by consumers is a level of pigment which is not too light nor too dark (Forster *et al.*, 1996) but preferences are strongly shaped by regional preferences (Arthur and Sullivan, 2005). Breeders may need to apply selection aimed at reducing variation rather than implementing selection in one direction if the market demands it. However, for some markets such as free-range hens, very brown eggs are seen as a differentiating factor for marketing (Hutchings, 1994) and selection will be applied where eggs are principally destined for this market. Given the relatively defined nature of the pathways that lead to the deposition of pigment in hen's egg shell (Lang and Wells, 1987), it would seem likely that it will be possible to define the contribution of genetic variation in the haem enzymes to the trait. However since consumers in different countries prefer different pigment phenotypes, it may be that traditional rather than molecular methods of selection will be of most utility combined with the creation of specific hybrids to give the colour desired by the consumer in the different markets.

11.3.3 Shell

The shell is the most obvious structure related to the safety and integrity as well as the appearance of the egg and has been the focus of most attention for genetic selection. Indeed it is observed that when comparing across traditional and commercial breeds, eggshell traits have been maintained in comparison to other traits suggesting the breeding goals have been successful (Hocking *et al.*, 2003).

The structure and formation of the shell have been extensively reviewed in this volume and elsewhere (Nys *et al.*, 1999) and its form in biological terms is a relatively well-defined and structured system. However, the avian shell gland that produces the shell remains a complicated structure, and we are just beginning to understand the important genes expressed in it (Dunn *et al.*, 2009b; Jonchere *et al.*, 2010; Mann *et al.*, 2006). Selection for eggshell quality has followed the efforts by breeders to improve overall production of eggs. The single biggest problem for breeders is that all measurements of eggshell quality are proxies for the real thing, the 'real thing' being damage to the eggshell on its journey to the consumer or processor (Hamilton *et al.*, 1979) or penetration by pathogens (Messens *et al.*, 2005). Methods to quantify and estimate the genetic parameters for quality have included, in roughly

Table 11.1 A selection or heritability estimates made in the last 20 years for traits involved in egg quality and where available the genetic correlation with other production traits

Trait	Heritability estimate	Genetic correlations	Breed	Method if known	Reference
Shell colour					
Colour	0.49–0.53±0.06	Egg number, –0.29 to –0.03, Egg weight 0.00 to 0.30	Penedesenca Negra, Prat Lleonada, and Empordanesa Roja	Percentage light absorbance	Francesch *et al.* (1997)
Colour	0.46±0.09	Egg weight –0.12±0.16	Dwarf ISA-Vedette × female CAU brown egg	Reflectrometry	Zhang *et al.* (2005)
Colour	0.3–0.5		Rhode Island Red	Lab Minolta Chromameter	Forster *et al.* (1996)
Egg white quality					
Albumen height	0.51	Albumin weight 0.34±0.15 Egg weight 0.32±0.15 Haugh units 0.98±0.01	Dwarf ISA-Vedette × female CAU brown egg	Micrometer	Zhang *et al.* (2005)
Albumen height	0.29–0.31		White leghorn	Micrometer	Ledur *et al.* (2002)
Haugh units	0.41±0.10	Albumin weight 0.13±0.17 Egg weight 0.10±0.17	Dwarf ISA-Vedette × female CAU brown egg	Micrometer	Zhang *et al.* (2005)
Haugh units	0.30±0.28	Yolk percent 0.6 Egg weight –0.62	Taiwanese local breed	Micrometer	Chen *et al.* (1993)
Haugh units	0.12–0.19		White leghorn	Micrometer	Ledur *et al.* (2002)
Shell quality					
Breaking strength	0.24±0.08	Egg weight –0.19±0.20 Eggshell thickness 0.77±0.1 Albumin weight –0.41±0.19	Dwarf ISA-Vedette × female CAU brown egg	Veterinary and Livestock Instruments	Zhang *et al.* (2005)

Breaking strength	0.23–0.27		Male and female lines for a modern brown egg commercial hybrid	N/A	Besbes and Gibson (1999)
Breaking strength	0.37±0.23		Taiwanese local breed		Chen *et al.* (1993)
Breaking strength	0.18		Rhode Island Red	LR50 material testing machine	Dunn *et al.* (2005)
Shell thickness	0.34±0.09	Egg weight 0.32±0.16 Eggshell strength 0.77±0.10 Eggshell weight 0.59±0.12	Dwarf ISA-Vedette × female CAU brown egg		Zhang *et al.* (2005)
Shell thickness	0.29±0.1		Rhode Island Red	Electron microscopy	Dunn *et al.* (2009a)
Shell thickness	0.14±0.24	Egg weight –0.94 Breaking strength 0.87	Taiwanese local breed		Chen *et al.* (1993)
Specific gravity	0.11–0.30		White leghorn	Flotation	Ledur *et al.* (2002)
Dynamic stiffness	0.53	Egg weight –0.06±0.25 Breaking strength 0.49±0.22 Egg production –0.19 to –0.36	Rhode Island Red	Acoustic resonance	Dunn *et al.* (2005)
Proportion of cracked eggs	0.50–62		Rhode Island Red/White	Threshold model	Wolc *et al.* (2005)

chronological order, shell weight, specific gravity which estimates shell weight, quasi-static compression such as deformation or the most currently used measurement, breaking strength (Hunton, 1982; Wells, 1967). Breaking strength tests the shell to destruction and estimates the load required to reach that point. This measurement along with the other measurements of eggshell traits have reasonable heritabilities at around 0.2–0.3 (Table 11.1). More recently acoustic resonance has been used to estimate the dynamic stiffness of the egg shell, because of its non-destructive nature it was possible to correlate the dynamic stiffness measurement with the likelihood of an egg being damaged in a packing plant. This showed clearly that as the dynamic stiffness decreases, the risk of the egg being damaged increases (Bain *et al.*, 2006). Heritability of dynamic stiffness was high (Table 11.1), allowing genetic progress to be made rapidly if desired (Dunn *et al.*, 2005).

Very precise measurement of shell thickness has indicated that the heritability of the total thickness which comprises predominately the palisade region is moderate (Dunn *et al.*, 2009a) in line with other measurements (Table 11.1). In an effort to understand the basic factors which contribute to the measurement of quality of eggshells it would be desirable to measure the basic components that determine the eggshell's strength and structure. Encouragingly one of the largest heritabilities observed for egg shell traits is $CaCO_3$ crystal size (Bain *et al.*, 2010). Given that the trait appears to be correlated with other quality traits this may mean that the measurement will have value in the future. Alternatively, if the promise of using a threshold model to estimate heritability for the number of cracked eggs ($h^2 = 0.50–0.62$) (Wolc *et al.*, 2005) (Table 11.1) was to be demonstrated in commercial populations then it might be possible to implement direct selection for the 'real thing' rather than relying on indirect measures.

Because many eggshell traits are relatively easy to measure in large numbers of eggs without killing the birds that laid them, a number of studies have been performed seeking the genetic loci that control the traits, so-called quantitative trait loci (QTL). QTL with genome-wide significance that can explain variation in shell quality can be found in Table 11.2. Many other loci with lower or suggestive significance can be found in these and other publications, and of course among these will be QTL that do genuinely contribute to economically important egg quality traits. Perhaps not surprisingly, given the different crosses or pure lines used in the studies in Table 11.2, it can be seen that there is little or no agreement between studies and in some cases, although the traits may appear similar, there will be between-lab differences in how they are measured. Since there is limited progress towards defining the precise regions underlying these QTL, with the exception of the medium density single nucleotide polymorphism (SNP) scans, it seems likely that results for some of these QTL will start to appear as genotyping costs reduce.

There has been a number of candidate gene studies for egg shell characteristics using both genes from QTL regions and from those in genes

Table 11.2 QTL for traits involved in egg quality with chromosome wide significance

Trait	Cross	Chr.	Position or SNP ID	Reference
Egg white				
Albumin height 34 weeks	Cornish × White Leghorn	1	ADL0314-UMA1.126	Hansen *et al.* (2005)
Albumin height 34 weeks	Cornish × White Leghorn	2	ADL0157-ADL0236	Hansen *et al.* (2005)
Haugh units at 40 weeks	Rhode Island Red × White leghorn	1	ADL188-MCW43	Tuiskula-Haavisto *et al.* (2004)
Haugh units at 40 weeks	Rhode Island Red × White leghorn	2	MCW247-ADL217	Tuiskula-Haavisto *et al.* (2002)
Haugh units at 60 weeks	Rhode Island Red × White leghorn	2	MCW247-ADL218	Tuiskula-Haavisto *et al.* (2002)
Early and late albumin height	White Leghorn and Brown egg layer	5	rs14350738	Abasht *et al.* (2009)
Early albumin height	White Leghorn and Brown egg layer	18	rs15817690-rs15817992	Abasht *et al.* (2009)
Late albumin height	White Leghorn and Brown egg layer	19	rs14116385	Abasht *et al.* (2009)
Late albumin height	White Leghorn and Brown egg layer	23	rs14288801	Abasht *et al.* (2009)
Early albumin height	White Leghorn and Brown egg layer	Z	rs13677045	Abasht *et al.* (2009)
Colour				
Egg colour 35 and 55 weeks	White Leghorn × broiler	2	BCL2-LEI0147	Schreiweis *et al.* (2006)
Shell				
Shell thickness at 33 weeks		2	MCW0051	Wardecka *et al.* (2002)
Shell thickness at 33 weeks	Rhode Island Red × Green-legged Partridge	4	MCW0170	Wardecka *et al.* (2002)
Mean shell thickness	White Leghorn × red junglefowl	5	MCW081-ROS330	Wright *et al.* (2006)
Late shell quality	White Leghorn and Brown egg layer	7	rs16589956	Abasht (2009)
Early shell quality	White Leghorn and Brown egg layer	7	rs15864122-rs14617583-rs14618292	Abasht *et al.* (2009)
Late shell quality	White Leghorn and Brown egg layer	12	rs15656643	Abasht *et al.* (2009)
Shell strength at 40 weeks	Rhode Island Red × White leghorn	Z	MCW154-MCW128	Tuiskula-haavisto *et al.* (2002)

derived from knowledge of the eggshell organic matrix or its formation (Nys *et al*., 1999). Selecting between candidate genes from a QTL detected in a Rhode Island Red (RIR) and Green-legged Partridge (a Polish national breed) cross (Wardecka *et al*., 2003) on chromosome 4 identified an expressed sequence tag (EST) (CR523443) of unknown function that was demonstrated to be differentially expressed between hens laying thick and thin egg shells (Sazanov *et al*., 2007). Chromosome 4 also harbours the eggshell matrix genes osteopontin and ovocleidin 116, which are in close proximity to each other at around 47 Mb; this is some distance from the area examined by Sazanov *et al*. (2007), which was around 16 Mb. Particular attention has focused on chromosome 9 which contains the genes for ovocalyxin-32 and ovotransferrin, known components of the eggshell organic matrix. Reports of QTL on this chromosome for eggshell quality seem to be principally derived from changes in egg weight (Takahashi *et al*., 2009) but these QTL are coincident with the candidate gene association observed for ovocalyxin 32 (Dunn *et al*., 2009a). The QTL in this study which seem to be related to shell quality was for shell thickness and is separate from those related to egg weight, but it was only at chromosome-wide significance and was at the start of the chromosome (Takahashi *et al*., 2009), perhaps more in the region of ovotransferrin. The use of candidate genes found in the eggshell matrix in commercial lines has suggested potentially useful association between alleles of ovocleidin 116 and aspects of shell thickness as well as the association mentioned with ovocalyxin 32 (Dunn *et al*., 2009a). The polymorphism in ovocalyxin 32 was, however, not observed in an F2 cross of White Leghorn and RIR strains (Uemoto *et al*., 2009). There was also an effect of oestrogen receptor (Dunn *et al*., 2009a) on the novel measurement of quality, dynamic stiffness, although this measurement is easy to make and has high heritability (Dunn *et al*., 2005) as already mentioned. Efforts to derive candidates on the Z chromosome have identified a number of candidates but none has been tested (Ankra-Badu and Aggrey, 2005).

11.3.4 Egg white

In terms of egg usage the egg white has perhaps one of the largest impacts. Quality has traditionally been measured by albumen height of the broken out egg or Haugh units which include the effect of egg weight to equalise the measurement between eggs of different size (Haugh, 1937). However, the Haugh unit's ability to do that, compared with simple albumin height, has been questioned, although for selection within a genetic line it may be appropriate (Silversides *et al*., 1993). These measurements were introduced as a way of determining freshness rather than the properties desired by the user or catering processor, for example in custard production (Ericson, 1943). However, the phenotypic correlation between albumin height and protein content is not large, at around 0.32, while protein level is well correlated with gelling properties (Hammershoj *et al*., 2001). If selection

to control the internal quality was not maintained it would seem likely that selection for increased egg production would increase the content of water in the egg. This would be the metabolically cheapest way for the hen to increase production and there is some data to corroborate that (Silversides and Budgell, 2004), although a study in leghorns did not (Tharrington *et al.*, 1999). Estimates of heritability for either the albumen height or Haugh unit trait are relatively high, with the lowest estimate at 0.11 (Table 11.1). Possibly as a consequence little effort has been made to find new ways of assessing this trait and, although some non-destructive approaches have been attempted, the results were not an improvement on existing destructive measurements (Kemps *et al.*, 2007).

In view of the poor relationship of Haugh units or albumin height with protein content it would appear that selection for Haugh units or albumin height traits may be more to maintain the aesthetic appearance of a fresh egg than it is for improving its nutritional or processing quality. Therefore continued pursuit of methods that estimate protein content non-destructively or quickly may well have value to ultimately improve those qualities desired by industry. Similarly meat spots are an aesthetic problem for which there are some indication of between breed differences (Hall, 1939) possibly due to the relative ease of detection meat and blood spots in white shelled eggs by candling and past successful selection. This seems to be supported by the observation that the incidence of blood spots in white leghorns was increased from less than 1% to 24% in nine generations by positive selection (Lerner *et al.*, 1951). This suggests a genetic solution should work but heritability estimates are low in hens laying brown shelled eggs (Noda *et al.*, 2007), probably due to poor measurement and analysis problems. However, progress was made in reducing the incidence in an experiment using the size of meat spots as a selection criterion rather than incidence (Noda *et al.*, 2007).

A number of workers have identified QTL for egg white properties using a variety of crosses (Table 11.2). Amongst the loci with genome-wide significance there has been little evidence of any concurrence between the studies. Candidate gene approaches, although appearing attractive given the limited number of major proteins comprising egg white (Mann, 2007) do not appear to have been reported. Attempts to target alleles of vimentin to explain a QTL observed for Haugh units on chromosome 2 were not successful (Honkatukia *et al.*, 2005b). One exception is the chicken FMO3 gene, although not a gene for the mechanical quality of albumin, it is responsible for the fishy taint in eggs when hens carrying the mutant allele are fed rapeseed meal (Honkatukia *et al.*, 2005a). This has allowed the eradication of the mutant allele from commercial lines of hens.

Of course the egg white has not evolved to make cakes and custard but to protect the embryo. As such it is full of peptides and proteins with antimicrobial properties. It is also obvious that there is considerable variation in the rate of bacterial growth in egg white (Baron *et al.*, 1997) and indeed heritability estimates suggest a low to medium proportion of variation

in the antimicrobial properties of these proteins is genetic (Sellier *et al.*, 2007). Attempts to define the role of individual proteins in contributing to this variation have had limited success, only lysozyme showed genetic association with antimicrobial activity (Ian Dunn, unpublished observation). In the future it may be necessary to find a simple reproducible measurement of antimicrobial activity to make progress with this trait.

11.3.5 Yolk

The yolk is probably the component which has the least attention in terms of the genetics of quality. The majority of attention on quality has been devoted to the aesthetic trait of colour which is primarily determined by dietary carotenoids (Nys, 2000). However, it seems likely that there is some genetic component at least in the transport of pigments or their metabolism although it is difficult to find evidence for this. One area which has not received attention, but which is of importance to the processors in particular, is the strength of the vitelline membrane. This determines the likelihood that the yolk will leak during separation. In the processing industry where separation of egg components is critical this can be an issue. Although methods have been used to measure its strength using hydrostatic pressure since at least 1936 and despite postulation that genetics would influence the trait (Moran, 1936), there is still little evidence as to what extent this may be genetically determined. Preliminary studies suggested there were strain differences (Curtis *et al.*, 2005; Jones and Anderson, 2007) but this may have been confounded by other factors. Proteomic studies suggest the number of major proteins is relatively small (Mann, 2008) and it has been possible to determine that one of the major proteins degradation is associated with age-related decay (Schäfer *et al.*, 1998) and may be a good candidate for any genetic differences. A number of methods of applying force to determine rupture strength have been applied (Berardinelli *et al.*, 2008; Fromm and Matrone, 1962; Kirunda and McKee, 2000) that might be suitable for appraisal for selection to make progress for this trait, but as yet this has not been reported.

Because of scares surrounding dietary cholesterol, eggs were viewed with some mistrust as a source of food. Fortunately this has been reversed by more balanced appraisal (Herron and Fernandez, 2004); however, in the intervening period efforts were made to investigate the role of genetics in reducing levels of cholesterol as summarised by Elkin (2006). Despite heritability of around 0.2 (Cunningham *et al.*, 1974; Washburn and Nix, 1974), in practice little progress was made and selection to reduce yolk proportion was also unsuccessful while selection in the opposite direction was successful. Although commercial leghorns had a lower percentage of egg yolk than their unselected controls this seems to reflect increased white production (Tharrington *et al.*, 1999). This may be due to the importance of the yolk to the chick which means that changes to yolk composition results in increased embryo mortality as has been observed (Cunningham *et al.*, 1974).

11.4 New genetic selection methodologies and their potential impact

There are two main areas where selection technologies may increase quality and efficiency of production above that of current methodology. On one hand it seems that scientists should continue to research measurements that are better predictors of the traits which are commercially important. These I believe will be aimed at the trait that has been a major focus throughout and that is the resistance of the shell to damage. There may also be a role for methods that prevent bacteria entering or growing in the egg to prolong shelf-life. I believe there is also a requirement to reduce wastage in the food industry by improving measurement of the traits that determine the nutritional or processing potential of eggs such as protein content of egg white and vitelline membrane strength. All of these may be given some greater meaning because we appear to be on the brink of a revolution in the use of genetic markers in selection. If the promise is delivered it will allow measurements that are hard to carry out to be included in selection programmes as measurements only need to be made on one generation of a commercial pedigree flock.

The method in question is genome-wide selection, first proposed by Meuwissen *et al*. (2001). In contrast to the studies discussed previously that use F2 populations and relatively small numbers of markers to detect loci controlling traits using linkage or candidate association studies; this approach utilises the availability of large numbers of SNP genotypes available from chip technology and takes more of a 'black box' approach to selection. Prior knowledge of chromosomal regions with association with important traits could allow inclusion of these regions at higher density in the genotyping chips. In other words, rather than locating regions or causative SNPs that explain relatively large amounts of variation for a trait, it is accepted that many loci all have a small effect. It is all these small contributions to variation that the method is trying to capture with very dense genotyping where the markers are each sufficiently close to the causative variation to be inherited with it. However, the level of genotyping density currently available in the chicken (Groenen *et al*., 2009; Muir *et al*., 2008) may be inadequate to achieve in practice the level of accuracy that is possible theoretically in predicting a trait. However, a number of consortiums (http://www.quantomics.eu, http://www.reeis.usda.gov/web/crisprojectpages/215006.html) are working to increase the available density on assay platforms to around 50 000 SNP markers. Amongst chickens, White Leghorns have much larger areas of the genome which are inherited as a block, so that the linkage disequilibrium between marker and causative variation is likely to be higher and so less dense SNP genotyping may be required. On the other hand broilers may require a higher density genotyping as they have lower linkage disequilibrium between similarly spaced markers (Abasht *et al*., 2009; Aerts *et al*., 2007; Muir *et al*., 2008).

Genome-wide selection tries to utilise the predictive value of SNP marker genotypes at large number of positions to assign a genome-wide breeding value to an individual animal. The information to do this is gained from so-called training sets where the phenotype and genotype are measured on a large number of animals. Of course just as with any breeding value the accuracy is correlated positively with the heritability of the trait (Hassen *et al*., 2009; Luan *et al*., 2009) as well as density of the markers (Solberg *et al*., 2008). The models of Solberg *et al*. (2008) suggest for the chicken an informative marker approximately every 0.20 Mb would be required to achieve accuracy of selection around 80% for a relatively highly heritable trait. In chickens, assuming that the SNPs on the new generation of chips have a not too high redundancy rate, it should be possible to reach that target and it is expected that results of trying this approach will begin to appear soon, with the expectation that it will have great advantage for sex limited traits, such as egg quality, which are measurable only from females and in reducing generation intervals by early selection of males to speed up genetic progress and for expensive to measure traits (Albers, 2010; Preisinger, 2010). If these results can deliver even close to what they promise they will provide impetus to both the development of denser and hopefully cheaper SNP genotyping technologies for chicken and the development of tools to better measure the traits that really matter.

11.5 Conflicts in selection goals

Genomic selection or any other form of selection will not change the negative genetic correlations between traits that are concerned with producing more eggs and those ensuring the quality of the eggs produced (Table 11.1). Breeders will continue to select for traits that address both of these objectives. Hopefully some of the advances that are discussed in this chapter will help them in this pursuit and the challenges will be taken up by scientists to find the best measurements to maintain and improve egg quality.

11.6 References

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12

Hen nutrition for sustained egg quality

I. Bouvarel, ITAVI, France and Y. Nys and P. Lescoat, Institut National de la Recherches Agronomique, France

Abstract: Hen nutrition is crucial for optimising egg quality. Hen's weight at point of lay, energy and protein densities of the diets, amino acid and fatty acids contents but also the mode of feed delivery throughout the day affect egg weight and also, but to a lesser extent, the proportion of yolk and albumen. Egg profile in fatty acid and in trace elements (iodine, selenium, manganese) and fat-soluble vitamins (E, D, A and K) reflects the dietary supply of these nutrients. The yolk colour also depends on the dietary carotenoids even if effectiveness of colouring varies according to the source of the xanthophyll. Finally, this chapter describes the effects of dietary calcium and other minerals and vitamins on shell strength.

Key words: hen, eggs, yolk, eggshell, energy, protein, mineral, calcium, vitamins, carotenoids, yolk colour.

12.1 Introduction

A laying hen produces more than 300 eggs a year. The very high feed efficiency of conversion of hen diets, which are mainly composed of raw vegetable material, into animal proteins of high biological value is a real metabolic challenge. The diet of the laying hen is crucial in the optimisation of the excellent genetic potential of modern chickens for both production performance and egg quality. Appropriate management of diet is thus a true challenge for the industry.

The egg contains all the nutrients required for the development of an embryo in an external environment (Chapter 11, Volume 2). There are

variations in the composition of these constituents, mainly related to the bird itself (Chapter 13) and its genetic origin (Chapter 11) and age, but also resulting from the diet and the system of production. Egg macro-constituents such as lipid, protein and mineral macro-element contents are stable. The main dietary influence when feeding pullets and laying hens is on the overall egg mass, i.e. the number and weight of eggs, with little effect on the main constituents of the egg except perhaps the proportions of albumen and yolk. However, some feed constituents do have a direct effect on their levels in the egg, e.g. fatty acids and nutrients such as vitamins, trace elements and yolk carotenoids. Finally, changes in the mode of diet distribution and in the composition of the ration within each 24 hour period offered to birds may also affect the production and quality of eggs, possibly related to the daily egg cycle and phase of egg formation. This chapter presents the different criteria associated with egg quality which are likely to be modified by the diet. These are egg weight and shell strength, albumen and yolk ratio, fatty acid content, mineral and vitamin content, and yolk colour.

12.2 Variations in egg weight

Egg weight depends mainly on bird intrinsic factors (genetic origin and age) and on diet during the laying period. Pullet feed contributes indirectly to this by influencing sexual maturity, live weight and body composition at onset of lay.

12.2.1 Influence of pullet diet

Pullet feeding programmes influence the growth profile and hence bird live weight gain and body composition at onset of lay. Keshavarz (1984) reported the effects of different protein levels at various bird ages (18% at 0–6 weeks of age, then 15/12%, 12/12% or 12/15% at 6–14, 14–20 weeks, respectively). Keshavarz and Jackson (1992) tested other sequences (20, 16 and 14%, or 16, 13.5 and 11.5% at 0–6, 6–12 and 12–18 weeks, respectively). For the latter, feeds were supplemented with and without methionine, lysine, tryptophan and isoleucin. Effects of four protein levels from hatching to 16 weeks of age (20, 17, 14 and 11%) were also tested by Summers and Leeson (1994). These authors showed that the different levels of proteins provided during the growth phase influenced live weight at onset of lay (+32 g/supplementary protein point) ($r^2 = 0.94$; $p < 0.05$) at 16 weeks. Compilation of these results emphasises the relationship between live weight of the hen at onset of lay and the mean weight of eggs laid during this initial period ($r^2 = 0.85$, $p < 0.01$) (Fig. 12.1) without any effect on the total egg output for the whole laying period. The increase in egg weight during the onset of the lay period is 0.7 g for each 100 g gain in pullet live weight. The effects on egg weight during the remaining laying period are less significant (Fig. 12.1). Pullet body

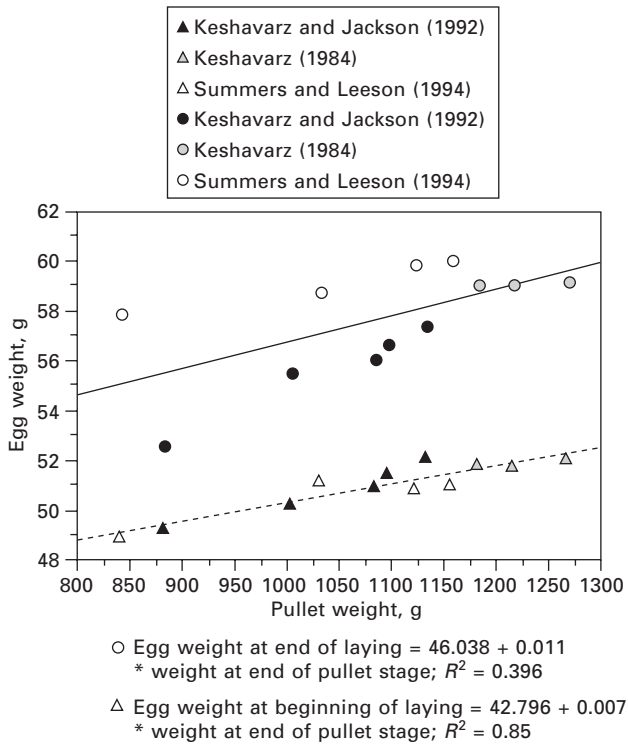

Fig. 12.1 Mean egg weight at beginning and end of laying according to live weight of chicken on reaching sexual maturity (triangles represent onset of laying; circles represents end of laying).

composition at onset of lay also influences the egg weight. Diets containing higher energy levels (+215 kcal/kg from 0 to 6 weeks and +357 kcal/kg from 6 to 18 weeks) do not affect live weight at 20 weeks but do increase the fattening score (+7%) and reduce mean egg weight from 20 to 64 weeks (55.0 vs 56.1 g) (Cheng *et al.*, 1991).

Moreover management of calcium supply between pullet and laying stages has a key role on the egg weight through its effects on food intake. Providing a feed with calcium in particulate form (free choice *ad libitum*) prior to the onset of lay, will improve future feed consumption and increase egg weight from 25 to 32 weeks (+1.6 g) compared with a feed low in Ca (0.89%) (Classen and Scott, 1982). A feed too low in calcium during the transition phase will result in excessive fattening due to overconsumption of feed (Roland, 1986). It is therefore common practice to introduce two to three weeks before the onset of lay an intermediate or pre-lay diet with approximately 2.5% calcium, possibly in a particulate form. This allows maturing hens to fit their increased calcium requirement for medullary bone formation.

12.2.2 Diets for laying hens

Effects of energy content in the feed

When there is decrease, e.g. introduction of sand, or enhancement of feed energy content, for example with fatty acids, dietary intake varies linearly in relation to the energy concentration of the feed (Peguri and Coon, 1991; Walker *et al.*, 1991; Joly and Bougon, 1997; Grobas *et al.*, 1999b; Harms *et al.*, 2000; Leeson *et al.*, 2001; Jalal *et al.*, 2006, 2007; Valkonen *et al.*, 2008; van Krimpen *et al.*, 2008, 2009) (Fig. 12.2).

$$\text{Variation in intake (\%)} = 1.452 - 0.685 \times \text{variation in energy (\%)},$$

$$R^2 = 0.875$$

According to this regression, the chicken adapts its intake to the feed energy content. However, the response slope is not proportional to a precise quantitative substitution. When there is a decrease in metabolisable energy due to introduction of a source of very low energy, e.g. sand, laying hens do not sufficiently increase their consumption to reach the same energy intake. Likewise, when there is an enrichment, hens do not reduce their intake proportionally to reach the same energy intake. Energy consumption is thus significantly higher with more concentrated feeds (on average 1.3%/100 kcal, $p < 0.01$) (Peguri and Coon, 1991; Walker *et al.*, 1991; Joly and Bougon, 1997; Grobas *et al.*, 1999b; Harms *et al.*, 2000; Leeson *et al.*, 2001; Jalal *et al.*, 2006, 2007; Valkonen *et al.*, 2008; van Krimpen *et al.*, 2008, 2009). Bulking up the feed with either sand or alternative raw materials does not affect the adaptative response of feed intake to the dietary energy level.

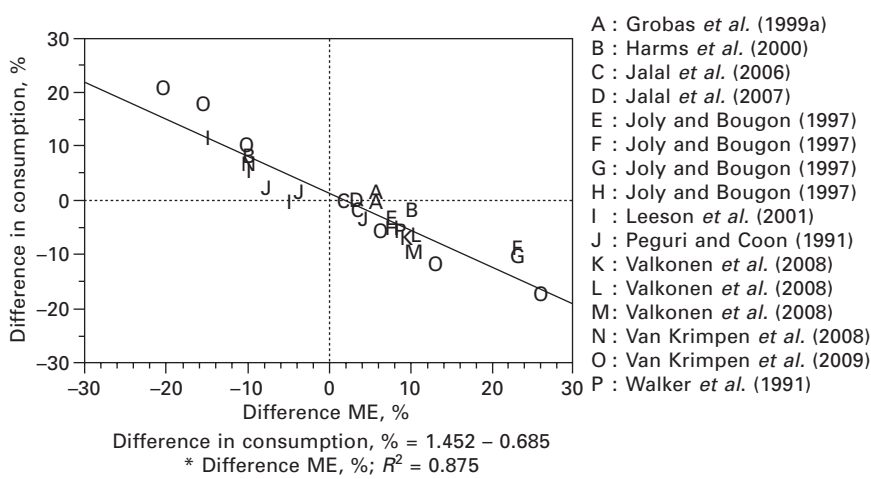

Fig. 12.2 Feed intake according to feed energy content (Difference metabolisable energy (ME) < 0 for dilution with sand or > 0 for addition of calorific raw materials including fats) (C, E, F, J and L represent onset of laying; A, B, D, G, H, I, K, N, O and P represent middle of laying; M represents end of laying).

Mean egg weight increases slightly with energy intake ($R^2 = 0.33$ (Peguri and Coon, 1991; Walker *et al.*, 1991; Joly and Bougon, 1997; Grobas *et al.*, 1999b; Harms *et al.*, 2000 ; Leeson *et al.*, 2001; Jalal *et al.*, 2006, 2007; Valkonen *et al.*, 2008; van Krimpen *et al.*, 2008) (Fig. 12.3). The mean variation is 0.96 for each additional intake of 10 kcal. Within these experiments recorded on hen with different genetic background (large change in egg production for the last 20 years), the same trend was observed (see linked points in Fig. 12.3), with a lower effect for the highest energy intakes.

Effects of protein and amino acid content in the diet

Mean egg weight is also related to the quantity of protein consumed ($R^2 = 0.59$) (Peguri and Coon, 1991; Walker *et al.*, 1991; Joly and Bougon, 1997; Grobas *et al.*, 1999b; Harms *et al.*, 2000; Leeson *et al.*, 2001; Jalal *et al.*, 2006, 2007; Valkonen *et al.*, 2008; van Krimpen *et al.*, 2008) (Fig. 12.4). Consumption of an extra 1 g of protein per day results in an average increase in egg weight of 1.4 g.

Methionine is the main limiting amino acid in the diet of laying hens. Several studies have evaluated the effects of methionine content on egg weight at peak production by adding DL-methionine (Schutte *et al.*, 1994; Bertram *et al.*, 1995a, 1995b; Dänner and Bessei, 2002; Narvaez-Solarte *et al.*, 2005), to feeds with sufficient protein content. Egg weight follows a curvilinear relationship with methionine content, with a plateau at 0.36–0.38% methionine in the feed (Fig. 12.5).

Calderon and Jensen (1990) reported similar results at two stages of laying (32–35 weeks and 59–63 weeks), with a dietary protein content of 16 and 19%, respectively. However, egg weight remained the same at lower

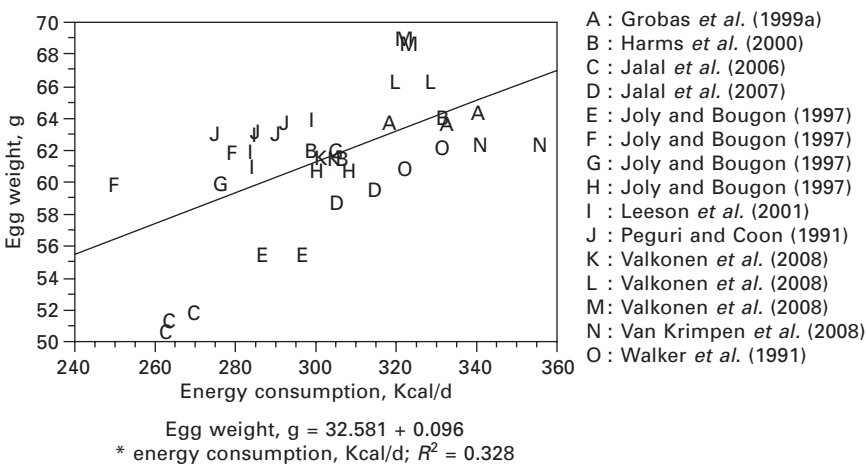

Fig. 12.3 Effects of energy consumption on egg weight (C, E, F, J and L represent onset of laying; A, B, D, G, H, I, K, N and P represent middle of laying; M represents end of laying).

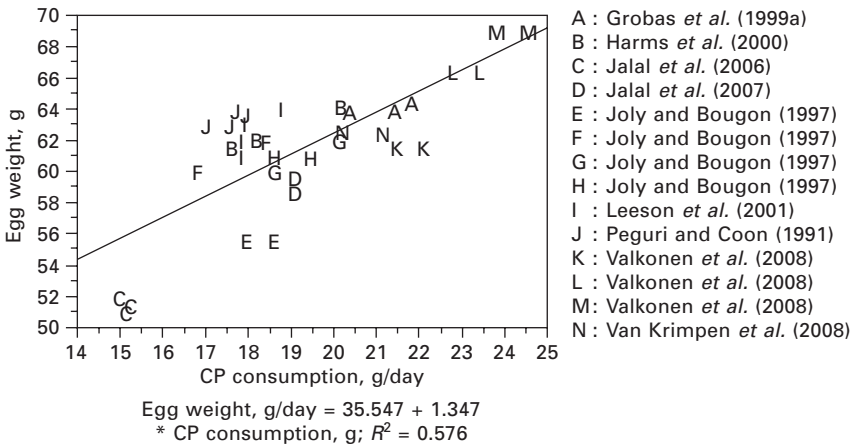

Fig. 12.4 Egg weight (g) according to protein consumption (g/d) (C, E, F, J and L represent onset of laying; A, B, D, H, I, K and N represent middle of laying; M represents end of laying).

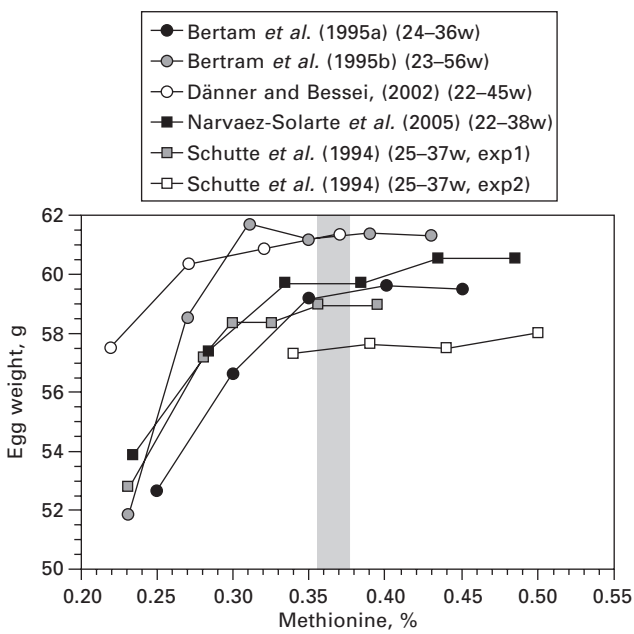

Fig. 12.5 Egg weight (g) according to total methionine content in the feed (%).

protein concentrations (13%) for lower methionine content (around 0.30%), indicating that other amino acids were limiting in these conditions.

Bregendahl *et al*. (2008) recently measured the responses of 28–34 week old Hy-Line W-36 chickens to a low protein feed (12.3%) with varying amino acid content (lysine, isoleucine, methionine, threonine, tryptophan

and valine). The amino acid that had the greatest effect on egg weight when it was limiting was methionine, followed by threonine, valine and finally lysine. Tryptophan had no effect.

Specific effects of fatty acids

Most dietary lipids are used for synthesis of the lipids of the yolk, through their actions both on vitellogenesis and the fatty acid profiles. Dietary fatty acids in turn influence egg weight. The best known effect is that of linoleic acid (Balnave and Weatherup, 1974) with a recommended level of linoleic acid of approximately 1% in the diet (Whitehead, 1981; Grobas *et al.*, 1999b). Nevertheless, the most meaningful response is found when the diet is enriched with both linoleic acid and oleic acids, which are found in olive oil (Whitehead, 1981) or soya oils (Dänicke *et al.*, 2000). These easily-to-absorb fatty acids increase the retention of the others (Whitehead, 1981). Effects of linoleic acid enrichment on egg weight are more pronounced in hens at the onset of laying (22–32 weeks) (Whitehead *et al.*, 1991) with no effect on older birds (94–106 weeks) (Yousefi *et al.*, 2006).

Effects of raw materials

Corn, wheat and soybean meal are the main raw materials used in feeds for laying chickens. The use of leguminous seeds can result in reduced egg weight. Incorporation of peas at levels of 10, 15, 20 and even 30% does not affect the total number of eggs but can slightly decrease egg weight (up to 3.1%). Such an effect is not associated with anti-trypsin factors which is present in winter varieties (Lacassagne, 1988a). The negative effects of faba beans on egg weight are linked to the presence of anti-nutritional factors such as vicine-convicine (Lacassagne, 1988a; Castanon *et al.*, 1990; Grosjean *et al.*, 2000; Lessire *et al.*, 2005). The reduction in egg weight is reported to be directly proportional to the level of faba beans above 7% inclusion in the diet (Lacassagne, 1988a). Inclusion of 15% faba beans significantly increased egg weight when the faba beans did not contain vicine and convicine but egg weight remained lower (–1.4%) compared with the control according to Grosjean *et al.* (2000). However, Dänner (2003) did not find differences in egg weight when a different variety low in vicine and convicine (Divine) was compared with a conventional one (Condor) at an inclusion of 30%.

Usually, the inclusion of rapeseed in feed reduces egg weight (by around 8–9% Lacassagne, 1988b) with a decrease in egg weight proportional to the inclusion level of rapeseed meal in the feed. There are more marked effects when the rapeseed contains high levels of glucosinolates (Lacassagne, 1988b). However, Ciurescu (2009) recently showed that 15% rapeseed meal can be included for a short period (40–47 weeks) without having a negative effect on laying rate or mean egg weight.

Eggs from brown egg laying strains have been reported to develop a fishy taste if rapeseed levels are higher than 5%, which is the maximum level recommended for brown eggs strains. This unusual feature has been related

to a polymorphism of the gene of the enzyme degrading trimethylamine (originating mainly from sinapine present in rapeseed) in the liver (Honkatukia *et al.*, 2005). It tends to disappear, however, through selection for this criterion using this association between the gene polymorph and undesirable egg taste.

Effects of feed presentation

Laying hens feed themselves in respond to their needs but also according to their preferences and ability to recognise particular components of the diet. Interactions occur between feed preferences and physiological regulation, thus affecting food intake and consequently egg production and egg weight.

Poultry feed particles must be sufficiently large to be picked up with the beak (Rogers, 1995). Such preferences correspond to energy optimisation (cost/benefit) of feeding behaviour (Collier and Johnson, 2004). Laying hens thus select their food intake according to the relative size of the particles in relation to the beak. Using feeds presented as crumbs (72% of particles > 1.18 mm) (Portella *et al.*, 1988) demonstrated an immediate preference by laying hens for the largest particles. In a mix of whole wheat and meal diet formulated on complete diet, birds prefer particles larger than 2 mm (Dezat *et al.*, 2009). On the other hand, hens do not demonstrate a preference for the largest particles when the feed is presented as a fairly homogeneous meal in which 'mean sized' particles are mainly between 0.60 and 2.34 mm and when it contains substantial quantities of calcium and proteins. This observation suggests that calcium is a key factor in feed consumption (Portella *et al.*, 1988). Moreover, small particles (<0.60 mm) disappear slowly from the trough whatever the presentation (Portella *et al.*, 1988). These small particles often contain micro-nutrients such as vitamins, minerals and amino acids and percolate to the bottom of the feeding trough (Tang *et al.*, 2006).

Joly (2004) showed that distribution of a fine meal compared with a more coarse one (31% vs 9% particles <0.5 mm) resulted in a reduction in feed consumption, laying rate and egg weight (–0.9 g). Addition of small quantities of fat or water can solve this problem by sticking the particles together, facilitating uptake (Tang *et al.*, 2006).

The use of moistened feed has been scarcely studied in laying hens. It stimulates consumption of dry matter (Tadtiyanant *et al.*, 1991; El Kaseh and Forbes, 1995). In contrast, the use of fermented moist feed, which is characterised by a low pH (4.5), reduces ingestion of dry matter when fed to birds between 18–37 weeks (110 vs 125 g/day) (Engberg *et al.*, 2009).

The physical form of the feed interacts with the ability of the hen to fit its energy intake to the energy concentration of the feed. Hens will more willingly eat feeds diluted with fibre (45% wheat bran) when presented as pellet rather than as meal (Vilariño *et al.*, 1996). Diet presentation therefore makes it possible to improve the energy intake of low energy diets.

Moreover, it has been shown that poultry are able to learn and recognise their feed which improves their ability to change their feeding behaviour.

A sudden change in feed, for example from flour meal to large particles (>2.35 mm), can result in an immediate reduction in consumption (–28%) which in turn will affect egg weight (Portella *et al.*, 1988).

Effects of mode of distribution

Staggering the provision of protein amount over the day

The first studies performed on the staggering of protein sources over the day were performed in the 1970s. They were governed by the hypothesis that, following the example of calcium, an appropriate time point in the day can be determined when the amino acids necessary for the formation of albumen should be provided. In fact, whereas the lipoproteins of the yolk are synthesised constantly by the liver, the proteins in the albumen are synthesised in the magnum over 24 hours even though they are specifically secreted into the oviduct during the morning. Leeson and Summers (1978) separated the provision of proteins and calories (morning) from the provision of calcium (afternoon). Reichmann and Connor (1979) provided a calorific feed in the morning, and protein and calcium in the afternoon. Feed consumption and egg weight were reduced in both cases.

More recent studies with more efficient genotypes have indicated that it is possible to separate the provision of proteins without affecting egg weight, when low levels of proteins are supplied in the morning and higher levels in the afternoon. Providing lower protein levels in the afternoon (13% in place of 16% from 14.00h until 08.00h, including the night) resulted in lower egg weights (–1.8 g), regardless of the dietary protein levels distributed in the morning (13 or 16%) (Penz and Jensen, 1991). On the other hand, lower levels of proteins in the morning and higher levels in the afternoon maintained egg weight.

Keshavarz (1998b) also studied alternating feeds varying in protein content during the day (10 and 16%). Compared with the control protein diet (16%) which was distributed throughout the day, the provision of a higher protein diet in the afternoon maintained egg weight, but this was not the case when it was provided in the morning (Keshavarz, 1998b). The difference observed between the morning and afternoon may be explained by lower feed consumption in the morning (40%) which might result in amino acid deficits.

These approaches require further investigation, including study of the provision of lower levels of protein in the morning and higher levels in the afternoon, and evaluating nitrogen elimination.

Use of whole grains

Other studies have sought to evaluate the use of whole cereal grains in feeds. Whole grains can be provided with a complete feed, concentrating the supply of proteins, minerals and vitamins as a mixture, or sequentially. Blair *et al.* (1973) reported that the sequential supply of cereals (wheat, barley and corn) in the morning and a balancer diet (diet enriched in protein, minerals

and vitamins to provide the daily requirement of hens) in the afternoon, did not result in a deterioration in egg weight. The same occurred with the simultaneous provision of cereals and balancer diet. Umar-Faruk *et al*. (2010) obtained similar results with the distribution of whole wheat in the morning and a balancer diet in the afternoon, as well as with a mixture of the two fractions. It should be emphasised, however, that the feed effectiveness was markedly improved with the sequential distribution of wheat and a balancer diet (+5%, $p < 0.01$), this was not the case with a mixture. Robinson (1985) reported decreased egg weight with the distribution of a concentrated protein feed in the morning and whole cereals in the afternoon. This can be explained by an inadequate diet (in terms of proteins and energy), as already reported by Keshavarz (1998b).

The use on farm of whole cereals with a supplemented feed has several advantages, e.g. reduced transportation costs of the cereals, lower energy consumption related to grinding, and lower production costs due to the improved feed effectiveness. However, such a system requires the availability of two silos and the ability to control precisely the amount of balancer diet and cereals distributed.

12.3 Variations in the proportions of albumen and yolk

The proportion of yolk to albumen is mainly related to age. For example, in brown hens, yolk content increases from 23.1 to 28.1% between ages of 20–26 weeks and 54–60 weeks while albumen decreases from 63.8 to 59.2% (Zita *et al*., 2009). Nutritional effects are limited.

12.3.1 Effects of protein and amino acid content

The relative proportions of the egg constituents can be slightly modified by the protein content of the feed. The percentage of albumen is reduced whilst that of the yolk is increased (0.4 %) with feeds with lower protein content (13 *vs*. 16%) (Penz and Jensen, 1991). According to Novak *et al*. (2006), if the dietary protein content is reduced (14.4 vs 18.9% from 20 to 43 weeks, 13.8 vs 16.3% from 44 to 63 weeks) the percentage of albumen and the protein content of albumen and yolk are slightly reduced (60.6 vs 61.1%, 9.67 vs 10.37% and 15.70 vs 15.95%) but the yolk percentage is unaffected (26.2%).

Increased provision of methionine (0.28 and 0.43%) or lysine (0.70 to 1.58%) resulted in improved egg weight without affecting the percentages of albumen and yolk (Shafer *et al*., 1996). When egg weight is increased, albumen and yolk proportions are not affected (Prochaska *et al*., 1996). On the other hand, with increased lysine level in the diet (0.72 to 1.37%) from 26 weeks of age and at higher temperatures, the proportion of albumen was

found to be reduced, while the yolk increased (with a 0.5% of difference between the two criteria for the extreme diets), whereas egg weight was not affected (Prochaska *et al.*, 1996). These results suggest that there is some scope for slightly changing the proportions of albumen and yolk through dietary manipulation.

12.3.2 Effects of fatty acids

Linoleic acid enrichment of feeds also results in a slight reduction in the proportion of yolk, and hence an increase in albumen (Whitehead *et al.*, 1991; Grobas *et al.*, 1999a; Dänicke *et al.*, 2000) (Fig. 12.6).

12.3.3 Effects of method of distribution

Sequential distribution of two feeds of different protein content (16 and 13%) resulted in a slight reduction in albumen percentage (–0.6 point) and

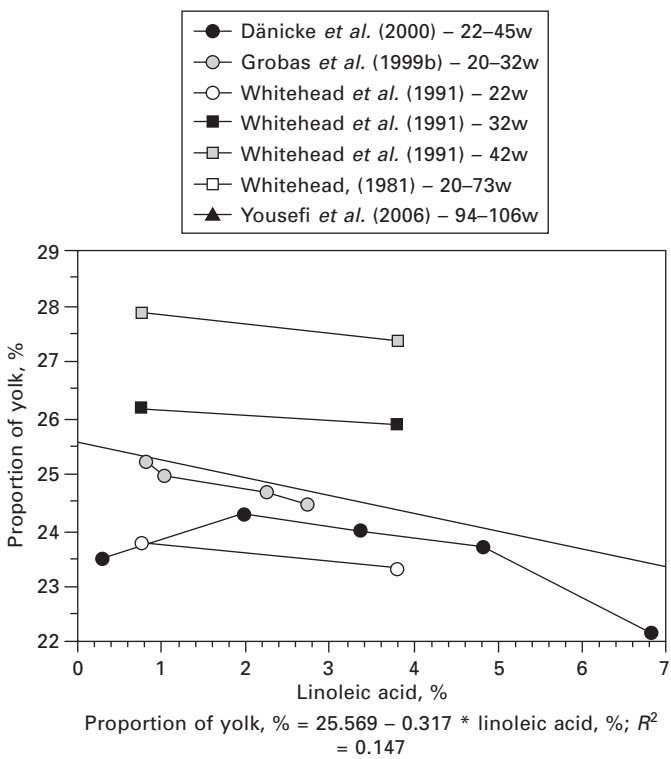

Fig. 12.6 Proportion of yolk according to linoleic acid content of feed. The proportion of yolk in the egg was measured for varying levels of dietary linoleic acid. Each spot corresponds to the mean level reported in a publication (for a given period or age).

a reduction in egg weight when the low protein diet was distributed in the afternoon (Penz and Jensen, 1991). However, no change in egg weight or percentage of albumen and yolk were reported when feeds of varying protein content were alternated during the day (Keshavarz, 1998b).

Umar-Faruk *et al*. (2010) showed that the use of whole wheat grains mixed with a balancer diet or their sequential use, with whole grains in the morning and balancer diet in the afternoon from 19 to 46 weeks of age had no effect on the percentage of albumen. The percentage of yolk, however, was reduced between 27 and 37 weeks with sequential distribution compared with a mixed feed containing the same raw materials.

12.4 Variations in fatty acid composition

The fatty acid profile of an egg, which includes triglycerides and phospholipids, reflects the hen's consumption of fatty acids. As a result, it is fairly easy to modify the fatty acid composition of the egg through dietary manipulation. For example, modifying the saturated and unsaturated fatty acid content of the hen's diet is directly dependent on the inclusion of raw materials rich in oil or sources of oil incorporated into the feed. More precisely, saturated fatty acids (palmitic acid (C16:0) and stearic acid (C18:0)) are the most stable in the egg, whereas mono- and polyunsaturated fatty acids are the most modifiable by substitution between these unsaturated fatty acids. This process, which has been known for 50 years, is described in detail in Volume 2, Chapter 14, and in the chapter on the nutritional value of the egg (Volume 2, Chapter 11).

12.5 Variations in mineral and vitamin composition

One of the main characteristics of the egg is the stability of its major components in contrast to its minor components such as trace minerals and vitamins. The mineral macro-element content of eggs (calcium, phosphorus, sodium and potassium) may be considered as constant with a coefficient variation < 12% (Volume 2, Chapter 11). In contrast, the trace element and vitamin contents of eggs are much more variable and are directly influenced by the amounts ingested by the chicken (Stadelman and Pratt, 1989; Sirri and Barroeta, 2007). This high variability and the possibility to enrich eggs in vitamins and trace elements is exhaustively discussed in Volume 2, Chapter 15. Numerous reviews underlined the feasibility of improving the nutritional values of egg by enrichment in fat soluble vitamins (A, D, E, K), in water soluble vitamins (folate, B12, pantothenic acid and at lower magnitude B1, riboflavine, B5, thiamine and B8, biotin) and in trace minerals (selenium, iodine and at lower magnitude iron, zinc, fluorine and

manganese). Vitamin A for example is transferred to the egg with a very high efficiency varying from 60 to 80% (78% until a supplementation of 8000 IU). The enrichment in the yolk is proportional to the dietary level and can be enhanced 10-fold from its initial value when hens are supplied with 30 000 IU retinol. Egg yolk can also be enriched in vitamin D_3 by dietary supply (1000 IU to 15 000 IU) more than 15 fold (2–5 µg/100 g until 34 µg/100 g in hens fed 2500 and 15 000 IU, respectively) but when vitamin D_3 is provided as 25 OH D_3, the increase in yolk content is limited to 2-fold (Mattila *et al.*, 1999). Vitamin D_3 is more effectively transferred to yolk than vitamin D_2 (Mattila *et al.*, 2004). Numerous authors have also demonstrated the ability to increase the vitamin E content in the yolk by dietary supplementation (3 to 20-fold depending on basal diet content and dietary supply). There are also a few studies which have shown that the levels of vitamin K can also generally be increased in eggs. For water soluble vitamins, the magnitude of increase in eggs due to dietary supply tends to be lower. Of these folate (more than two-fold), B_2 (riboflavin), B_{12} (cobalamin) are the most responsive to dietary supplementation. Thiamine, biotin and panthotenic acid can also be increased but at a lower magnitude than B_6 (pyridoxine) and niacin.

The trace minerals which have been most studied in terms of egg enrichment are selenium, iodine, iron and zinc. Selenium is the most frequently studied trace element, with feed content values of 0.1 to 0.6 mg/kg. Egg selenium content can increase 3 to 6-fold (12-fold in the albumen and 4-fold in the yolk) compared with base levels observed in different geographical areas of production (in the order of 0.15 mg/kg in the yolk and 0.05 mg/kg in the albumen) (Surai and Sparks, 2001). The use of an organic source of selenium provides selenium enrichment up to 30 µg/egg (0.5 mg/kg), representing half the daily requirements of humans (Surai *et al.*, 2007). However, it should be noted that European legislation limits its use to a maximum of 0.5 mg/kg feed.

The iodine egg content of an egg is typically 4–10 µg. This occurs mainly in the yolk but this can be multiplied by a factor of 5 to 12 when the dietary iodine supply is largely increased relative to the requirement of hens (0.3–0.4 mg/kg) up to 30 mg/kg. However, the level of iodine should be limited to less than 12 mg to avoid negative effects on feed consumption and egg production (Yalcin *et al.*, 2004). These authors observed a level of 26 µg I/egg when hens are fed 6 mg/kg dietary iodine.

The magnitude of iron enrichment in eggs is limited to 10–20% but is favoured over zinc and copper when hen's diets are supplied with 80 mg/kg Zn, 120 mg/kg Fe and 25 mg/kg copper (Skrivan *et al.*, 2005). These trace elements, however, can accumulate in the soil (Skrivan *et al.*, 2005). Egg magnesium and manganese can also be influenced by dietary supply of the hen.

12.6 Variations in yolk colour and carotenoid content

12.6.1 Factors influencing the effectiveness of feed carotenoids in birds

The use of carotenoids and the ability to manipulate pigmentation varies from species to species. Ruminants mainly accumulate carotene (Dunne *et al.*, 2009) and bird's oxycarotenoids. Birds cannot synthesise carotenoids; skin or egg pigmentation depends directly on dietary content and the polarity of carotenoids (Na *et al.*, 2004). Animals are, however, able to metabolise them, to a variable degree. In poultry, β-carotene does not contribute to pigmentation (Hencken, 1992) and birds mainly accumulate xanthophylls (Surai *et al.*, 2001; Sinanoglou *et al.*, 2011) which correspond to the group containing one or more oxygen groups. Deposition of carotenoid in the yolk is rapid (<48 h) but 8–10 days are needed for the colour to remain stable, since this is the mean time required for the formation of the yolk (Romanoff and Romanoff, 1949; Marusich and Bauernfeind, 1981).

Variations in the effectiveness of carotenoids in birds originate from different parameters but in birds this is mainly due to their ability to generate vitamin A by splitting the molecules, especially the non-pigmenting carotenoids (α and β-carotenes, Fig. 12.7). Carotenoids lose their pigmenting properties when they are metabolised to vitamin A; all carotenoids possessing an structural extremity similar to that of β-carotene have this property. However, the degree of intestinal conversion into vitamin A depends on the characteristics of the carotenoid and is influenced by the dietary provision of vitamin A

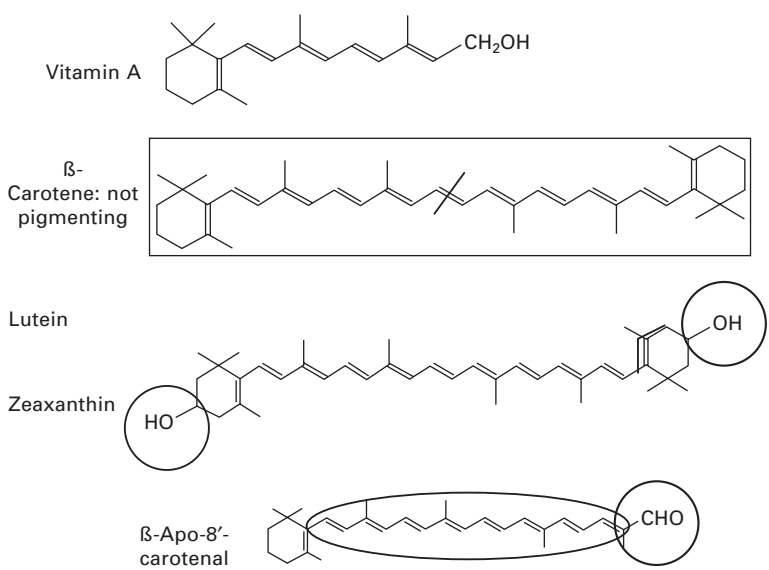

Fig. 12.7 Carotenoid structure. Only oxycarotenoids are effective in hens. Carotenes can be split to provide two molecules of vitamin A.

(Lietz *et al*., 2010). The effectiveness of yolk colouring also varies from one carotenoid to another, because it is influenced by intestinal absorption, plasma transfer and/or transporter, the effectiveness of transfer between tissues and the mechanism for degradation of carotenoid (Hamilton, 1992; Hencken, 1992; Nys, 2000). The absorption of lutein in the bird is more effective in the free form than in the esterified form, although it is in the latter form that it is found in marigolds (*Tagetes*) and in other flowers rich in lutein used in poultry feeds. Saponification, which converts lutein diester to lutein, improves the digestibility of the pigment by about 40 to 60% in chicks. Saponification also improves the absorption of zeaxanthine and capsanthin (from red pepper) in hens and the efficiency (×1.5) of numerous natural yellow or red carotenoids for yolk coloration (Galobart *et al*., 2004). The most effective carotenoids in birds are those that contain an oxygen atom (xanthophylls), and this is why the most commonly used carotenoids of vegetable origin are lutein, zeaxanthin and capsanthin (Nys, 2000).

Dietary fat influences xanthophyll absorption. Short-chain, saturated fatty acid and long-chain unsaturated fatty acids favour lutein absorption and yolk coloration (Huyghebaert, 1993). In contrast, mycotoxins such as aflatoxine or ochratoxine markedly depress the absorption of carotenoids and decrease its tissue accumulation in chicks. Disease (coccidiosis, Newcastle disease, infectious bronchitis) can also have negative effect (Hamilton, 1992).

The effective deposition of carotenoids in the egg yolk will occur in proportion to the initial content in the feed underlining the importance of the dietary carotenoid source and of its stability (Surai *et al*., 2001). The effectiveness of deposition in the yolk has been estimated to 14% for astaxanthin, 25% for zeaxanthin and 30–40% for canthaxanthin when measured by radioactive carotenoids (Hencken, 1992). The yield of transfer to yolk of xanthophylls from corn gluten is the highest for lutein and zeaxanthin (25–30%) but is slightly lower for cryptoxanthin (14–17%) (Looten *et al*., 2003).

12.6.2 Dietary sources of egg carotenoids

The main vegetable sources of carotenoids are corn, corn gluten, lucerne, lucerne concentrates and flower (marigolds) and plant (paprika) extracts (Tables 12.1 and 12.2). Carotenoid concentrations are higher when the protein contents of the feedstuff are increased, whether they are corn, lucerne or flower extracts. Wheat and sorghum are very low in carotenoids and so contribute little to egg yolk pigmentation.

Corn and its derivatives are characterised by being rich in lutein and zeaxanthin, and also by the presence of cryptoxanthin (Table 12.2). The concentration of total xanthophylls in maize or gluten is very variable between strains and with duration and conditions of storage as shown in Table 12.1. The proportion of the various xanthophylls can also vary to some extent but are typically 10% carotene and 90% xanthophylls including lutein and

Table 12.1 Xanthophyll concentrations in various foodstuffs (Nys, 2000)

Ingredient	Mean level (mg/kg)	Range (mg/kg)
Yellow maize*	17	10–40
Corn gluten meal (41%CP)*	130	90–180
Corn gluten meal (60% CP)*	260	180–400
Dehydrated lucerne (20% CP)*	310	200–380
Dehydrated lucerne (25% CP)*	480	350–540
Lucerne concentrate (45% CP)*	800	450–1000
Lucerne concentrate (59%CP)**	1560	900–2100
Marigold meal*	9000	4500–14 000
Marigold meal concentrate*	50 000	40 000–55 000
Marigold meal concentrate***	26 000	12 000–40 000

*Marusich and Bauernfeind (1981), **Coulmier, personal communication, $n = 465$, ***Huyghebaert (1993), $n = 7$.

Table 12.2 Oxycarotenoid content of corn gluten (protein: 59.2%; $n = 28$) and corn ($n = 11$) (Looten *et al.*, 2003)

	Corn gluten (mg/kg)		Corn (mg/kg)
	Mean	Range	
Total xanthophylls	361 ± 46	278–440	31 ± 4
Lutein	155 ± 19	117–205	15 ± 2
Zeaxanthin	91 ± 18	60–161	9 ± 2
β-Cryptoxanthin	14 ± 5	7–35	1 ± 1
Other carotenoids	84 ± 7	71–99	5 ± 1
β-Carotene	7 ± 2	3–12	1 ± 0
Other carotenes	9 ± 2	6–13	< 1

zeaxanthin and at a lower magnitude cryptoxanthin and zeinoxanthin (Table 12.2)

The corn gluten composition reflects that of the batch of maize used to produce the gluten (Table 12.2) as shown by tracing the composition of the derivatives produced throughout the steps of production (dry corn, steeped corn, full milk, protein cake, corn gluten, fibres). The follow-up shows that 85% of corn carotenoids are concentrated in gluten, lutein (42–45%) and zeaxanthin (20–30%) being the predominant carotenoids (Looten *et al.*, 2003). Fibres contain only 8% of the carotenoids. Other corn derivatives contain negligible quantities of carotenoids.

The yield of transfer of carotenoids is similar for corn and gluten as shown in Table 12.3 which is in agreement with the similarities in composition of maize and corn gluten. The yield of transfer to yolk of total xanthophylls, lutein or zeaxanthin and other minor carotenoids remains stable for a dietary supply between 5 and 10 total xanthophylls whatever the carotenoids.

Lutein is the main carotenoid in lucerne (45–75%). Zeaxanthin and cryptoxanthin are present in low concentrations (4–6% and 1–7%).

Table 12.3 Concentration of xanthophylls in laying hen diets (mg/kg), egg yolk (mg/kg) and yield (%) of transfer to the yolk

Level of corn gluten	White corn gluten (8%)			Yellow (4%) and white (4%) gluten			Yellow corn gluten (8%)		
Yolk colour*	5.9 ± 0.3			8.8 ± 0.3			10.5 ± 0.56		
	Diet	Yolk	Yield	Diet	Yolk	Yield	Diet	Yolk	Yield
Total xanthophylls	5.45	8.5	22.7	17.3	27.4	25.8	28.5	46.8	25.1
Lutein	3.25	5.6	24.8	8.05	14.9	29.9	13.3	24.3	27.8
Zeaxanthin	1.1	1.4	18.8	4.9	7.6	25.1	7.95	13.5	25.7
β-cryptoxanthin	0.1	0.1	14.4	0.85	0.7	13.3	1.3	1.5	17.4
Other carotenoids	1	1.4	21.9	3.5	4.3	20.3	5.95	7.6	19.8
Total carotenes	<0.1	<0.1	ND	1	1.2	6	1.4	1	5

* DSM scale was calculated using the Cielab system (L*, a*, b*; $n = 60$) (Nys, 2000).

Violaxanthin and neoxanthin which can represent 7–10% and 3–14% of the total xanthophylls in lucerne are ineffective for pigmenting yolk (Nys, 2000). However, the decrease in violaxanthin content is higher during storage (84% and 28% at 12 weeks for violaxanthin and lutein, respectively) so the proportion in lutein increases with duration of lucerne storage.

The carotenoid content of raw materials varies according to the genetic origin of the plant, its maturity at harvest, dehydration process and storage duration and conditions. Maize can lose 25–50% of its initial xanthophylls after being respectively dried or stored for one year. Similarly, gluten xanthophyll level is the highest after maize harvest until January then declines slowly to a minimum in June (Fig. 12.8, Looten *et al.*, 2003). However, the corn gluten carotenoid content remains unchanged for a shorter period when stored for two months at 4 or 20 °C.

The xanthophyll content of lucerne depends on the order of harvesting during a season (maximum at first harvest then can decrease by 10%) and the maturity of the plant (Table 12.4). This is why large-scale mixes are performed in the industry between harvests throughout the year to homogenise the product. The drying conditions for lucerne concentrates are also an important factor.

Marigold extracts are rich in lutein. They are prepared from flower petals from which carotenoids are extracted by solvents that are saponified and protected from oxidation by antioxidants. They are rich in esters which must be hydrolysed to obtain the free form of lutein. Their effectiveness depends on the preparation process, in particular the degree of saponification which can increase effectiveness by 40%, and their stability during storage. There is considerable variability between different commercial preparations. The relative effectiveness of tagetes varies between 13 and 20% (Grashorn and Seehaver, 1999). The values of commercial sources can, however, change, particularly in view of the fact that preparation processes are constantly being improved.

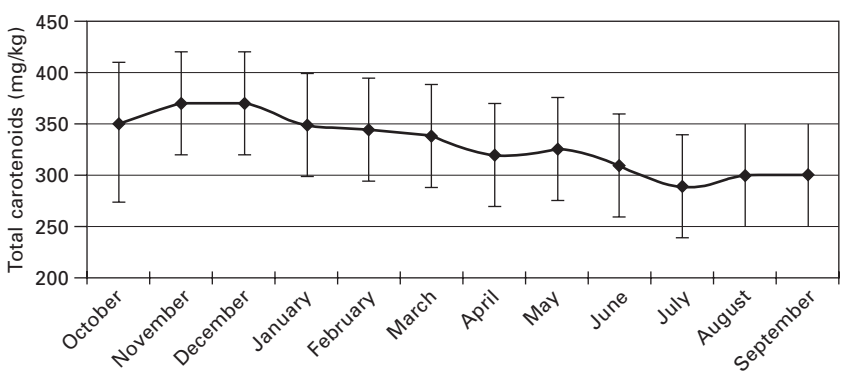

Fig. 12.8 Change in total carotenoids content in corn gluten over the year (monthly means from 1994 to 1999, $n = 290$) (Looten *et al.*, 2003).

Table 12.4 Variation in proportion of pigmenting xanthophylls with harvest order and plant maturity of lucerne extract (Nys, 2000)

Harvest	Plant maturity	Xanthophylls	Lutein and zeaxanthin (%)
1	Onset	1756 ± 64	77 ± 2
	mid	1757 ± 50	80 ± 3
	end	1268 ± 53	86 ± 4
2	Onset	1442 ± 132	80 ± 4
	mid	1472 ± 46	82 ± 0.7
	end	1332 ± 146	87 ± 2
3	Onset	1295 ± 72	83 ± 5
	mid	1254 ± 112	86 ± 5
	end	1362 ± 30	84 ± 4

Vegetable sources provide mainly yellow carotenoids. Only paprika, used in small quantities, provides red carotenoids. Paprika oleoresin is prepared from the dried fruits of *Capsicum annuum* or *Capsicum frutescens* (red pepper) by a similar process to marigold involving dehydration, solvent extraction, saponification and stabilisation. The major component is capsanthin (32–38%) but numerous other xanthophylls are detectable (12–18% α-carotene, 8–10% violaxanthin, 4–8% cryptoxanthin, 6–10% capsorubin, 1–4% others: crytocapsin, zeaxanthin, antheraxanthin, capsanthin epoxide). Saponification considerably improves its effectiveness, which nevertheless remains fairly weak (1–6%). However, there are wide differences in effectiveness between preparations, with effectiveness ratios in relation to canthaxanthin of 3 to 5 for yolk deposition yield and 1 to 4 for pigmentation ability.

Asthaxanthin (3,3′-dihydroxycanthaxanthin) is widely distributed in the animal kingdom, and in some yeast or algae. It produces pink egg yolk when fed alone and increases yolk pigmentation 15–30 times more efficiently than lutein when combined with yellow xanthophylls but its rate of deposition is 5-fold less than that of canthaxanthin when concentrated in oil solution (Marusich and Bauernfeind, 1981). The supply of 0.5 to 3 mg/kg of asthaxanthin from algae spore (*Haematococcus pluvialis*) in addition to a low (3 mg/kg) yellow xanthophyll diets largely increase yolk coloration (4 to 12 DSM scale; Elwinger *et al.*, 1997). The microalgae *Chlorella vulgaris*, which contains 360 mg/kg canthaxanthin and 550 mg/kg astaxanthin, greatly increases yolk coloration when incorporated in the diet (Gouveia *et al.*, 1996). Dietary supply of photosynthetic bacterium *Rhodocytus gelatinosus* increases yolk colour (Ponzano *et al.*, 2004). Fish oil, rich in astaxanthin, (35 mg/kg), can also contribute to yolk colour (Hammershoj, 1995). It has also been shown that hens deposited carotenoids issued from crab meal (Anderson *et al.*, 2008). Dried carrot increases yolk colour slightly when introduced at high levels (4 and 8%), possibly as a consequence of its lycopene content (Sikder *et al.*, 1998) which is incorporated in the egg yolk (Olson *et al.*,

2008). Orange skin (40 g/kg) has the capacity to colour egg yolk but with a lower efficiency than marigold flowers (Chowdhury *et al.*, 2008).

The concentration of xanthophylls and their pigmenting efficiency show wide variation in natural feedstuffs and therefore synthetic oxycarotenoids have been produced and commercialised since 1962 for β-apo-8′-carotenoid ethyl ester (apo-8-ester), since 1964 for canthaxanthin, since 1968 for citranaxanthin and since 1984 for asthaxanthin (Huyghebaert, 1993). Zeaxanthin and lutein can also be synthesised but are not produced for commercial reason. Synthetic carotenoids corresponding to natural carotenoids (β-apo8′-carotenoid acid, ethyl ester, canthaxanthin, citranaxathin) are currently introduced into laying chicken feeds. The colouring effectiveness depends mainly on the chemical structure of the molecule and also on its stability during storage. These carotenoids have been chosen for their colouring effectiveness, which is two to three times better than carotenoids of vegetable origin, and the absence of conversion of these molecules to vitamin A. Coating of the active molecule also enhances the stability of synthetic carotenoids. For example, the deposition rate is 3 fold higher for apo-8-ester (42–50%) relative to marigold extracts (13–20 %) in egg yolk (Grashorn and Seehaver, 1999). Its higher efficiency is confirmed in egg product at high level of supplementation (Sirri and Barroeta, 2007). Dietary xanthophyll supplementation in chicken is limited to 80 mg/kg in Europe, with the exception of canthaxanthin (8 mg/kg). Eight synthetic carotenoids are authorised either for red carotenoids (capsanthin, canthaxanthin and cryptoxanthin) or for yellow carotenoids (β-apo-8′-carotenal, β-apo-8′-carotenoic acid, lutein and zeaxanthin) but only canthaxanthin is commonly used (Breithaupt, 2007).

Perception of the intensity of the colour of the yolk depends directly on the quantity of carotenoids consumed by the chicken, its effectiveness and the chemical composition of the sources used (vegetable or synthetic). The dietary supply of oxy-carotenoids depends on consumer demand for yolk coloration. It varies greatly among countries. For table eggs, satisfactory colour can be obtained with small amounts of xanthophylls (15–25 mg/kg yellow carotenoids combined with 1–2 mg/kg red carotenoids), but the requirement is higher when the egg is cooked or used in prepared foods (noodles, bakery products, mayonnaise, etc.). A similar yolk colour can be reached by different combinations of yellow and red carotenoids. The optimal ratio depends on technical considerations and on economical constraints. Sources are the main origin of variation as illustrated in Fig. 12.9. It also demonstrates the proportional increase in colour due to hen dietary intake and the existence of a plateau in the colour response of the yolk above a relatively low quantity of carotenoid ingested.

The efficacy of xanthophylls for coloring yolk clearly declines at larger dietary supplementation. The coloration capacity of apo-8-ester is the most efficient. That of corn gluten is slightly higher than that of alfalfa (90% lutein) because of the presence of a higher levels of zeaxanthin and cryptoxanthin in gluten which are more reddish than the yellow lutein. It also illustrates the

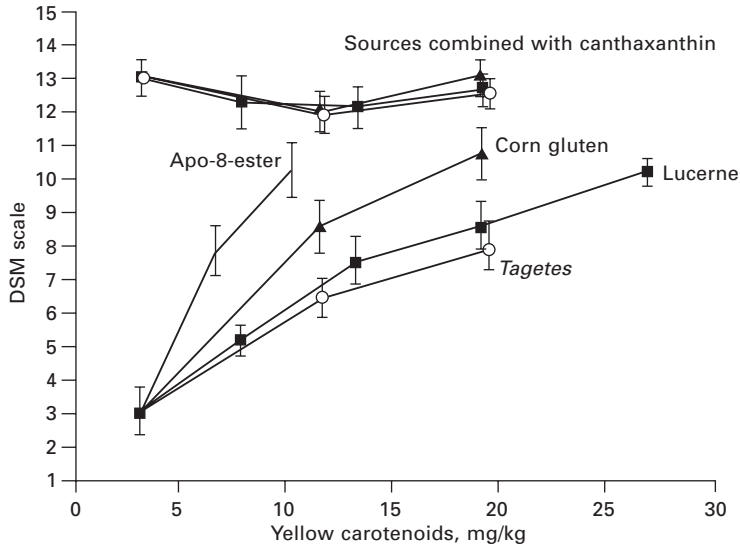

Fig. 12.9 Change in yolk colour with the source and level of dietary carotenoids combined with or without 2 mg kg^{-1} canthaxanthin (Nys, 2000).

necessity of introducing a red source when hens are fed plant feedstuffs rich in yellow carotenoids to meet the demands of the consumer for a strongly coloured yolk. Whatever the source, the yellow parameter (b*, L-a-b system) plateaus at dietary concentration of xanthophylls higher than 10–15 mg/kg but the reddish parameter (a*) still responds to higher dietary level of yellow carotenoid sources especially for apo-8-ester. Supplying only red carotenoids (1–2 mg/kg) allows high yolk colours but defined as off-colours (out of the DSM scale and the consumer demand). A substantial base of yellow is indeed required to prevent off-colouring and to improve the homogeneity of yolk colour (Hernandez *et al.*, 1999).

In conclusion, a supply of xanthophylls is crucial to reach the demand of consumers for a coloured egg yolk. The enrichment of egg yolk, in particular by carotenoids, has recently gained a lot of interest since it has been shown that retina contains lutein, zeaxanthin and meso-zeaxanthin. Eggs are a good source of zeaxanthin and lutein with higher availability for human than other foods (Thurnham, 2007). Therefore eggs enriched in lutein and xanthophylls might contribute to preventing age-related macular disease (Mares-Perlman *et al.*, 2002; Wenzel *et al.*, 2006).

12.7 Variations in shell quality

The structure and the formation of the shell are described in Chapter 8 in this volume. The strength of the shell depends on the quantity of material

deposited and on the fabric of this protective material controlled by the organic matrix (Nys *et al.*, 1999) (Chapter 8). Several studies have demonstrated that the hen's diet influences shell quality.

12.7.1 Dietary level of calcium in pullets and laying hens

Calcium is the key feedstuff for shell strength (Nys, 1999). Classen and Scott (1982), Sauveur (1987) and Hartel (1990) have reported that calcium requirements are in the order of 0.9 to 1.2% in the growth period of the pullet, from 2 to 2.5% for medullary bone formation, which occurs about two weeks before laying, and from 3.5 to 4% for egg formation during the laying period. The transition phase from the 'immature pullet' to that of the 'laying hen' is crucial. It is essential to increase calcium levels in the feed from 1 to 2.5% two weeks before the appearance of the first eggs (14 to 16 weeks) to enhance formation of medullary bone and that of the shells of the first eggs. Introducing a laying hen diet too soon can encourage under-consumption, whereas delayed introduction reduces both the quality of the first eggs and that of subsequent eggs, even if the hen then receives a diet rich in calcium (Leeson *et al.*, 1986; Roland and Bryant, 2000). Nowadays, sexual maturation occurs earlier in the hen, and so the likelihood that the first eggs will be laid before the hen is transferred to the laying house (which usually coincides with the introduction of the layer diet) is therefore very high. It is therefore recommended to introduce a higher calcium diet (25 or 3.5%) before the onset of egg production (14–16 weeks of age). Any risk of lowered feed consumption due to excess of calcium can be alleviated by improving the presentation of the diets (size of particles, use of crumble) or timing of distribution of the diets toward the end of the rearing period. Moreover, these procedures will also optimise the weight of the pullets and consequently that of the egg.

The hen requires on average 2.2 g calcium per egg and, taking into account the mean calcium retention, must therefore consume 4 g per day. A limited supply of calcium (feed content lower than 3%) reduces shell strength and egg production, and increases chicken mortality (Hartel, 1990; Keshavarz, 1998a, 1998b; Liu *et al.*, 2007). Shell strength is at a maximum when hens consume 4 to 4.5 g calcium per day (Scott *et al.*, 1999). Attempts to model the relationship between calcium intake and calcium retained in body and eggs has highlighted the effect that calcium intake can have on both calcium and phosphorus retention in laying hens (Kebreab *et al.*, 2009). Some authors have recommended progressive increase in calcium intake during the laying year (3.5, then 4.5, and finally 5.5% calcium in the feed) to limit deterioration of shell strength with laying hen age, but experimental evidence of improved shell strength is not convincing, and only bone reserves increase (Guinotte and Nys, 1993). In hens of 80 weeks of age, a large increase in dietary calcium (5%) has been shown to have little effect on eggshell quality (Keshavarz, 1986). Likewise a step-up phase feeding system (3.5, 4.5, 5.5% Ca) does

not improve eggshell quality (Keshavarz and Nakajima, 1993). Similarly, in conventional and furnished cages, Valkonen *et al*. (2009) observed no effect on egg shell quality or bone strength when the calcium supply was increased (3.7–4% to 4.4–5 % in three feeding phases during the laying period) but there was an improvement in egg production. However, in brown egg-laying hens in the late phase of production, the supply of 4% dietary calcium has been shown to improve the shell quality compared to hens fed 3.5% calcium (Safaa *et al*., 2008).

The selection for increased egg numbers is associated with a daily production of an egg which is laid very early in the morning or even before the lights come on (80% of eggs are laid 11 h after extinction, i.e. 3 h after light on, 16L–8D (Joly and Alleno, 2001). Consequently, the morning feed intake cannot contribute to the supply of calcium required for eggshell formation as this is completed 1.5 h before oviposition. The introduction of midnight feeding improves synchronisation of calcium intake and eggshell formation and eggshell quality is increased when an intermittent lighting supplement (2 h) is introduced in the middle of the night (Grizzle *et al*., 1992), especially when midnight feeding is combined with food distribution using an automatic feeder (Harms *et al*., 1996). However, in Europe, legislation relating to bird welfare does not permit the introduction of a light period during the night. Studies looking at the effects of feeding hens during the morning and afternoon, with alternative diets containing low and high calcium levels as a means of improving the synchronisation of calcium intake and eggshell formation and eggshell quality are inconclusive (Sauveur, 1991; Keshavarz, 1998a, 1998b).

The hourly kinetics of intestinal calcium retention throughout the day is of great importance because of the lack of coincidence between the period of uterine deposition of calcium for shell formation in the uterus during the night and the period of calcium intake during the day. Hens show a specific appetite for calcium a few hours before calcification takes place, i.e. a few hours before lights off (Mongin and Sauveur, 1979), resulting in storage of feed including calcium in the crop. This results in increased acid secretion because of the consequent dilatation of this organ (Ruoff and Sewing, 1971; Lee *et al*., 1988). This specific appetite for calcium in hens therefore favours the storage and solubilisation of the dietary calcium throughout the night especially when available as coarse particles and this compensates partly for the gap in time between calcium dietary supply and its requirement for shell formation. The timely provision of coarse calcium particles in this way also limits the hens' needs to mobilise calcium from the bone reserve and therefore also decreases the associated phosphorus elimination (Whitehead, 2004).

Particulate calcium (in the form of calcium carbonate) comes from quarries (chalk, marble) or it is of marine origin (oyster and other marine bivalve shells). It is available in various particle sizes and it can have different physico-chemical properties (Guinotte and Nys, 1991). Chemical analysis of sources demonstrates wide variability of calcium and oligo-element composition (Reid

and Weber, 1976; Guinotte and Nys, 1993), but the crystallographic type (calcite, aragonite, amorphous) does not influence the composition and makes no difference to shell quality in the chicken (Brister *et al.*, 1981). Among the numerous trials published since more than 60 years, sources of marine origin show a positive effect on shell quality, in 13% of the trials compared with those using limestone irrespective of the particle size. A comparison of most frequent European calcium sources introduced at different particle sizes (Richter *et al.*, 1999) shows no influence of source on eggshell mass or mechanical properties. The main factor influencing eggshell is therefore the size of the particle (gross or ground). Indeed the positive effect of particulate calcium compared with ground calcium has been demonstrated in more than 50% of the studies (>350 assays since 1927; Guinotte and Nys, 1993).

The percentage of experimental positive responses among all experimental groups increases (51 vs 19%) when particles, whatever the source origin are larger than 1 mm compared with smaller particles (0.2–1 mm, or inferior to 0.2 mm). This is in agreement with the observation that particles have to be greater than 0.8 mm to be retained in the gizzard (Rao and Roland, 1990). The probability of observing a positive effect is also increased (53 vs 41%) when half to two-thirds of the calcium is supplied as coarse particles instead of 33 or 100% or when the large calcium particles are distributed completely separately from the diet. Hens fed mixed particle sizes seem to have greater feed consumption than those fed 100% ground calcium source (Saunders-Blades *et al.*, 2009). The positive effect on feed intake of calcium particle size is well established when hens are subjected to heat stress (see Chapter 13). In addition, the positive response of eggshell quality to particle size depends not only on the size but also on the density and solubility of the limestone source, i.e. to the quarries where the limestone is mined. It will be, therefore, very useful to predict by an *in vitro* test the efficiency of calcium source with a particular size. The solubility of various calcium sources decreases in proportion to the size of the particles and depends also on the density and porosity of the source (Cheng and Coon, 1990; Richter *et al.*, 1999; Saunders-Blades *et al.*, 2009). Attempts to predict optimal particle size for improving eggshell quality from its solubility are inconclusive because under experimental conditions, very large differences in physical properties are needed to demonstrate statistical differences or because of interaction between origin of the source and size of the particles. However, as a general rule, a coarse particle with a low solubility can be introduced to supply two-thirds of the calcium as particles of 1 to 2.5 mm, and a marine source highly soluble as particles of 2–4 mm. Large, poorly soluble particles are poorly absorbed and appear in the manure and, consequently, are of little benefit for eggshell quality when used as a single source.

The use of coarse calcium particles completely separated from the food requires an additional silo of calcium carbonate to distribute calcium a few hours before lights off. More frequently, coarse particles of calcium carbonate are incorporated into the diet as laying hens preferentially eat the calcium at

the onset of shell formation. It is noteworthy that pelleting of diets and the use of crumbs reduces the size of the calcium carbonate particles (Guinotte and Nys, 1993) and, therefore, coarse calcium should be mixed after pelleting and crumbling rather than incorporated earlier in the diet.

The supply of coarse particles of calcium shows the greatest improvement in eggshell quality when given to hens towards the end of the laying period (62% in older hens vs 30% in younger hens), in a hot climate (78% vs 43%) or when hens are fed low to medium levels of dietary calcium (Guinotte and Nys, 1993).

Finally, the use of coarse calcium carbonate particles induces in most of the experiments (in excess of 80%) an increase in bone mineralisation in medullary bone content and improves bone strength in older hens (about 20%; Whitehead and Fleming, 2000) as was confirmed in a recent study (Saunders-Blades *et al.*, 2009).

12.7.2 Effects of dietary lipid content on calcium retention

Fatty acids can combine with calcium and magnesium in the gastro-intestinal tract and form insoluble soaps, particularly with saturated fatty acids. The supplementation of polyunsaturated fatty acid in hens is considered to have limited effect on calcium retention. Conjugated linoleic acid (CLA) supplemented at 2% in hen diet favours accumulation of yolk CLA and that of saturated fatty acid: it decreased shell thickness and strength (Kim *et al.*, 2007). This negative effect was however corrected when CLA was combined with oleic acid or linoleic acid. Diets high in polyunsaturated w3 fatty acid supplied during the period of pre- and post-moult has little effect on the decline in skeletal integrity during moult (Mazzuco and Hester, 2005). Increased calcium levels in the diet and the incorporation of large quantities of fatty acids (particularly unsaturated fatty acids; 10%), however, resulted in the production of soaps in the chicken intestine and in reduction in calcium retention (Atteh and Leeson, 1985). The absorption of calcium and lipids, and shell quality, was not affected by the formation of soaps when the levels of fatty acids in the diet were lower than 6%. Indeed, calcium absorption appears to precede that of lipids, and soap formation takes place with the non-absorbed calcium in the distal parts of the gastro-intestinal tract (Gueguen, 1992). The weak effect of lipids on calcium might also result from high secretion of bile salts in the adult bird compared to the younger bird as bile reduces the formation of soaps (Krogdahl, 1985). Finally, gastric emptying and intestinal transit are slowed down by the presence of fats (Krogdahl, 1985), and this might facilitate the solubilisation of calcium and its absorption.

12.7.3 Dietary phosphorus

Variations in dietary phosphorus during the rearing period of pullets (range of 0.3 and 0.2% respectively) have no influence on the quality of the first egg

or on bone mineralisation at 18 weeks of age (Keshavarz, 2000). In laying hens, however, numerous publications (Nys, 1999) have established that too much phosphorus in the diet can have a negative effect on shell quality. This effect is most significant when the dietary non-phytate phosphorus is higher than 0.35–0.4%. Hens fed on a marginal calcium diet seem more sensitive to high dietary phosphorus (Scott *et al.*, 1999). The recommendations for phosphorus intake were therefore substantially reduced 25 years ago (0.28% non-phytic phosphorus), under the guidelines of the European Group on Mineral Supplementation of the World Poultry Science Association (Vogt *et al.*, 1984). The question then was how far was it possible to lower the supplementation of inorganic phosphorus, especially when using phytase which liberates phytic phosphorus from raw vegetable matter and therefore limits phosphorus excretion and alleviates environmental pollution. Without phytase, a dietary supply lower than 0.15% non-phytic phosphorus will have a negative impact on egg production and egg mass and may even increase mortality (Boling *et al.*, 2000; Snow *et al.*, 2004; Francesch *et al.*, 2005; Liu *et al.*, 2007). The effects are most severe when no inorganic phosphorus is supplied to the basal diet (Boling *et al.*, 2000; Francesch *et al.*, 2005). When using phase feeding, performance is normal when using a progressive decrease in dietary phosphorus from 0.25% (20–35 weeks), 0.15% (36–51 weeks) and 0.1% (52–63 weeks) but production traits may be reduced when only 0.1% non-phytic phosphorus was fed during the second phase (Keshavarz, 2003b). In such conditions, supplementation of phytase does not restore hen performance. Similarly, phytase (300 FTU or higher supplementation) can only partially alleviate the negative performance when no inorganic phosphorus is supplied. Feed supplementation with microbial phytase is commonly carried out as this allows a reduction in the provision of mineral phosphorus. Phytase supplementation (300 FTU) improves the digestibility of phosphorus (Francesch *et al.*, 2005) but also that of calcium (9.8%) and that of amino acids (2–8%) (Liu *et al.*, 2007). It is generally accepted that supplying 300 FTU (phytase units) is the equivalent of 0.8 or 1 g (0.1%) mineral phosphorus. The supplementation of 0.1% non-phytic phosphorus (inorganic NPP) is sufficient when supplying a diet with phytase to avoid any possible reduction in egg production related to phosphorus deficiency. Alternatively, a supply of 0.25–0.2 and 0.1% at three phases of laying hen period, 20–36, 37–51 and 52–63 weeks, combined with at least 150 FTU phytase has been shown to maintain performance similar to a control diet containing 0.45% NPP (Keshavarz, 2003b). This may need adjustment depending on the strain of hen being used.

There is evidence that the phosphorus requirement of laying hens slightly increases in hot environments. NPP levels below 0.25% increase mortality in hens exposed to high temperatures (Garlich, 1978; Nys, 1995) and when supplied at 0.3% decrease feed production and egg production (Cabuk *et al.*, 2004). Garich (1978) and Nys (1995) recommend 350 mg/day/hen to sustain skeletal mineralisation. Usayran and Balnave (1995) have demonstrated

a reduction in phosphorus retention at high temperatures. A low dietary phosphorus level (0.3% available phosphorus) associated with low calcium intake, as is frequently observed at high temperatures, causes weak bones and may facilitate the appearance of cage fatigue in layers. That can be partly alleviated by incorporation of particulate calcium as it decreases mobilisation of bone reserves and permits lower dietary phosphorus levels (Nys, 1995, Whitehead, 2004).

12.7.4 Vitamins D and C and shell quality

Vitamin D_3, the only form that is effective in birds, has a role in the control of calcium metabolism in the chicken, in particular in the intestinal absorption of calcium which is directly dependent on its active metabolite, 1.25 dihydroxy-cholecalciferol (Nys, 1993; Bar, 2008). However, transfer of calcium to the uterus for the formation of the shell does not demonstrate such dependence on vitamin D_3 and its metabolites. Vitamin D_3 nevertheless is essential for maintaining egg production and shell quality, 400 IU being necessary according to studies performed before 2000 (Whitehead, 1986; 300 IU/kg, NRC, 1994) but, in practical terms, levels of incorporation are maximised in feeds for hens producing more than 300 eggs in a laying year (Weber, 2009). High levels of vitamin D_3 (6000 to 15 000 IU) have no effect on production parameters but improve bone strength (Mattila *et al.*, 2004). The biological activity of 25(OH) D_3 is greater than that of its precursor, vitamin D_3, and this metabolite can be substituted for vitamin D_3 (Soares *et al.*, 1995). A positive effect on shell quality has been reported in chickens in the late production stage (Koreleski and Swiatkiewicz, 2005), but this has not been confirmed by other authors (Keshavarz, 2003a). 25(OH) D_3 results from hydroxylation of vitamin D in the liver, and its levels reflect the dietary provision of vitamin D_3. Production of 25(OH) D_3 can be reduced in cases of hepatic metabolic disorder, and in such a case it is obviously valuable to supply dietary 25(OH) D_3. The active metabolite of vitamin D_3 (calcitrol or $1.25(OH)_2D_3$) increases the intestinal absorption of calcium in proportion to dietary provision, but the high cost of this metabolite and the absence of it having any direct effect on uterine calcium transfer (Nys, 1993) prohibits its use in poultry feeds. Pre- and probiotics have been mentioned as affecting levels of cholesterol.

In hens exposed to heat stress, the supply of vitamin C has been reported to have beneficial effects on eggshell quality and bone strength (Njoku and Nwazota, 1989; Chung *et al.*, 2005) but the results in the literature are rather inconsistent.

12.7.5 Electrolyte equilibrium of feeds

The electrolyte equilibrium of feeds influences the bird's acid–base metabolism, which itself is also strongly dependent on shell formation (Mongin, 1978). The

initial attempts to define the optimum ratio of Na, K and Cl in the chickens are not conclusive. Changes in Na + K/Cl ratio as high as 0.4 to 7.7, obtained by modifying dietary provision of sodium and chlorine, considerably affect blood pH and plasma concentrations of bicarbonate, but they have no effect on shell quality (Mongin, 1978; Hamilton and Thompson, 1980; Kurtoglu and Balev, 2007).

In contrast, it has been clearly established that a significant excess of chlorine has a negative effect on shell quality, weak shells being obtained when chlorine supply is in excess of 0.75% (Austic, 1984). However, shell quality is not affected when the level of chlorine is lower than 0.3% (Hess and Britton, 1989). High levels of dietary sodium (0.35 and 0.45%) combined with high (0.47%) or low (0.12%) levels of chlorine also reduce shell strength (Hughes, 1988).

On the other hand, sodium or chlorine deficiency (less than 0.1%; Vogt, 1977; Sauveur and Mongin, 1978) decreases shell production and quality. This is sometimes observed when the addition of salt to the feed is overlooked!

12.7.6 Trace elements

The importance of trace elements (copper, zinc, manganese) has been demonstrated in the normal formation of the shell membranes and the shell. Copper deficiency in hens affects the formation of lysine-derived cross-links and, consequently, the biochemical and mechanical properties of eggshell membranes (Chowdhury, 1990). This results in egg shape abnormalities. Eggs from hens fed on manganese-deficient diets (less than 7 mg/kg) also have thinner shells, translucent areas due to alterations in eggshell ultra-structure in the mammillary layer and a decreased concentration in polysaccharide precursors of the eggshell matrix (Leach and Gross, 1983). Zinc is a component of carbonic anhydrase, a key uterine enzyme in supplying the carbonate ions for shell formation. One kilogram of standard laying chicken feed based on corn and soya contains 30 mg zinc, 6 mg copper and 20 mg manganese. Such levels avoid deficiencies leading to significant shell abnormalities. Nevertheless, it is current practice to supplement the chicken diet with these three elements.

The absence of supplementation of these elements decreases eggshell weight but this is mainly due to an absence of manganese (Abdallah *et al*., 1994). This was confirmed by Sazzad *et al*. (1994) who supplied 80 mg/kg manganese to a 25 mg/kg basal diet. Manganese supplementation (50–80 mg kg^{-1}) increases the weight of the shell but also improves the shell mechanical properties independently of the effect of quantity of eggshell material (Mabe *et al*., 2003). Manganese when provided at levels higher than 70–100 mg/kg is of no additional benefit in terms of eggshell quality (de Faria *et al*., 1999). A zinc deficiency in laying hens (<10 mg/kg in a soybean-type diet) reduces hen weight, egg production, hatchability; retards feathering but has no effect on

eggshell thickness (Kienholz *et al.*, 1961). No negative effects however are observed on these parameters when the diet contains 28–34 mg zinc/kg (Stahl *et al.*, 1986). When supplied at high level (90, 130 mg/kg), zinc did not affect shell thickness (Kienholz *et al.*, 1992). However, when hens are exposed to high temperatures or to water containing 2 g/l NaCl, both of which result in poor eggshell quality, the supplementation of 100 mg/kg zinc increased eggshell weight and decreased the percentage of shell defects (Balnave and Zhang, 1993). In another study, however, poor eggshell quality induced by heat stress was not corrected by zinc-methionine supplementation (40 mg/kg zinc; Kita *et al.*, 1997). The general consensus, however, is that dietary zinc might be a useful tool to combat the negative effects on egg quality when hens are exposed to heat stress (Sahin *et al.*, 2009). The effect of commercial sources of chelated inorganic sources of zinc or manganese on eggshell quality remains controversial with, some studies reporting positive results while others demonstrate little or no effect (Dale and Strong, 1998; Mabe *et al.*, 2003).

The eggshell quality is not affected when hens are fed moderately high dietary copper (70–140 mg/kg Cu SO4) (Christmas and Harms, 1983). Shell thickness, however, is reduced when copper is supplied at higher levels (250 and 500 mg/kg; Harms and Buresh, 1986). This negative effect results partly from the use of copper acetate rather than copper sulphate when 250 mg/kg is used (Al Ankari *et al.*, 1998).

Attempts to improve eggshell quality by supplying boron (100 mg/kg; Qin and Klandorf, 1991), vanadium (20 mg/kg; Ueberschär *et al.*, 1985), fluoride (6 to 20 mg/l; Coetzee *et al.*, 1997) or germanium (0.5% germanium biotite, Lee *et al.*, 2003) are inconclusive. Other metal cations such as nickel (100–500 mg/kg), chromium (500–2000 mg/kg) and lead (20–100 mg), decreased eggshell weight (Meluzzi *et al.*, 1996). Dietary selenium supplementation can increase the percentage of cracked egg when supplied as a selenium enriched yeast (3 m/kg; Payne *et al.*, 2005) but at lower level (0.4 or 0.8 mg/kg) this does not influence shell thickness or breaking strength (Pavlovic *et al.*, 2010).

12.7.7 Clays

Several studies have investigated the value of supplementation with natural or synthetic clays to enhance the formation of granules in order to improve dietary effectiveness to control moisture, ammonia content of manure and its odour. Sodium aluminosilicates (zeolites incorporated at 0.75 or 1.5% in the feed) have been reported to be able to form complexes with calcium and to improve shell quality (specific gravity) in 77% of 35 of trials analysed, particularly when the calcium provision was marginal (2.75%; Roland, 1986) or when chickens were exposed to heat stress (Ingram and Kling, 1988; Keshavarz and McCormick, 1991). However, the use of these zeolites is limited by the fact that only 10–20% of aluminium and 40% of silicium in

clay is absorbed by the chicken (Roland *et al*., 1993). There is no evidence that natural clays have any positive effect on eggshell quality.

12.8 Conclusion

Analysis of published references emphasises the importance of nutritional factors as determinants of egg quality. Genetic selection today provides a high level of productivity, but the genetic potential of the stock is realised only if their nutritional requirement is equally matched. Many studies have been performed on the nutritional 'requirements' of laying hens and on the relationships between nutrients and the nutritional value or organoleptic (colour) qualities of the egg. The literature often described trials carried out more than 20 years ago when performance was lower so adjustment to some observations may be needed even if the main conclusions remain valid. The challenge for the future will be to define sustainable feed systems, i.e. those which impact least on the environment while guaranteeing profitability for all stakeholders involved in this highly integrated industry without compromising on the quality of the final product by the time it reaches the consumer. A greater emphasis in the future must be placed on the nature of the raw feedstuffs used in the formulation of layer diets, for example favouring use of local raw materials, as well as redefining the modes of feeding. For example, sequential feeding (using whole cereals or a protein source) might be one feeding system used more widely in the future.

12.9 References

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13

Effect of hen age, moult, laying environment and egg storage on egg quality

A. Travel, ITAVI, France, Y. Nys, Institut National de la Recherches Agronomique, France and M. Bain, University of Glasgow, UK

Abstract: Egg production and quality are greatly influenced by the physiological status of the laying hen. In this chapter the effects of bird age, moulting, temperature, lighting and housing system are discussed in relation to how consistency in lay and egg quality can be optimised and maintained. The environmental conditions experienced by a freshly laid egg during transport and storage are also critical if the freshness of the egg is to be maintained throughout the logistical chain. Optimal conditions for egg handling and storage of eggs are therefore discussed in the final section of this chapter along with new technologies which may be implemented to ensure and extend the shelf-life of eggs in the future.

Key words: egg quality, bird age, induced moulting, temperature, lighting programmes, housing system, egg handling, egg storage.

13.1 Introduction

The modern commercial layer has been selected for a light mature body weight and an extended egg laying period, and is capable of laying an egg almost every 24 hours such that in excess of 320 eggs are produced in any one calendar year.

The formation of the egg is under the control of precise physiological mechanisms and occurs in two steps following a precise temporal and spatial sequence: the yolk is produced in about 10 days from precursors synthesised in the liver, the oocyte is then released from the ovary into the oviduct. This ovulation of the oocyte takes place only a short time after expulsion of the

preceding egg from the distal oviduct (oviposition). During its journey through the oviduct, the ovum acquires the albumen in the magnum, the paired shell membranes in the isthmus and the shell in the uterus (or shell gland pouch) which takes approximately 18 hours to form. Just prior to oviposition, the egg shell acquires the pigment and the cuticle. More detailed information on egg formation is available in Chapter 6 in this volume.

The egg has a high nutritional value (Volume 2, Chapter 11). Consumers expect a homogeneous and stable product. Therefore the breeders and farmers strive to find the best means to control variability in egg quality. The quality of the egg is influenced by a range of factors including the strain and the age of the bird, the diet as well as the housing system. The environmental conditions within the house can also modify the birds' physiological status and thus affect egg quality. Numerous studies have therefore been carried out to identify the conditions which optimise egg quality and which minimise the negative effects of, for example, hen age or unfavourable rearing conditions. Finally, the way in which a freshly laid egg is subsequently handled and stored will ultimately determine if quality is maintained throughout the logistical chain.

13.2 Egg quality and the effect of increasing bird age

13.2.1 The effect of bird age on egg weight and on the different compartments of the egg

Egg weight is the primary criterion used in the grading of eggs and, as a result, this will influence an egg's retail value. The weight of an egg varies between 50 and 70 g depending mainly on the age of the hen and, to a lesser extent, on its genotype. Egg weight increases with hen age, thereby increasing the heterogeneity of this product. Therefore, as egg weight is a highly heritable trait (H^2 = 0.5–0.6) (Chapter 11), selection programmes have been used for many years to limit this change in egg weight. Thus, most modern commercial strains are now capable of achieving egg weights of 60 g by 26 weeks of age and 65.5 g by 50 weeks, and sustaining this until the end of production, which is typically around 72–74 weeks of age. Twenty years ago, only a small proportion of the laying flock would have been capable of achieving egg weights within the range of 53 to 67 g (Nys *et al.*, 2008).

The increase in egg weight during a normal production cycle is associated with an age related change in the proportion of the different components of the egg (Table 13.1). The % contribution made by the yolk increases throughout lay resulting in a decrease in the proportion of yolk to albumen over time (Ternes *et al.*, 1994). According to the same authors, the % shell remains relatively consistent (10%), a trait which could account for the reported increase in the number of these larger eggs being downgraded at the end of lay due to cracked and broken shells.

Table 13.1 Mean values of egg compartments at different hen ages (900 eggs per group)

Hen age (weeks)	34/35		50/51		70/71	
	(g)	(%)	(g)	(%)	(g)	(%)
Egg	61	100	66	100	68	100
Yolk	16	26	19	29	20	29
Eggshell	6.1	10	6.6	10	6.7	10
Egg white	39	63	41	61	41	61
Ratio white/yolk	2.4		2.2		2.1	

Source: Ternes *et al*. (1994).

The importance of egg weight on profitability has resulted in the development of a range of management techniques which promote larger egg size. These include lengthening the period birds remain in lay and promoting egg size through dietary manipulation (e.g linoleic acid). An alternative strategy, and one which is widely used in the USA, is to induce laying flocks to go through a moult and keep them for a second laying cycle, as discussed below.

13.2.2 The effect of bird age on egg shape

Egg shape is usually expressed in terms of the ratio of an egg's length to its breadth (SI), with values at the beginning of lay typically being between 1.2 and 1.3. At the end of lay, the SI values increase as the eggs become more elongated (Fig. 13.1a–b).

This age-related change in shape seems to be caused by a weakening of the muscular tone of the shell gland but may also arise from a change in the proportion of thick to thin albumen. Occasionally, double yolked eggs, abnormally large eggs or very small rounded eggs lacking any albumen may be produced by young birds. These usually disappear over time and are associated with multiple ovulations which can occur in young birds coming into lay. An abnormally high proportion of small eggs, however, may also persist if sexual maturity has been induced too early (Sauveur, 1988).

Other defects in egg shape which can arise in young flocks include the target egg, the slab sided egg and the soft shelled or shell-less egg (Fig. 13.1c–e). These forms often occur in tandem and can result from an early ovulation occurring prior to the oviposition of an egg which is still forming egg within the uterus.

Eggs exhibiting an equatorial bulge (Fig. 13.1f) may also result if a bird is stressed during the early stages of shell formation (early evening). In this case, a change in muscle tone causes the shell to fracture and then undergo repair as shell formation continues throughout the night. This type of defect can occur at any time in the laying year but is more common in older flocks. Older flocks also lay more eggs with sandy deposits or pimples on their shells

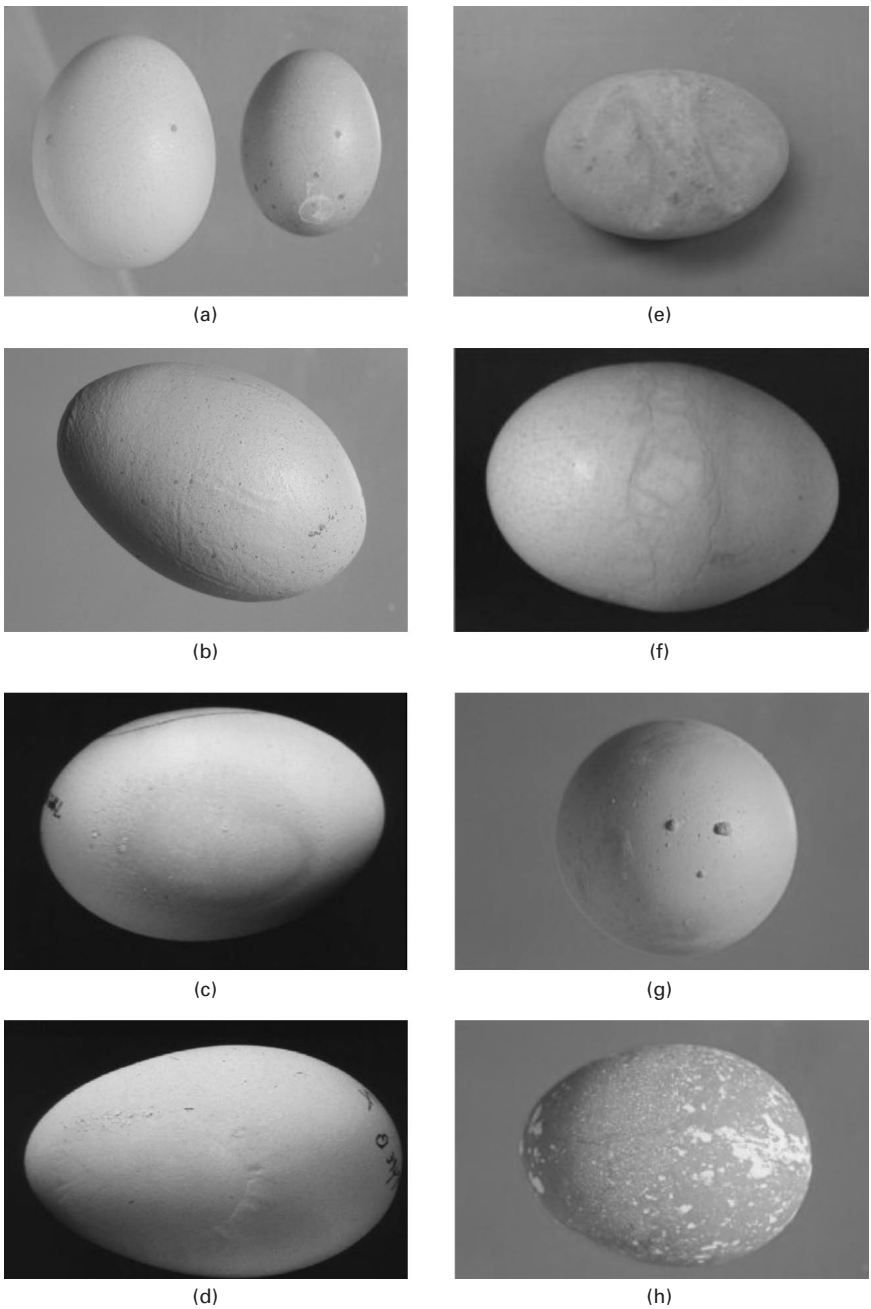

Fig. 13.1 Examples of common egg abnormalities: egg variation in size (a) and shape (b), the target (c) and slab-sided egg (d), the soft shelled egg (e), egg with an equatorial bulge (f), an egg with accretions or pimples (g) and (h) a calcium splashed egg. Source: University of Glasgow.

(Fig. 13.1g and Chapter 10). The latter is usually caused by a localised defect in the shell membranes. The aetiology of the former is not well understood but, in younger flocks, this type of defect has been associated with certain disease conditions including mycoplasma infections.

13.2.3 The effect of bird age on egg colour

Consumers prefer brown shelled eggs to white shelled eggs in many European countries and other parts of the world although, in terms of nutritional value, there is no difference between the two. In brown egg laying flocks, the colour of the shell decreases with bird age (Mills *et al*., 1991) due to an increase in egg size with no proportionate change in the quantity of pigment deposited over the shell surface (Odabasi *et al*., 2007). Many other factors can also influence shell colour. If a laying hen is subjected to environmental stress, just prior to oviposition for example, the fully formed egg in the shell gland may be retained beyond its normal time for oviposition (Hughes *et al*., 1986). During this time, the egg often receives a superficial white chalky coating (Fig. 13.1h). Certain disease conditions and heat stress can also cause a reduction in the amount of shell pigment produced (Lang and Wells, 1987).

13.2.4 The effect of bird age on eggshell quality

The percentage of eggs downgraded due to cracked and broken shells increases with bird age. At the beginning of lay, the incidence of cracked eggs is in the region of 2–5% but this can reach levels of between 12 and 20% depending on the strain of bird, the nutritional status of a flock and whether the flock is stressed (environmental or disease related) (Sauveur 1988; Nys *et al*., 2008). The recorded level may also be high partly because techniques to record shell breakage are more sensitive nowadays. In addition, not all cracked eggs are due to shell quality related problems. Malfunctions in the collection and grading equipment are commonplace and should be considered if the percentage cracks suddenly increases above normal levels for any given flock.

A decrease in percentage shell has been reported to occur in the larger eggs laid by older flocks due to a proportionately greater increase in egg weight than in that of the shell (Sauveur, 1988; Curtis *et al*., 2005; Curtis, 2008). It is noteworthy that the duration of shell formation, which influences the amount of shell, is similar in young and older hens even if the interval between two oviposition increases (Nys, 1986). However, shell thickness per se is not the only important criterion relating to the mechanical properties of an eggshell (Bain, 1992), even though there is a high correlation between the shell weight and strength (Chapter 10). The relative thickness of the mammillary layer, that of the palisade layer, and the degree of contact made between individual mammillae and the shell membranes also vary with bird age. Changes with age also affect the size of the individual calcite crystals

(increase with age) and their orientation throughout the eggshell (Rodríguez-Navarro *et al.*, 2002). All of these criteria contribute to the structural integrity and strength of an eggshell and will therefore influence an egg's ability to withstand insult during routine handling.

13.2.5 The effects of bird age on egg composition

The percentage of albumen, yolk and whole egg solids significantly changes during the production cycle (Sauveur, 1988; Curtis *et al.*, 2005; Curtis, 2008). These variations are at the origin of the main change in egg composition because of the very different composition of albumen and yolk. Genetic selection has contributed to reducing this variability in albumen/yolk ratio when current strains are compared to old lines (Curtis *et al.*, 2005).

Albumen solids decrease with age (loss of 0.5 to 1.5%). Consequently, the increase in albumen weight with age is associated with very limited changes in total albumen dry matter. In contrast, the yolk solids remain stable throughout the laying period (Sauveur, 1988).

In the egg product industry, the current percentage of dry matter of egg white (10.5 to 11%) and of whole egg (albumen and yolk together, 22.5 to 23%; SNIPO, 2008) are lower than values reported in the literature 20 years ago (12–13% for albumen dry matter and 25% for whole albumen and yolk; Sauveur, 1988). These observations might be related to the selection for higher egg number even if breeder companies take care to maintain egg composition.

The reported changes, related to bird age, in chemical composition of whole eggs are inconsistent from one study to the next. The protein and lipid content of the egg compartments (albumen and yolk) have been described as being stable whilst the cholesterol and essential fatty acids have been found to vary inconsistently in studies where the yolk weight has not been taken into account. However, when yolk weight is taken into account, the total lipid content of the egg has been reported to increase from 6 to 9 g with bird age when comparing a small and large egg at onset and end of the egg laying period (Sauveur, 1988).

The total amount of fat in the egg cannot easily be changed; however, value added products can be produced by altering the fatty acid composition of the egg through dietary manipulation (Volume 2, Chapter 14). These so-called enriched eggs are marketed on their potential benefits to human health and have become popular with consumers. Bird age, however, is again important as this can influence the rate of transfer of fatty acids into the egg contents. Scheideler *et al.* (1998) reported that the deposition of n-3 fatty acids into the egg was significantly higher (5.61%) in layers aged 58 weeks than in those aged 36 weeks (2.52%).

Albumen quality (expressed in terms of Haugh unit) decreases with flock age (Sauveur, 1988; Curtis *et al.*, 2005) from an average of 89.6 to 68.8 (Curtis, 2008). The vitelline membrane strength is also influenced by bird age.

At the end of lay, this membrane is more easily ruptured (2.33 g compared with 1.92 g) probably due to structural changes induced by the larger yolk size and, as a consequence, the yolk more often is broken during the process of albumen and yolk separation in eggs from older hens (Chapter 10).

Age-related changes to the emulsifying properties of the yolk have also been reported (Kerth *et al.*, 2005). The strength of a mayonnaise made from the eggs of a flock increases throughout the first five months of the laying period (386 to 512 g) then remains stable for the following 7 months (438 to 508 g).

13.3 Induced moulting

Wild birds naturally moult and renew their plumage after each breeding season. During this process the reproductive tract naturally regresses. In the USA, it is common practice to put commercial laying flocks through an induced moult at between 60 and 80 weeks of age. This makes it possible to prolong the life of a laying flock to 110 weeks of age or 140 weeks of age if a second moult is used (Bell, 2003). Following the moult, there is a renewal of feathering and reactivation of the reproductive organs. The birds come back into lay and both production levels and egg quality are improved to levels similar to those in younger flocks (Fig. 13.2). Food efficiency and mortality rates are also improved (Mansoori *et al.*, 2007). An induced

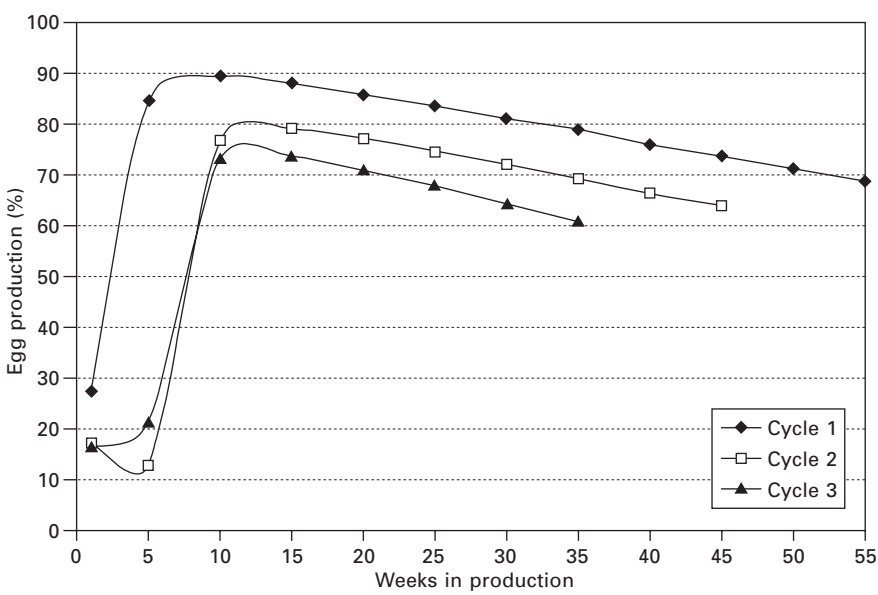

Fig. 13.2 Production curves for a laying flock during the first, second and third lying cycles after moulting. Source: Bell (2003).

moult is usually carried out in response to a decline in flock performance and deterioration in egg quality. The price of feed, eggs, replacement pullets and a reduced investment in breeder farms, rearing farms and hatcheries, particularly in the USA, provide further justification for prolonging the life of laying flocks in this way.

The process of moulting a flock and the birds' subsequent recovery is physiologically highly complex as it involves the endocrine system, the reproductive tissues, lymphoid tissue and the immune system (Berry, 2003; Webster, 2003). The duration of the moult, the level of mortality experienced, and the period of time taken to bring a flock back into production is also dependent on the age of the flock when the moult is initiated, the treatment used and the length of the treatment (Berry, 2003).

A moult of 4 to 6 weeks' duration can be induced in a laying flock in a number of ways. Methods used include feed removal or limitation in energy level or diet rich in fibre (sometimes used in combination with a short photoperiod); the provision of a feed low in sodium and calcium or low in energy and/or a feed rich in fibre (by products of other crops, wheat and rice bran, cottonseed); the provision of a feed in which the levels of zinc, iodine and aluminium are in excess (Bell, 2003). An alternative strategy is to treat the birds daily with thyroxine T4 for about 12 days (Kuenzel *et al.*, 2005).

Methods which require feed or water to be removed or limited for a period of time can result in birds losing up to 30% of their body weight and can cause mortality rates of between 1 and 1.5%. Severe rationing of laying hens in this way is prohibited in most European countries and in the USA (United Egg Producers) as it is considered to be cruel. The provision of a feed low in sodium and calcium or low in energy or high in trace minerals also considerably decreases feed intake and body weight and, similarly to feed withdrawal, perturb hen physiology and behaviour (Webster, 2003). Therefore numerous experiments have recently been conducted to develop alternative moulting programmes that do not rely on withdrawal of feed (Landers *et al.*, 2005; Koelkebeck *et al.*, 2006; Yousaf and Chaudhry, 2008; Aygun and Yetisir, 2010; De Souza *et al.*, 2010). These studies indicated that qualitative feed restriction by providing wheat middlings or bran, soybean hulls, barley or oats combined with alfalfa and rice hulls could be successfully used to moult laying hens.

During the moult, egg production typically falls to about 5% of the possible per day. This continues for about 3 weeks after which time the production gradually improves to a level higher than that observed before the moult. Peak production (75–85% in the second laying cycle and 74.7% in the third laying cycle) is typically reached after about 13 weeks, but persistency in lay is lower than in the first laying cycle. The production curves follow a similar trajectory for the first and second laying cycles; however, in the third cycle, the rate of decline in production is usually more rapid (Fig. 13.2).

Egg quality is also improved for months following the moult but the

decrease in quality with age is often more rapid during the second laying cycle (Bell, 2003; Gordon *et al*., 2009). Egg weight is typically between 63 and 65 g following a moult, which is 4–5 g heavier than that observed in the same flock at the beginning of their first laying cycle (Sauveur, 1988; Ahmed *et al*., 2005). Egg weight typically reaches a maximum in the second and third laying cycles by about the 4th month following the moult (Fig. 13.3).

The eggs laid at the beginning of the second laying cycle tend to have larger yolks and as a result they retain the same low yolk to albumen ratio as those laid prior to the moult. Eggs from a second cycle are therefore of interest when looking for eggs with large amounts of yolk. Albumen quality is also initially improved but the rate of decline is more pronounced in the second laying cycle (Sauveur, 1988).

There are many published accounts which show an improvement in eggshell quality after an induced moult (Garlich *et al*., 1984; Bell, 2003). Most studies report an improvement in shell thickness but the improvement can sometimes be very subtle. Roberts and Brackpool (1994), for example, found an increase in early fusion at the level of the mammillary layer rather than an overall change in the total thickness of eggshells following a moult. Likewise, Ahmed *et al*. (2005) reported an improvement in the mechanical properties of eggs following a moult and linked this to a decrease in the size of calcite crystals within the shell. A change in the composition of the organic matrix following the moult was also reported by these authors who observed an increase in the proportion of 'eggshell-specific matrix' proteins relative to other matrix proteins.

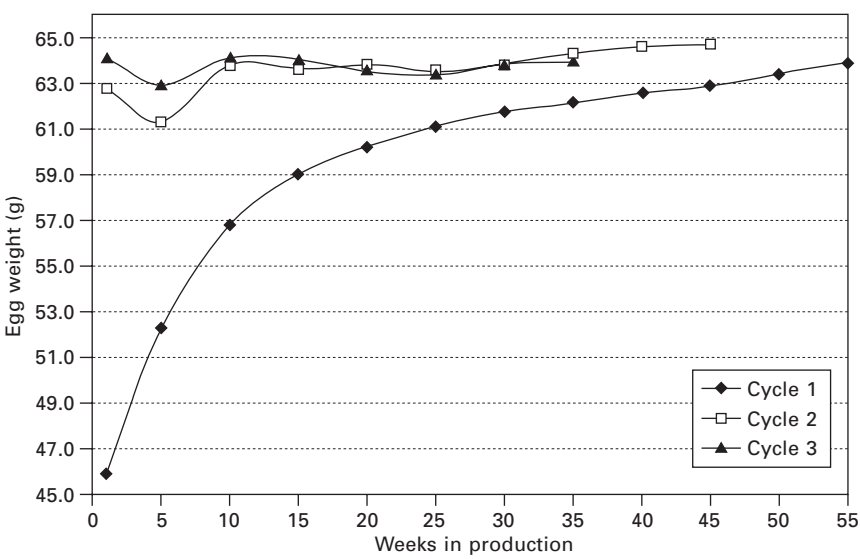

Fig. 13.3 Change in egg weight during the three consecutive laying cycles in a molted flock of laying hens. Source: Bell (2003).

13.4 The laying environment

Temperature, lighting programme (intensity and duration) and housing system can influence the bird's physiological status and as a consequence will influence the quality of eggs produced. Part of the effect of these parameters, however, is often explained by a concomitant change in feed consumption.

13.4.1 Temperature

The optimum temperature (or thermal neutral zone) for laying hens is in the region of 22–24 °C (Mardsen and Morris, 1987). Hens have a better tolerance to lower temperature than to high temperature even though low temperatures can cause digestive pathology and higher mortality. Exposure to temperatures greater than 29 °C, even for a short period, will result in a change to the bird's metabolism and a reduction in food intake. This is accompanied by a decrease in egg production and egg quality (Mardsen and Morris, 1987; Sauveur, 1988; Picard *et al.*, 1993). Moreover, prolonged exposure to excessively high temperatures (42 °C) can be lethal (Yahav, 2009). The detrimental effects of high temperatures are most pronounced when combined with a high degree of humidity but both can be alleviated by increasing the ventilation of the poultry house. Both these parameters influence the ability of the hen to eliminate high body heat by respiratory hyperventilation (Balnave and Brake, 2005).

Effect of temperature on bird physiology

The normal body temperature of a laying hen is 41 °C. The thermal neutral zone (defined as the environmental temperature which does not modify the body temperature) ranges between 10 and 20 °C. Commercial layers therefore have a broad tolerance within which their body temperature will remain stable. In terms of optimising productivity, however, the temperature range is restricted to the temperature at which the bird will consume sufficient feed. At low temperatures (<10 °C), hens tend to overeat and, conversely, to reduce feed consumption at high temperature (>22–25 °C).

Birds use a variety of ways to control their body temperatures but, unlike mammals, this does not involve the production of sweat. When exposed to a warm environment, a hen will initially redirect up to 45% of its total blood flow away from the visceral organs to the skin, especially featherless areas where excess heat can be lost through radiation and convection (Sauveur, 1988; Yahav, 2009). Prolonged exposure to high temperatures (>29 °C or at 27 °C when the relative humidity is also elevated) results in an increase in heart rate and respiratory rate (160 cycles per minute compared with 30 cycles per minute during normal breathing). Excessive heat is thereby lost through evaporative cooling (6 kcal per 1 g of evaporated water) from the upper respiratory tract. This mechanism is more efficient when hens are in a low humidity atmosphere. Prolonged respiratory hyperventilation (thermal

panting) can lead to hens becoming dehydrated (5–18 g of water/hour are lost) if they are not given access to sufficient water to compensate for this water loss.

Hyperventilation also causes excessive CO_2 to be eliminated from the body which in turn results in an increase in blood pH (respiratory alkalosis). To compensate for this, the kidneys eliminate bicarbonate ions. This, together with the increased loss of CO_2 due to hyperventilation, reduces the availability of bicarbonate ions for use as precursors of the carbonate fraction required for shell formation (Mongin, 1978). A decrease in blood flow to the uterus (40%, Wolfenson *et al.*, 1981) at the same time means that shell formation will be disrupted when birds are exposed to high environmental temperatures.

Birds have a limited capacity to eliminate heat and therefore reduce their own metabolic heat production (thermogenesis) when they experience heat stress. This is achieved mainly through a reduction in feed consumption which results in lower heat production during intestinal digestion of feed. This process is regulated by thyroid hormones and the sympathetic nervous system and will decline in response to the duration and the level of heat stress to prevent excessive accumulation of heat in the body (Yahav, 2009). When birds experience acute heat stress, there is an immediate reduction in the production of the main metabolism stimulating hormone (triiodotyronine, T3) since there is less deiodination of thyroxine hormone (T4) to T3 (Yahav, 2009) in the peripheral tissues. Food intake and the digestibility and metabolic use of the nutrients in the food are therefore depressed under these conditions (Picard *et al.*, 1993).

Effect of temperature on feed consumption

The maintenance requirement for laying hens, which influences feed consumption, decreases by 4% per °C at temperatures higher than the thermal neutral zone. The decrease, in feed consumption, results in a decrease in egg weight which is more pronounced when hens are exposed to ambient temperatures higher than 30 °C.

The relationship between environmental temperature and energy balance in laying hens is curvilinear (Mardsen and Morris, 1987). According to these authors, there would be a 1–1.5% decrease in feed consumption for each degree increase between 20 and 30 °C and this would rise to as much as 5% per degree increase between 32–38 °C. The magnitude of decrease in feed intake is therefore four-fold higher when birds are exposed to high temperatures compared with those maintained in their thermal neutral zone (Picard *et al.*, 1993). Birds also consume more water when the temperature is elevated higher than 20 °C. Between 21 and 32 °C, the consumption approximately doubles, and above this it triples (Balnave and Brake, 2005). The effects of temperature on production parameters and feed consumption are most pronounced when high temperatures are maintained but will be much less when the exposure is only of short duration (cyclic). Efficient ventilation in combination with evaporative cooling can help to minimise the

negative effects of sustained high temperatures by varying the temperature gradient within the poultry house (Balnave and Brake, 2005).

Effect of temperature on the rate of lay and on egg weight

Egg production is maximised in the thermoneutral zone, provided the birds are fed an optimum diet, but this changes dramatically when hens are exposed to elevated temperatures (drop of 20% of egg production at 30 °C; Sauveur, 1988; Balnave and Brake, 2005). Production peaks at a lower level and the age-related changes in production are also accelerated. Feed containing high levels of energy can alleviate the reduction in egg production until 25 °C and even 29 °C in white feathered birds. At moderate levels of thermal stress, it is also possible to compensate for the negative effects of high temperatures through dietary manipulation of protein levels and essential amino acids (Balnave and Brake, 2005). However, at very high temperatures, an increase in dietary energy or protein is not sufficient to prevent a decline in egg production (Picard *et al.*, 1993). Indeed, too much protein can worsen the situation if the essential amino acid content is not adjusted accordingly.

High temperatures (changing between 27 and 33 °C throughout the day) introduced at 18–28 weeks of age decrease egg weight relative to the standard performance of brown hens (Fig. 13.4) due to the concomitant decrease in feed consumption without affecting egg production (Travel *et al.*, 2009). At elevated temperatures, egg weight reduces in a curvilinear manner. Above 25 °C, the reduction in egg weight corresponds to 0.4–1 g/°C and, according to Smith and Oliver (1972), egg weight decreases according to the following formula:

$$\text{Egg weight} = 59.6 - 1.34\{0.2[(T - 32)*0.555] - 16\} - 0.313\{0.2[(T - 32)*0.555] - 16\}^2$$

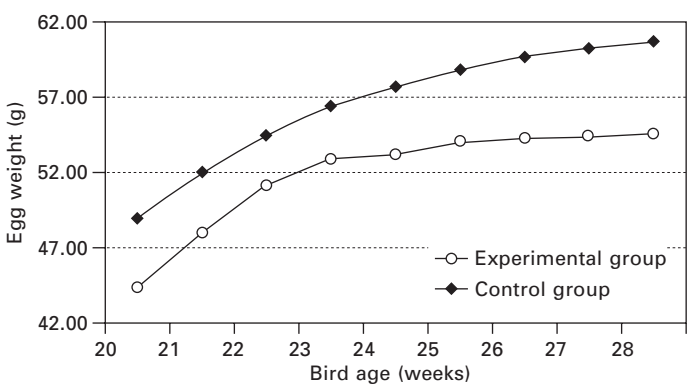

Fig. 13.4 Change in egg weight in a strain of brown laying hens submitted to high cyclic temperature of 27 °C and 33 °C in every 24-hour period. Source: Travel *et al.* (2009).

where egg weight is in grams and T is the temperature in degrees Celsius. The equation in this form looks complex but this comes from the need to integrate the relationship between °C and °F into the formula i.e. °C = (°F – 32)*5/9).

These authors, by using paired feeding of hens (control versus experimentally restricted to dietary level of hens submitted to high temperature), demonstrated that egg weight and production were depressed by the reduced feed intake rather than by any detrimental physiological change induced by the high temperature which caused changes to other quality parameters (including shell quality).

By manipulating the energy and crude protein content of the diet, the decrease in egg weight observed at higher temperatures can to some extent be attenuated (by 10 to 15%; Joly, 2003). Increasing the recommended levels of essential amino acids, of linoleic acid and supplementation of the drinking water with micronutrients can also have some beneficial effects when birds are kept in such conditions, although the results are not always consistent (Balnave and Brake, 2005; Lin *et al*., 2006). Interestingly because of the reduced food intake and despite the reduction in egg weight, the feed conversion ratio can actually be lower for heat stressed birds.

Effect of temperature on the albumen and yolk

At elevated temperatures, there is a rapid decline in albumen weight (Fig. 13.5). A noticeable effect on the yolk takes longer to become established (usually 6–7 days after the introduction of the heat stress), this being related to the hierarchical way in which the ova develop in the ovary (Sauveur and Picard, 1987). The relative proportions that the yolk and albumen each contribute to the egg's dry matter content are generally unaffected when laying hens are exposed to environmental temperatures lower than 35 °C. Albumen

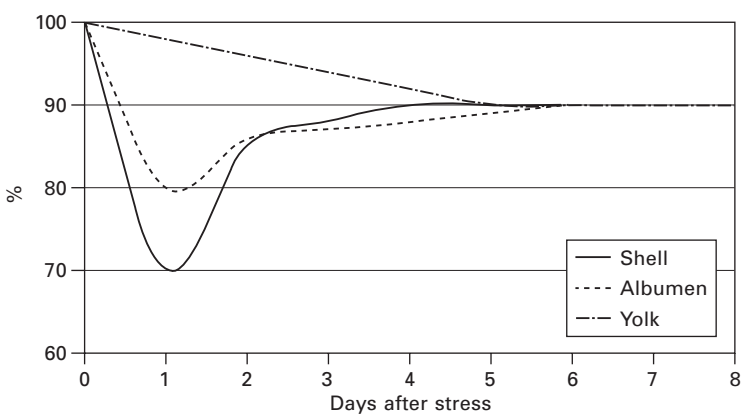

Fig. 13.5 Relative change (%) in the egg components (yolk, albumen, shell) in hens submitted to high environmental temperatures compared to the control level (100%). Source: Sauveur and Picard (1987).

quality (measured in Haugh units) at oviposition is also comparable but if the egg remains in the hot environment for too long, then the albumen quality rapidly deteriorates. This deterioration, however, is due to inappropriate egg storage conditions rather than due to a change in the bird's physiology (Sauveur and Picard, 1987).

Effect of temperature on eggshell quality

Nys (1995) provided an estimate of the effects of high temperatures (32–35 °C compared with 21–24 °C) on eggshell quality, food consumption and egg weight by comparing the results of 10 different studies reported in the literature from 1972 to 1993. These were as follows: shell quality decreased by between 6 and 30% (with an average of 9% $n = 10$ studies), food consumption from 17 to 34% (with an average of 24.5%, $n = 9$) and egg weight 6–13% (with an average of 8.4% $n = 8$ studies). The decrease in calcium intake associated with reduced feed consumption explains the lower shell quality. Eggshell quality is less affected if birds are subjected to cyclical rather than constant high temperatures (Balnave and Brake, 2005). However, large and rapid fluctuations in temperature should be avoided as this will have an immediate and noticeable effect on eggshell quality, long before any changes in feed intake become apparent. That is due to metabolic change such as respiratory alkalosis or reduced blood supply to the uterus.

Feeding a calcium coarse particle source can help to stimulate the appetite of birds suffering from heat stress (Picard *et al.*, 1993). Indeed, there are over 300 studies in the literature on this topic, and of these 75% report an improvement in shell quality when heat stressed birds were provided with calcium particles between 1.5–3 mm in diameter (Nys, 1999). A short burst of light during the dark period when the egg is forming can also improve both egg weight and shell quality in heat stressed hens (Grizzle *et al.*, 1992). The burst of light allows the birds to eat food and calcium during the dark hours when the eggshell is forming and favour supply of dietary mineral precursors to the shell.

Supplementing the diet with sodium bicarbonate (1%) has little beneficial effect on eggshell quality in heat stressed hens (Balnave and Muheereza, 1997) but some improvement has been reported when carbonated drinking water is provided (Odom *et al.*, 1985). The addition of vitamins A and C to the drinking water may also be beneficial but the supporting evidence for adding vitamin E is more convincing (Lin *et al.*, 2006).

13.4.2 Lighting programmes

There have been many reviews on this topic in the literature, including those by Lewis and Perry (1995), Sauveur (1996) and Morris (2004). The information presented in this section summarises the main findings of these reviews which relate specifically to the effects of lighting on egg quality.

Modern commercial layers have been specifically selected to have an

extended egg laying season, a trait which makes them less sensitive to seasonal effects than wild birds; however, they still respond to changes in both the duration and intensity of lighting received during the rearing and production period. Sexual maturity and the timing of oviposition reflect the previous ovulation and are dependent on lighting programmes, but a modern hen selected for high egg production will not stop laying even if the daily photoperiod is shortened. However, the interest in ahemeral lighting cycles which can contribute to egg quality has declined due to EU rules banning the introduction of light in the middle of the conventional night.

Birds perceive light intracranially, although ocular pathways also play an important role in establishing the synchronisation rate and pattern of ovipositions within a flock via the use of periods of light and darkness. The intensity of the light as well as the number of hours of light received is important, with most flocks receiving between 15 and 17 hours of light within each 24 hour period at an intensity of between 10 and 15 lux. This level of lighting helps to minimise the activity of the birds so that more energy can be directed towards producing eggs (Sauveur, 1996; Morris, 2004). However, a minimum of 20 lux is compulsory in the EU.

Effect of lighting programmes on controlling sexual maturity

The importance of controlling the sexual maturation of pullets during the rearing phase cannot be overstressed. If pullets become sexually mature before they have attained the optimum body weight during the rearing phase, then egg production and quality will be irreversibly affected. Birds which come into lay too soon typically produce small eggs with brittle shells, they exhibit disorders in oviposition such as double ovulations, and they also have higher mortality rates. The age at which sexual maturity is reached is therefore strictly controlled during the rearing period using a combination of lighting and nutritional targets set by the breeding company.

Effect of lighting programmes on egg production

Standard lighting programmes make use of only one photoperiod in every 24 hour period and work best in windowless houses. Egg production is then dependent on the birds receiving a clearly defined period of between 13 and 15 hours of constant light once the lights are turned on. There does not seem to be any benefit in extending the light period to beyond 15 hours nor does there seem to be any benefit in having intermittent dark periods during the light phase (Sauveur, 1996). Indeed, the frequency of body checked eggs (equatorial bulge) is higher when birds were exposed to an 18 hours lighting programme, probably because the birds were still active during the initial stages of shell formation (Roland, 1982). Any sudden surge in activity during this phase will cause the then fragile shell in the uterus to crack. With a 15 hour programme the hen would normally be in darkness during this critical phase (Sauveur and Picard, 1987).

There are benefits to progressively increasing the length of the light period

(step up) when young pullets are first coming into lay. A step up programme during this phase will result in peak production lasting longer and a higher egg weight at the end of lay (Morris, 2004).

Ahemeral lighting programmes

These types of lighting programme make use of light and a dark cycles which have a periodicity of either more than 24 hours (e.g 18L:10D) or less than 24 hours (e.g. 14L:7D).

Ahemeral lighting programmes > 24 hours

The number of eggs laid decreases linearly as the photoperiod increases (Sauveur, 1996). However, egg weight increases as each egg spends longer in the oviduct. Sauveur and Picard (1987) estimated that the increase in egg weight would be 4.5% for a 26-hour cycle, 6.8% for a 27-hour cycle and up to 11% for 28-hour cycle. The weights of the different components of the egg also increase but albumen quality (expressed in Haugh units) can decrease (Shanawany, 1990). The reduction in egg number has limited the use of ahemeral programmes which are >24 h in the commercial sector. However, the benefits of employing this type of programme at the end of the laying cycle to correct for a problem in shell quality should not be overlooked.

Ahemeral lighting programmes < 24 hours

This type of lighting pattern is of benefit to those individual hens within any given flock which are capable of producing an egg in less than 24 hours. These individuals cannot express their ability to lay an egg in a shorter interval than 24 h when they are kept on traditional 24 hour lighting programmes because the stimulus to ovulate in these individuals will eventually be too late to elicit ovulation of the mature ovocyte. By shortening the lighting programme, these individuals can realise their full potential. Ahemeral lighting programmes < 24 h are particularly useful for identifying individual pedigree layers to use in selection programs for improved productivity (Shanawany, 1990).

Split lighting programmes

There was considerable interest 30 years ago in using split lighting programmes as a means of exerting some control over the amount of food layers consume. Two types of split lighting programmes exist, examples of which are presented in Fig. 13.6 and described in detail by Sauveur (1996).

Type 1 Split lighting programmes (SLP1)

Also known as symmetrical split programmes, these consist of light and dark sequences of short duration which are regularly repeated every 24 hours. As there is no defined day or night period, oviposition becomes asynchronous. A reduction of about 2–4% in total production can occur if this type of programme is applied at the onset of lay, but egg weight, as well as the relative weights of the yolk, albumen and shell are all increased. Programmes

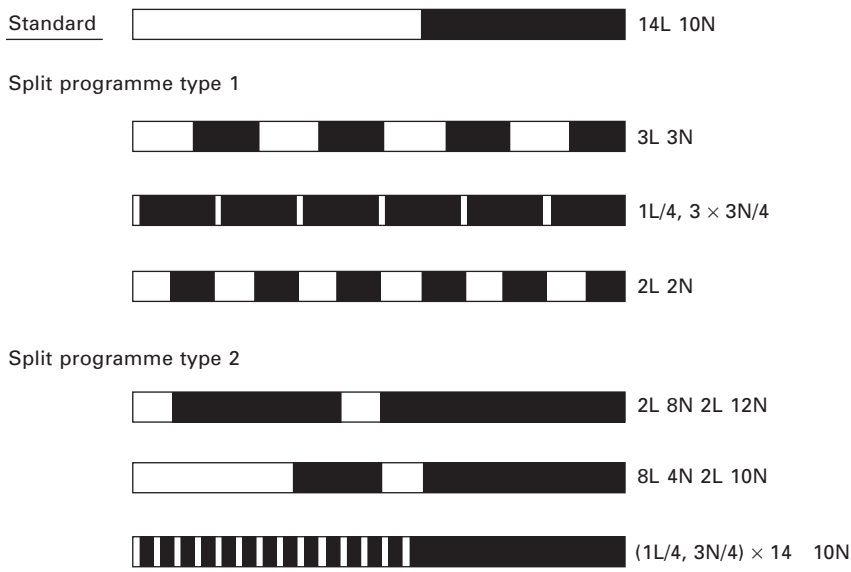

Fig. 13.6 Examples of split lighting programs for layers. All these examples fit into a 24 h period or sub-multiple thereof (white = light period, black = dark period). Source: Sauveur (1996).

of this type allow the bird to have continuous access to dietary sources of calcium when the eggshell is forming (a process which normally takes place in the dark) and, as a result, the birds is less reliant on the mobilisation of calcium from the skeleton to augment this process. One might therefore expect osteoporosis to be less of a problem under these conditions.

SLP1 programmes are relatively easy to set up in windowless houses and do not have any major drawbacks in terms of the stock worker performing routine tasks in a normal working day. The hens' response (Table 13.2), however, very much depends on the house being completely light and sound-proof. From a practical point of view, it is also possible to incorporate this kind of lighting programme for a limited time to correct a problem with egg weight or shell weight without noticeably disturbing egg production.

Type 2 Split lighting programme (SLP2)

Also known as asymmetrical programmes, these are characterised by having short bursts of darkness while the lights are on and a clearly defined dark period of about 8–10 hours to allow for synchronisation of oviposition (as in traditional programmes). These programmes were originally developed as a tool to control feed intake rather than to improve productivity or egg quality. The most popular example of an SLP2 programme is known as the biomittent programme in which the 16 hours of lights on is subdivided into periods of 15 min lights on and 45 min of lights off. Compared with SPF1 programmes (Table 13.3), egg weight and shell thickness are slightly depressed when a

Table 13.2 Change in hen performance and egg quality in hens submitted to different split lighting programs (SLP1) for a whole laying period of 20–28 and 60–72 weeks (% change relative to control)

Programme	Eggshell colour[1]	Laying rate	Eggshell quality			Haugh unit
			Eggshell % density or index	Eggshell weight or thickness	Egg breaking strength	
2L:2D[2]	W	– 6.7		+ 6.0*		– 2.5*
2L:4D[2]	W	– 4.1		+ 5.4*		– 1.9*
2L:6D[2]	W	– 2.0		+ 4.2*		– 1.7*
4L:2D[3]	W	– 11.0	+ 5.6*	+ 9.7*	+ 7.8*	– 1.5
4L:2D[3]	W	– 11.0*	+ 3.0*	+ 7.5*	+ 1.1	0
8L:4D[3]	W	– 6.8*	+ 4.3*	+ 7.5*	+ 3.7*	– 1.5
1.5L:4.5D[4]	B	– 6.5*	+ 6.7*	+ 9.6*	+ 13.6*	
3L:3D[4] [5]	B	– 5.5*	+ 4.3*	+ 7.7*	+ 11.0*	

**Statistically significant change compared to control.*
[1]*B =white shell; C = brown shell.*
[2]Cooper and Barnet (1977); [3]Torges *et al.* (1981; [4]Sauveur and Mongin (1983).
[5]*Progressive evolution of the lighting programme until 1.5L:4.5D.*
Source: Sauveur and Picard (1987).

Table 13.3 Performance and egg quality of hens submitted to three split lighting programmes between 23 and 72 weeks of age

	Lighting programmes		
	14(1/4L:3/4D) 10D Asymmetrical split programmes	4(3L:3D) Symmetrical split programmes	24(0.25L:0.75D) Symmetrical split programmes
Egg production (%)	73.9	72.1*	72.3*
Egg weight (g)	62.1	63.6*	63.5*
Daily egg output (g/hen)	49.5	49.4	49.5
Feed intake (g/hen/d)	109	112*	108
Shell thickness at 65 weeks of age (mg/cm^2)	79.3	82.3*	81.5*

*Significant differences among lighting programmes.
Source: Morris (2004).

biomittent programme is used, but the rate of lay is increased. The overall egg mass is therefore comparable between the two different types of split programme (Morris, 2004).

Split lighting programmes (Types 1 and 2) have been shown to improve bird health by decreasing their susceptibility to tick and lice infestation. There is also evidence that, under these conditions, the birds are more physiologically able to cope with thermal shock. Within Europe, however, Directive 1999/74/CE requires birds to receive at least 8 hours of uninterrupted darkness in every 24 hour period. This has effectively prohibited the use of symmetrical split programmes in Europe. Asymmetrical split programmes

have also fallen out of favour mainly because rationing of feed is no longer necessary now that hens have considerably lower feed consumption due to selection.

13.4.3 Housing system

In response to criticism relating to the welfare of laying hens, the European Commission (Directive 1999/74/EC) is introducing a ban on the use of conventional cages by 2012 in favour of furnished cages and floor-based systems with or without access to an open range. At the time of writing, about a third of the European (27 countries) laying hen population is held in free-range systems but this proportion varies from country to country (see Chapter 1). Furnished cages are also being phased in but the optimum design of these cages is still under investigation. The influence of alternative systems relative to conventional cages on the hygienic quality of egg is analysed in Chapter 15.

Comparison of conventional cages with floor-based systems with and without access to the outdoors

As long as the feed is consistent, the total composition of the egg should not vary between systems of management. However, where there is access to an outdoor range, soil, grass insects and worms often supplement the standard ration and differences in quality are to be expected. In some cases, however, access to range may also expose the birds to persistent organic pollutants (Volume 2, Chapter 4) leading to contamination of the contents.

Eggs from conventional cage systems are typically 1–2% heavier than those from alternative systems but this is as high as 10% in some farms due to differences in management (Sauveur, 1991). When present, a reduction in egg weight is concomitant with a lower yolk weight. The major components of the egg including the lipid, protein and dry matter content, however, appear to be fairly consistent between systems (Sauveur, 1991; Hidalgo *et al.*, 2008) but the fatty acid composition and some of the minor components of the egg are much more variable (Sauveur, 1991; Rossi, 2007).

There is a lot of inconsistency when it comes to comparing albumen quality in eggs from cages and floor-based systems. This could be due to a variation in the time spent by the hens in contact with their eggs. In cages, the sloping floor prevents prolonged contact, but in floor-based systems the time spent by hens inside the nest can vary, and so the newly laid egg will take longer to cool to ambient temperature.

The yolk colour is directly dependent on the laying hens' diet which is principally provided via the ration, which can contain a large amount of carotenoids, but is also influenced by whether the hen has access to outdoor pastureland (Chapter 12). The colour of the yolk is therefore generally more intense when hens have access to pastureland. However, conventional eggs have a more intense yolk colour in countries where consumers are used

to highly coloured yolk because of larger addition of dietary carotenoids including red carotenoids (Chapter 12). Organic eggs are often less coloured due to the restrictions placed on the supplementation of the standard feed in these systems with the red carotenoids.

The data relating to shell colour, and the number of egg inclusions (blood and meat spots), in eggs from different housing systems are difficult to interpret (Sauveur, 1991). It is unrealistic, however, to expect the eggs from any system of management to be a uniform colour, as the amount of pigmentation deposited into the shell has been shown to be highly dependent on the individual hen laying the egg. Nevertheless, instruments are available to measure the colour of the shell and for some niche markets this will be used to meet the standards set by the retailer.

The data from different studies comparing shell quality are also difficult to interpret (Sauveur, 1991). However, there is some evidence that the number of cracked or broken shells is attenuated in free-range and floor-based systems at the end of the laying period (Sauveur, 1991). Indeed, a small but significant increase in breaking strength in free-range eggs has been reported in some cases probably due to the birds consuming more feed to compensate for the exposure to lower temperatures in these systems. This, however, has not been confirmed in more recent work (EFSA, 2005; Michel *et al.*, 2007). In floor systems, the incidence of floor eggs and dirty eggs in aviaries, which is usually in the region of 2%, can also vary considerably between individual sites (Fiks-van Niekerk, 2005).

In summary, differences in strain, age and nutrition (both in terms of the ration provided and that available to the birds when they are on range) make scientific conclusions about the effects of housing system on egg quality exceedingly difficult. Consumer perceptions of these alternative systems delivering a better product are therefore scientifically unjustified in terms of there being any nutritional or quantifiable difference. Moreover, the lack of consensus in the literature highlights the fact that the most important criteria determining egg quality are often the standard of husbandry delivered by the stockman rather than the system itself.

Comparison of conventional cages and furnished cages

At the time of writing, the EU still intends to ban the use of conventional cages for laying hens by 2012. Cages equipped with perches, a nest and a dust bath will replace the majority of conventional cages, although some producers are switching to alternative floor-based systems. Zootechnical information and egg quality data are available from furnished cage systems set up in France (Mirabito *et al.*, 2005, 2007; Michel *et al.*, 2007), Sweden (Tauson *et al.*, 2002), Germany (Rauch *et al.*, 2002) and the United States (Appleby *et al.*, 2002). These studies confirm that productivity and mortality are comparable in furnished cages to that in conventional cages but there is a higher risk of death from the outbreak of injurious feather pecking in furnished cages where the colony size is large (Michel *et al.*, 2007). Mirabito

et al. (2005, 2007) reported a decrease in egg weight of about 1.2% in their first trial in a new installation but this was not repeated in subsequent trials. This highlights the need to allow time to develop best practice when installing one of these new systems.

Some of the early studies on furnished cages also report an increase in broken and cracked shells (4–34%) and a higher incidence of dirty eggs (7–26%). This has usually been attributed to a large number of eggs being laid out of the nest and difficulties in maintaining cage hygiene (Rauch *et al*., 2002; Tauson *et al*., 2002; Mirabito *et al*., 2005, 2007, Michel *et al*., 2007). Differences in the design of the nestbox, the type of nest, its position in the cage, the light intensity inside the nest, and the nesting material all have an effect on whether the birds make use of the nestbox area. Over-use of the nest can also be problematic as this can lead to an accumulation of eggs in one area in front of the cage. This increases the risk of hairline cracks forming as a result of egg to egg contact and damage induced by the birds (Mirabito *et al*., 2005). The design of the cage, colony size, the height and position of the perches, the presence of a wire to reduce the speed at which the eggs roll away from the nestbox, and the speed and timings of the automated collection belts also have an impact on the frequency of dirty and broken eggs in furnished cages. Despite these shortcomings, most of the major cage manufacturers are now making furnished cages which include many of these features in their design. However, it still remains to be established what the optimal group size should be in order to maintain bird condition without the risk of birds becoming feather pecked, and how best to optimise cage hygiene. Strategies are also still being developed on how best to integrate the dust bath and optimise the use of the nest (Bignon *et al*., 2009) to maximise production and egg quality.

13.5 Effects of egg handling and storage on egg quality

An egg's physical appearance, namely its soundness, smoothness, uniformity in size and colour, and cleanliness, are the main criteria used by consumers to determine quality at the time of purchase. The freshness of an egg is more difficult to assess without first having to break open the egg. Even then the freshness of an egg is difficult to quantify and is usually judged by reference to colour of the yolk and the consistency and clarity of the albumen.

13.5.1 Incidence of downgrading due to cracked and broken shells

Misshapen, dirty, broken and cracked eggshells are usually downgraded either at the farm or at the packing station. Downgrading due to broken and cracked eggs can vary from as low as 1–2% to as high as 35% depending on the age of the flock but also on the insults experienced throughout the logistical chain.

Mertens *et al*. (2006) monitored the critical points for eggshell breakage in four logistic chains with different housing systems and found that the highest percentage of breakages occurred in cage based systems (6.73% furnished cage, 10.72% conventional cage, compared with 1.94% aviary and 1.99% free range). Further investigation revealed a design problem with the furnished cage system while an operational problem associated with the collecting belts was blamed for the higher than expected levels of breakages in the conventional cage system. This study served to highlight the need to minimise the impacts experience by an egg during routine handling as an egg is ultimately only as strong as the impact it receives.

Automatic crack detection technologies have improved the detection of both cracked and dirty eggs during grading but the throughput of the modern packaging station requires these machines to operate at very high speeds. Thus false rejects, errors in detection and further breakages can sometimes occur (from 1.50 to 2.65%) (Mertens *et al*., 2006; Chapter 10).

The relationship between the number of cracked eggs on sale in a number of retail outlets and the presence of bacteria in the albumen was recently investigated by Widdicombe *et al*. (2009). According to these authors there were substantial differences between supermarkets in the prevalence of eggs with cracked shells (average 7%, highest 17%). More importantly the incidence of Gram negative bacteria was higher in the cracked vs. non-cracked eggs. Cracked and damaged eggshells are therefore not just an inconvenience, they are also potentially hazardous to consumer safety.

13.5.2 Effects of storage on the freshness of the egg contents

An egg is at its optimum in terms of quality and freshness immediately after it is laid. As the egg cools to ambient temperature the contents contract, and the two shell membranes separate at the broad pole to form the air cell. Thereafter, water and CO_2 are continuously lost by evaporation through the gaseous exchange pores of the shell. As a result, the air cell gets larger, egg weight decreases and changes to the consistency and chemistry of the egg contents take place. Eggs which have been stored for a period of time therefore exhibit higher albumen and yolk pH, a lower Haugh unit, a lower yolk index, a decreased albumen viscosity and have a larger air cell. The strength of the vitelline membrane also declines, making the yolk more susceptible to breaking during egg separation (Kirunda and McKee, 2000; Jones *et al*., 2002).

13.5.3 The effect of storage temperature and relative humidity on egg quality

The age of the bird is the single most important factor affecting egg weight and the albumen quality of a freshly laid egg (Fig. 13.7). The maintenance of egg weight and albumen quality after oviposition is then dependent on

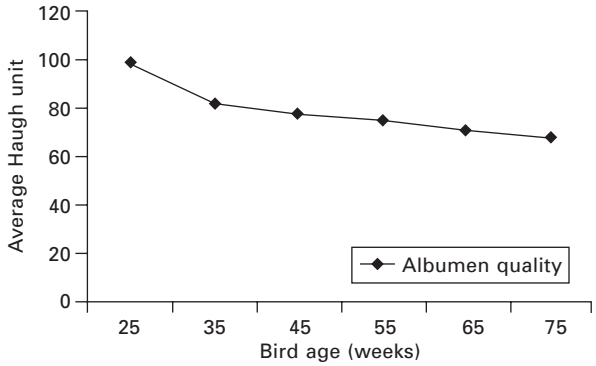

Fig. 13.7 The influence of bird age on albumen quality. Source: DEFRA (1983).

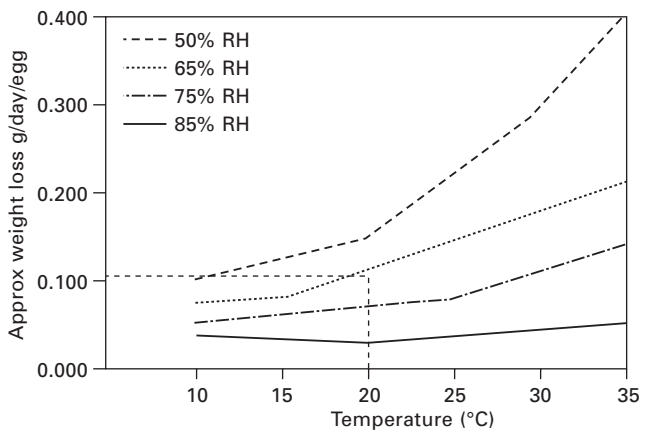

Fig. 13.8 The effect of different storage temperatures and relative humidities on egg weight loss. (g/day/egg). Source: DEFRA (1983).

the egg being cooled quickly and then held at an appropriate temperature and relative humidity (Figs 13.8 and 13.9).

Jones and Musgrove (2005) reported that albumen quality and vitelline membrane strength of eggs can be preserved for up to 10 weeks if eggs are stored at 4 °C and 80% RH, which is well beyond their normal shelf-life. Without refrigeration, the rate of albumen quality loss (Haugh unit) is greatly influenced by the storage temperature and the relative humidity. For eggs stored at an average temperature of 16 °C, the drop in Haugh units is about 2 units per day as compared with 0.5 units for eggs stored at 10 °C. It is therefore generally recommended to keep eggs at 10 °C or less (but not as low as 0 °C) and a relative humidity of 80–85% if they are to be stored for any length of time (Williams, 1992). A relative humidity greater than 85% is to be avoided as this leads to moisture condensation on the shell which encourages the growth of mould.

As the internal temperature of the egg increases above 7 °C, the protein

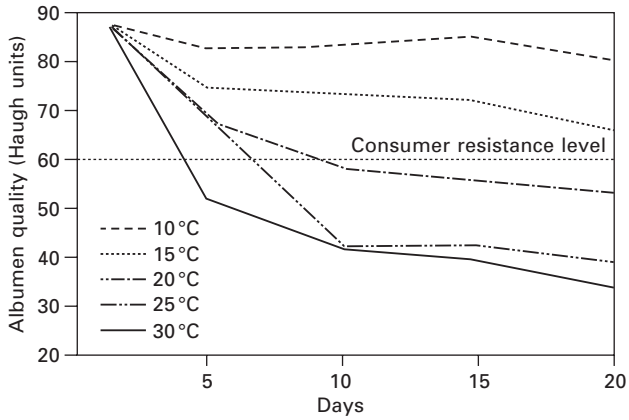

Fig. 13.9 The effect of different storage temperatures and relative humidities on albumen quality. Source: DEFRA (1983).

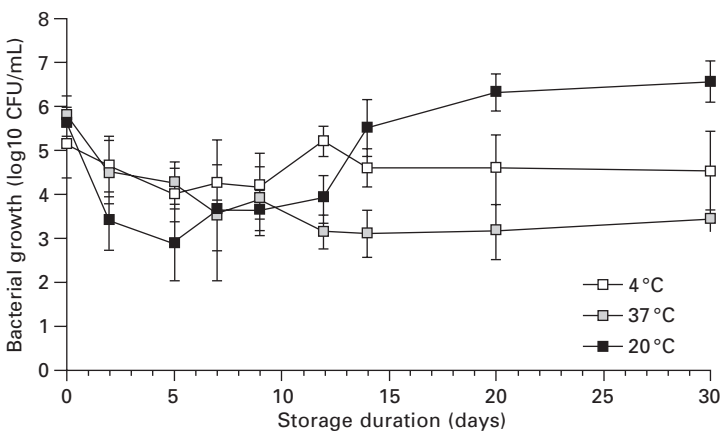

Fig. 13.10 Change in anti-*Salmonella* activity of egg white during egg storage at various temperatures. A higher bacterial growth corresponded to a lower anti-*Salmonella* activity. Source: Réhault-Godbert *et al.* (2010).

structures of the thick albumen and vitelline membrane break down faster. The processes involved are not fully understood but as the vitelline membrane degenerates, water enters the yolk causing mottling and after prolonged storage, albumen proteins also enter the yolk increasing the severity of mottling (Jacob *et al.*, 2000).

Biological activity of albumen protein is influenced by the duration and temperature of storage. Réhault-Godbert *et al.* (2010) demonstrated that egg white displayed higher anti-*Salmonella* activity after a few days of storage at 20 and 37 °C, compared with the level of an egg just laid or stored at 4 °C (Fig. 13.10). The rate of increase in the potential of egg white to inhibit growth of *Salmonella* Enteritidis was more rapid and pronounced at the

higher temperature of 37 °C but the bacteriostatic potential of egg albumen decreases after 12 days of storage at high temperature, in contrast to eggs kept at 20 °C which retained higher anti-*Salmonella* activity during the entire storage period (30 days).

13.5.4 Storage and the organoleptic properties of eggs

Fresh eggs have no odour but will acquire a characteristic stale odour after prolonged storage (Romanoff and Romanoff, 1949). Unpleasant odours generally result from the chemical breakdown of the egg's contents. This natural process will be exacerbated by adverse storage conditions, especially high temperatures, and by contamination of the egg contents by microorganisms. Eggs also readily absorb strong odours or flavours when they are incorrectly stored near strongly flavoured foods or chemicals.

13.5.5 Oiling eggs prior to storage

Coating eggs with mineral oil within 24 hours of the egg being laid, results in a reduction of carbon dioxide and moisture loss from the egg and as a result can help to preserve albumen quality (Hill and Hall, 1980). To be effective this coating must be evenly applied which requires the oil to be of the correct viscosity (Waimaleongora-Ek *et al.*, 2009). Oiling is typically carried out after eggs have been washed and sanitised. The application of a thin film of oil onto the surface of the eggshell helps to reseal the gaseous exchange pores which may have been exposed as a result of cuticle being damaged during the washing procedure (Hutchison *et al.*, 2003). In tropical conditions, oiling eggs can also be a very effective way of delaying albumen deterioration (Stephenson *et al.*, 1991) in the absence of refrigeration.

13.5.6 Storing eggs in modified atmospheres

The shelf-life of eggs can be extended for more than 14 weeks when they are rapidly cooled using CO_2 and then stored in an modified atmosphere of CO_2 (Keener *et al.*, 2000). However, thermal cracking can occur during the cooling process and as a result the widespread application of this technology has been limited (Jones *et al.*, 2002). Recent developments in modified atmospheric packaging, however, may see eggs being stored in this way becoming more popular, particularly in hot countries where refrigeration is not readily available (Rocculi *et al.*, 2009).

13.5.7 Detection technologies for assessing the freshness of stored eggs

In the egg products industry, the clean separation of the egg contents is critical. This requires the freshness of each egg to be accurately determined.

As a consequence of this, there has been considerable interest in applying new sensor technologies including spectroscopy methods and image analysis techniques to measure egg freshness. These new technologies are described in Chapter 10.

13.6 Conclusions

Egg quality is influenced by a wide range of factors in addition to bird strain and nutritional status. It is of importance for keeping a high quality of eggs to understand how the hens' physiology influences egg quality in favour of positive traits whilst limiting the more negative traits. Bird age is obviously the major factor and a tremendous effort has already gone into selection to limit age-related effects on the likes of egg weight, albumen quality and shell quality. This will continue to be of importance as we search for sustainable systems of production in the future. Environmental conditions within the poultry house, and the system of management, are also crucial components which can modify the bird physiological status at any time and change its feed consumption. This can lead to unpredictable changes in both egg production and quality. The tremendous increase in egg production (150 to more than 320 eggs in 72 weeks), for example, would never have been possible without an understanding of lighting programmes. Nowadays, change in climate, consideration of sustainability and novel regulations are currently increasing the importance of these environmental factors. Once an egg is laid, the way in which it is handled and stored becomes of importance if quality and safety are to be maintained throughout the logistical chain especially when considering the importance of safety of a product used sometimes as a raw material.

Finally, it should be mentioned that the literature presented here is often quite dated and, while measuring the effect of hen physiology or environmental conditions on egg characteristics, is not innovative but rather descriptive. There is a real need to perform new studies in this area. After all, the physiology and optimal conditions required by the modern commercial layer (which can lay in excess of 320 eggs per annum) may well have dramatically changed over this period.

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14

Egg and egg product microbiology

F. Baron and S. Jan, Institut National de la Recherche Agronomique, France

Abstract: This chapter reviews microorganisms responsible for egg contamination and their sources and gives a general description of current understanding of egg antimicrobial self-defence mechanisms. The methods limiting the presence and the development of microorganisms are discussed, both in the egg during development in the reproductive tract, and post-lay during egg storage and conditioning. In addition, an overview of the microorganisms contaminating egg products and their origins is given, and methods to preserve the microbiological quality of egg products are described.

Key words: Egg, egg product, microbiology, pathogenic and spoilage microbiota, antimicrobial properties, stabilization, storage, temperature control.

14.1 Introduction

In Europe, the microorganism responsible for the majority of cases of foodborne disease due to egg or egg product consumption is *Salmonella* (90%). *S*. Enteritidis was the predominant serotype causing salmonellosis in 2007 (EFSA, 2009). The risk of contamination by *Salmonella* is a major concern for the egg producing and the egg product manufacturing industries. The aim of this chapter is to highlight the types of microorganisms found in eggs and egg products and to comment on their origin and behaviour in the different parts of the egg and in egg products. The influence of breeding practices and egg product processing is also discussed.

14.2 Egg microbiology

Under healthy breeding conditions, an egg's contents are generally sterile just after laying. However, they can be contaminated by a diversified microbiota containing food spoilage microorganisms and sometimes pathogenic bacteria. Eggs can be contaminated both externally, on the eggshell, and internally, i.e. during development. The egg may therefore be a vector of bacteria causing foodborne illness in humans: this is particularly the case for *Salmonella*.

14.2.1 Endogenic contamination

Eggs can be contaminated during their formation in the oviduct. This type of contamination is essentially due to *Salmonella* and is termed vertical contamination. In the case of *Salmonella*, eggs may be contaminated during their formation in the ovary or in the oviduct of infected hens. Egg contamination has already been observed by different authors, following oral, intravenous or peritoneal inoculation of hens by *Salmonella* Enteritidis. However, this type of contamination is sporadic, occuring when high concentrations of bacteria are inoculated (from 10^8 to 10^9 cells per hen). Depending on the authors, the percentage of contaminated eggs varies from 0 to 8.1%, and the level of egg contamination is between 1 and 10 cells/ml (Gast and Beard, 1992; Keller *et al*., 1995; Gast and Holt, 2000b; Gast *et al*., 2002). Endogenic contamination by *Salmonella* Heidelberg (Gast *et al*., 2007) and *Salmonella* Pullorum (Wigley *et al*., 2001) has been observed. However, endogenic contamination by *Salmonella* Typhimurium (Keller *et al*., 1995; Reiber *et al*., 1995), *Salmonella* Infantis and *Salmonella* Hadar (Okamura *et al*., 2001) has never been observed, even though these species have been detected after laying (exogenic contamination).

Several studies on experimentally infected hens have shown that endogenic contamination may occur at each step during egg development. Gast and Beard (1990), Shivaprasad *et al*. (1990) and Keller *et al*. (1995) have observed a higher frequency of egg white contamination compared with egg yolk contamination, linked to the contamination of the magnum (the upper part of the oviduct), where the egg white is deposited. Gast and Holt (2000a, 2001) assume, however, that the contamination is associated rather with the ovary, since they observed high contamination levels in egg yolk and the vitelline membrane. It appears certain, though, that since *Salmonella* is more frequently isolated in egg contents than on eggshells, eggs are contaminated before eggshell deposit (Gast and Beard, 1990; Suzuki, 1994) so either the ovary or the upper part of the oviduct (magnum) is the location of contamination.

Keller *et al*. (1995) have observed a difference between the rate of tissue contamination of leghorns compared with Isabrown birds, the latter exhibiting the highest contamination level by the strain tested. The age of the hen is also important: its resistance to *Salmonella* generally increases with age, probably in connection with the development of a mature intestinal flora and

an effective immune system (Suzuki, 1994). However, eggshell contamination does not depend on the age for hens of 27 to 60 weeks (Protais *et al.*, 2003). Swayne and Beck (2004) have demonstrated vertical contamination of eggs by the type I avian Influenza virus, which causes an infection in several species of birds including hens. However, this virus does not represent a sanitary risk to humans, and effective measures have probably been put in place by authorities in case of outbreaks, To conclude, egg vertical contamination by microorganisms is possible but it remains sporadic, at low levels and far less of a concern than contamination after laying.

14.2.2 Exogenic contamination

Exogenic contamination of eggs (also termed horizontal contamination), by far more frequent than vertical contamination in terms of overall levels and diversity of bacteria, corresponds to the contamination of the eggshell surface. It occurs after laying, when the eggshells are in contact with hen's faecal microorganisms or with other microorganisms present in the farm environment, or elsewhere in the supply chain. Depending on the study, the level of eggshell contamination by mesophilic aerobic microbiota ranges from $10^{3.8}$ to $10^{6.3}$ CFU/egg, with an average of around $10^{4.5}$ CFU/egg (Moats 1980; Lucore *et al.*, 1997; Favier *et al.*, 2000; Jones *et al.*, 2004; De Reu *et al.*, 2005, 2006a; Musgrove *et al.*, 2005). Regarding the types of bacteria present, Moats (1980) isolated by conventional bacteriological methods the following Gram positive bacteria: *Streptococcus*, *Aerococcus*, *Micrococcus, Staphylococcus* and *Escherichia coli*. De Reu *et al.* (2006a) identified the major species present on eggshells by 16S DNA sequencing, after isolation of colonies on a non-selective nutrient medium. They observed a predominance of *E coli* (5.5×10^4 CFU/egg), and *Staphylococcus* (4.3×10^4 CFU/egg), the species *S. linens* and *S. equorum* being most prevalent.

The health of the pullets and hens and the sanitary state of the breeding environment influence the species of bacteria found and the level of contamination. Bacteria, including *Salmonella*, can survive in the breeding environment and contaminate whole flocks or spread between different flocks. Lice, insects and rodents are reservoirs and putative vectors of contamination. Other factors influencing the level of contamination are the quantity of dust, which may vary depending on hen behaviour, including laying behaviour, the use of cages and their design (conventional or furnished cages), the use of perches, and the flooring and the feeding systems (De Reu *et al.*, 2005). The contamination also depends on the season, the level of contamination being lower during the winter period (De Reu *et al.*, 2005; Wales *et al.*, 2007). Contamination may also occur during egg transport and/or packaging in farm or in the egg packaging centre. The source of contamination may either be the environment, or another egg. The presence of cracked or 'flowing' eggs increases the risk of contamination. The egg packaging step is for this reason critical in terms of contamination (Davies and Breslin, 2003). For

all the reasons outlined above, controlling the factors influencing eggshell contamination is essential to prevent the spread of microorganisms.

14.2.3 Penetration of microorganisms through the eggshell

Even if the egg surface is contaminated, the cuticle, shell and shell membranes are barriers preventing the penetration of microorganisms from the surface to the egg's contents. The cuticle is a film composed of mucoproteins. It is resistant to water and penetration of microorganisms. However, the effectiveness of this protective coating is limited, since it cracks rapidly over time during egg handling (Vadehra *et al.*, 1970; Tung *et al.*, 1979; De Reu *et al.*, 2006b). The shell is a calcified protein layer which represents a physical barrier that is rather ineffective because of the possible passage of microorganisms through its pores. However, it contains lysozyme and ovotransferrin, which may play a role in protection against penetration (Hincke *et al.*, 2000; Gautron *et al.*, 2001). The external and internal shell membranes represent effective antibacterial filters composed of glycoprotein fibres, organized into a network together with lysozyme, β-*N*-acetylglucosaminidase and ovotransferrin (Hincke *et al.*, 2000; Gautron *et al.*, 2001; Ahlborn and Sheldon, 2005).

Several authors have studied the factors favouring the penetration of microorganisms inside the eggs. The results obtained under different conditions lead to contradictory conclusions and the results of *in vitro* studies should be analysed with caution, the inoculation levels of eggshells being generally higher than those observed naturally. However, it appears from these studies that the weight of the egg, the thickness of the eggshell (Messens *et al.*, 2005, 2007), the number of pores, the quality of the cuticle (Messens *et al.*, 2005), and the presence of water condensation on the eggshell (Allen and Griffiths 2001; Ernst and Fuqua, 1998) are not correlated with the level of penetrating microorganisms. Even if the condensation generated by high humidity combined with temperature changes promotes penetration by retraction of the egg contents and absorption of water and microorganisms present on the shell (Miyamoto *et al.*, 1998; De Reu *et al.*, 2005), this does not influence the level of egg contamination (Messens *et al.*, 2006). Also, although the age of the hen has an impact on the characteristics of the eggshell, it does not seem to influence the level of penetrating microorganisms (Messens *et al.*, 2005; De Reu *et al.*, 2006b) and in addition, hen lineage (Warren Isabrown versus Bovan Goldline) and the type of hen housing are not influencing factors. The factors that do seem to influence microorganism penetration are mainly the level of contamination of the eggshell surface (Chen *et al.*, 1996; Miyamoto *et al.*, 1998; Messens *et al.*, 2006, 2007) and cracks in the eggshell or abnormal eggshell calcification (Ernst and Fuqua, 1998; Allen and Griffiths, 2001).

14.2.4 Bacterial survival and multiplication in egg white

Egg white, which is similar to an intracellular fluid, is an important line of defence against invading bacteria. It represents a nutritional reserve for the developing embryo and, through a broad spectrum of antimicrobial molecules and mechanisms, provides protection against invasion by microorganisms.

Role of antimicrobial molecules

Lysozyme

Lysozyme, present in many biological fluids, is an important component of the egg white's non-specific defence mechanisms. It is responsible for different types of bactericidal activity. The first, and also best known is hydrolysis of the peptidoglycan layer of Gram positive bacteria (degradation of the glycosidic β (1–4) bound between the *N*-acetylglucosamine and the *N*-acetylmuramic acid), which leads to cell wall hydrolysis, followed by lysis of the bacteria if the osmolarity of the environment changes. Gram negative bacteria resist this hydrolytic activity, owing to the composition of their peptidoglycan layer and its protection by an outer membrane. Some modified chemical structures of peptidoglycans also confer resistance to hydrolysis to several bacteria (Vollmer, 2008). A second non-specific and non-hydrolytic bactericidal activity for which lysozyme is responsible involves membrane disruption (Ibrahim *et al*., 2001a) and a third, identified by Ibrahim *et al*. (2001b), involves the induction of autolysins. Depending on the type of bacteria (composition and structure of the membranes and the cell wall, presence of a factor inhibiting lysozyme activity in the cytoplasm; Monchois *et al*., 2001) and on the environmental conditions, one of these three mechanisms may predominate and cause cell death.

Ovotransferrin

Ovotransferrin, a protein that chelates metal ions, belongs to the family of transferrins present in various animal fluids (e.g. blood transferrin and lactoferrin from milk). It has a bacteriostatic effect through the creation of an iron-deficient environment, This activity is its best known mechanism (Garibaldi, 1970; Baron *et al*., 1997). Some microorganisms, including the Enterobacteriaceae and *Salmonella*, are able to synthesize high affinity iron chelators, called siderophores (Neilands, 1981), thus counteracting iron unavailability. A second mechanism of action of ovotransferrin, proposed by Aguilera *et al*. (2003), is directed towards the biological function of the cytoplasmic membrane. Both mechanisms are dependent on iron concentration in the medium, with iron saturation eliminating the bacteriostatic effect.

Other proteins and the importance of the physico-chemical properties of egg white

The protease inhibitors found in egg white, such as cystatin (an inhibitor of cysteine proteases), ovomucoid and ovoinhibitor (inhibitors of serine proteases) and ovostatine (inhibitor of many proteases) (Li-Chan and Nakai,

1989; Stevens, 1991), the vitamin chelating proteins such as flavoprotein (fixing riboflavin), avidin (fixing biotin) and the thiamine binding protein (fixing thiamine) have also been shown to be involved in the antimicrobial activity of egg white, although their role appears minor. Egg white probably also contains yet unknown proteins or peptides involved in its bactericidal or bacteriostatic activities, It is also important to consider that there may be interactions between these molecules or synergistic effects between them and the physico-chemical properties of egg white, i.e. alkaline pH, viscous structure and heterogeneity. pH is a determining factor for many biological functions, particularly enzyme activity and membrane status. It is possible that the activity of certain proteins may change significantly at the basic pH of egg white.

Nutrients are poorly available to microorganisms in egg white. Furthermore the heterogeneity of egg white probably makes it difficult for bacteria to access the necessary nutrients for growth. Li-Chan and Nakai (1989) show, for example, that the iron–ovotransferrin complex is probably not distributed evenly within the white. Egg white undoubtedly also has a variety of mechanisms controlling bacterial growth that are more or less dependent on specific types of bacteria. It clearly appears that iron deficiency due to the action of ovotransferrin is a major part of the activity of egg white against *Salmonella* Enteritidis (Garibaldi, 1970; Baron *et al*., 1997; Kang *et al*., 2006), but other bacteria appear insensitive to this. Wang and Shelef (1991) highlight the role of lysozyme together with alkaline pH in the inhibition of *Listeria* in egg white. As the egg white has a highly selective effect on microorganisms, the high incidence of *Salmonella* Enteritidis in eggs could be explained by the fact that this bacterium is better adapted to survival in egg white or colonization of the hen reproductive tract (Clavijo *et al*., 2006).

Influence of environmental conditions

Bacterial growth in egg white and migration to the yolk depends not only on the microorganism but also on the quality of the egg and, more generally, the storage conditions (time and temperature). Duboccage *et al*. (2001) showed that the conditions in eggs less than 1 week old stored at 20 °C may be more favourable for *Salmonella* growth than those in eggs stored at this temperature after laying for 2 to 3 weeks. After three weeks, the conditions may also favour *Salmonella* growth, probably due to alteration of the vitelline membrane and a decrease in egg viscosity, both due to the dissociation of the ovomucin–lysozyme complex at alkaline pH (the pH changes from 7.6 to about 9.3 following the loss of carbon dioxide a few days after laying). These two factors favour the ingress of nutrients from the egg yolk into the egg white and the migration of *Salmonella* into the yolk (Humphrey and Whitehead, 1993; Chen *et al*., 2005).

The storage temperature also plays an important role. At temperatures below 8 °C, the growth of *Salmonella* seems to be impossible (Ruzickova, 1994; Schoeni *et al*., 1995). Moreover, a bactericidal effect is observed with

the disappearance of the whole population at 4 °C in a few days (Lock and Board 1992; Schoeni *et al*., 1995; Chen *et al*., 2005). The same phenomenon was observed at 10 °C by Clay and Board (1991) and Chen *et al*. (2005), while Schoeni *et al*. (1995) observed a slight growth. Between 20 and 30 °C, these authors observed a growth of one to four logarithmic units per ml, depending on temperature and incubation time (Clay and Board, 1991; Lock and Board, 1992; Ruzickova, 1994; Humphrey and Whitehead, 1993; Schoeni *et al*., 1995; Baron *et al*., 1997; Cogan *et al*., 2001; Duboccage *et al*., 2001; Chen *et al*. 2005; Murase *et al*., 2005). For temperatures above 37 °C, a bactericidal effect was observed depending on temperature and incubation time (Tranter and Board, 1984; Bradshaw *et al*., 1990; Ruzickova, 1994; Clavijo *et al*., 2006; Kang *et al*., 2006). These studies suggest that certain enzymatic activities may be amplified at temperatures above 37 °C and cause the death of bacteria.

14.2.5 Bacterial migration and multiplication in egg yolk

Over time, the chalazae and egg white liquefy and do not maintain the egg yolk in a central position. The distance between the egg yolk and the eggshell reduces, hence promoting the access of microorganisms (including bacteria derived from the egg shell) to the egg yolk. The vitelline membrane, which contains, among other specific proteins, lysozyme and ovomucin, represents the last obstacle to the migration of the microorganisms to egg yolk (Chen *et al*., 2005). The vitelline membrane is permeable to solutes such as egg yolk iron and amino acids. Different authors have shown that this permeability enables the growth of *Salmonella* in egg white when the cells are inoculated close to the egg yolk (Gast and Holt, 2000b; Grijspeerdt *et al*., 2005; Murase *et al*., 2005). The exchange of solutes depends on the length of egg storage, due to the alteration of the vitelline membrane structure mentioned in the preceding section, and can be controlled by egg storage at refrigeration temperatures (Gast and Holt, 2001; Gast *et al*., 2006). Similarly, it seems that penetration does not occur at 42 °C, the temperature of egg formation in the hen reproductive tract (Guan *et al*., 2006).

If bacteria reach egg yolk, their growth is very fast at temperatures equal to or higher than 25 °C (Gast and Holt, 2000b, 2001; Schoeni *et al*., 1995; Ruzickova, 1994; Gumadavelli *et al*., 2007), but less significant at 10 °C (Gast and Holt 2000b; Gumadavelli *et al*., 2007). *Salmonella* growth has never been observed at 7 °C (Gumadavelli *et al*., 2007). The storage of eggs at refrigeration temperatures is an effective way of reducing the loss of integrity of the vitelline membrane, the access of bacteria to egg yolk and their subsequent growth.

14.2.6 Controlling egg contamination

Various ways to reduce the level of egg contamination have been explored. These include both upstream methods (hen selection, breeding practices,

farm management) and downstream methods (packaging, transport and storage of eggs).

Genetic selection

Berthelot *et al*. (1998) have shown that hen resistance to caecal colonization by *S*. Enteritidis has a heritable genetic basis. Similarly, Sadeyen *et al*. (2006) comparing two lineages of hens have observed significant differences in the expression of several genes encoding proteins involved in the defence against colonization by *Salmonella*. Thus, hen selection may be an efficient way to improve resistance to colonization by *Salmonella*. Regarding eggs themselves, some authors consider that not only the bactericidal activity of egg white (Vidal *et al*. 2003; Sellier *et al*., 2007), but also the strength of the eggshell (Dunn *et al*., 2005) have a genetic component. These parameters could therefore be improved by a specific breeding programme, although more information on the antibacterial compounds involved in protection is essential to achieve this aim.

Husbandry methods

In general, stress experienced by hens on the farm is known to affect their susceptibility to infection by *Salmonella*. Stress is also involved in triggering abnormal eggshell calcification. During the egg production peak, stress seems to weaken the immune system and reduce the influx of macrophages to the reproductive organs, favouring infection of the ovaries (Wigley *et al*., 2005; Li *et al*., 2007).

Breeding practices

It seems that the size of the flock is the predominant risk factor for hen contamination by *S*. Enteritidis. The number of cracked or broken eggs also seems to relate to the type of hen housing system and the design of the cages (Mallet *et al*. 2005; De Reu *et al*., 2005). The impact of housing systems on egg quality is examined further in Chapter 15.

Cleaning and disinfection practices of the breeding environment

Since the level of the mesophilic microbiota present on the surface of eggshells is related to the level of air contamination in the breeding environment (De Reu *et al*., 2005), it seems crucial to limit the dust and to promote good hygiene practices. Building decontamination between two hen batches is now carried out routinely (Huneau-Salaün *et al*., 2005).

Hen vaccination against *S*. Enteritidis

Hen vaccination has been obligatory since 1 January 2008 in countries of the European Union in which *Salmonella* prevalence in laying hens exceeds 10%. This practice is probably an important preventive way of reducing the level of hen contamination but scientific evidence to verify its effectiveness in reducing egg contamination is lacking.

The use of feed additives

A wide range of anti-*Salmonella* food additives are available. Short chain organic acids (including propionic, butyric and formic acids) or medium chain organic acids (caproic, caprylic, capric and lauric acids) are often used (Van Immerseel *et al*., 2004; Thormar *et al*., 2006) and are intended to reduce colonization of the hen digestive tract and, consequently, faecal excretion of *Salmonella*. By decreasing the rate of contamination of the environment, the risk of horizontal and vertical contamination is reduced. An alternative strategy is the addition of pre or probiotics to the hen diet. Prebiotics are non-digestible food components supposed to improve health by positively influencing the intestinal flora through the selective stimulation of certain bacteria already present in the gut (Gibson and Roberfroid, 1995). Fructo-oligosaccharides are used to promote the growth of *Bifidobacteria*, which may reduce colonization by *Salmonella* and other substances are being studied or tested. Probiotics are living microorganisms which, when they are administered in adequate amounts, confer a health benefit on the host. Some studies, especially those with lactic acid bacteria, have already been carried out (Van der Wielen *et al*. 2002; Tayeb *et al*., 2007). However, the effects of these additives on levels of egg contamination are not yet clear.

Practices of egg collection, sorting and storage

Good practices for egg collection on-farm, sorting, packaging, storage and delivery must also be followed to reduce contamination. Cross-contamination must be avoided by preventing contamination by the staff and environmental contamination (e.g. by surfaces and pests). At each step, it is essential to preserve eggshell integrity. Specific sorting measures can be implemented, such as candling and exclusion of cracked and broken eggs. The analysis of micro-cracks and of the fragility of the eggshell remains a weak point. Several technologies of eggshell analysis have already been tested (De Ketelaere *et al*., 2004) and other techniques, currently under study, may be useful to reduce the production of weak eggs.

It is necessary to limit temperature changes during egg collection on the farm and delivery to the egg packaging centre. In France, eggs must be stored between 5 and 25 °C. The use of air-conditioned storage rooms and the practice of isothermal transport would minimize temperature variation. It could be interesting to investigate the effects of cooling eggs quickly after laying and maintaining the cold chain until delivery, in order to limit penetration, migration and proliferation of *Salmonella* in the egg content. We have already mentioned the importance of refrigeration in the control of *Salmonella*. This stabilization method would be easy to implement but involves a radical change in the distribution chain from the farmer to the consumer.

Practices of egg decontamination

Washing of table eggs (Class A eggs) is common in many countries but is not practised in Europe except in Sweden. On the one hand, it is argued that

egg washing decreases the level of eggshell contamination and, consequently, the level of internal and external egg contamination (Lucore *et al.*, 1997; Hutchison *et al.*, 2004; Jones *et al.*, 2004). On the other hand, egg washing is considered to be responsible for weakening the external barriers of the egg, such as the cuticle, and for an increase in humidity (Kim and Slavik, 1996; Wang and Slavik, 1998; Favier *et al.*, 2000), promoting the penetration of microorganisms and increasing the level of internal egg contamination (Haines and Morand, 1940; Lorenz and Starr, 1952).

According to an EFSA report (EFSA, 2005), the practice of egg washing cannot, alone, solve a public hygiene problem. However, taking into account improved egg washing technologies, the increasing number of recent studies supporting egg cleaning and washing, and their efficiency in countries that apply this practice on sorted eggs (e.g. in Sweden), egg washing could ultimately be authorized in Europe (Hutchison *et al.*, 2004; Jones *et al.*, 2004; Musgrove *et al.*, 2005).

Egg 'pasteurization' has been the subject of various studies and patented technologies are applied in the United States. Braden (2006) considers that heat treatment could reduce the occurrence of salmenellosis related to egg consumption in the United States. This hypothesis is valid in the American context, where egg washing and cooling are carried out after the heat treatment; however, extrapolation to *Salmonella* control in Europe is difficult.

Other methods of egg decontamination have been investigated or are under study, including 'flash' treatments based on hot water or steam, UV, ozone, radiation, ultrasound, electrolysed water or ionized air (plasma) treatments. It should be noted that it is necessary to validate all egg decontamination processes under natural contamination conditions at the industrial scale, to keep in mind the need to study the benefits of adopting the process in terms of public health.

14.3 Egg product microbiology

An increasing proportion of eggs are processed by the egg product industry (also known as the egg breaking industry). Egg products intended for human consumption are produced, after removal of the shell and the shell membranes, from the egg's inner components, which are either separated or mixed together. Several forms of egg products are available in the market, including liquid, concentrated, dried, granulated, frozen, boiled and natural egg products. Because of their various functional properties (for example foaming, binding, gelling, or dyeing properties), egg products are widely used in the food industry in pastries, meats, desserts, etc. Controlling their microbiological quality is imperative, especially when they are used as ingredients in raw or lightly cooked foods. Egg quality, hygiene practices and use of the appropriate processing and preservation technologies are critical.

14.3.1 Egg reception

The eggs to be processed may originate from farms controlled by veterinary services or egg packaging centres, or they may have been imported. At egg reception, quality control procedures should be in place to check the packaging, the appearance of the eggs and possibly their freshness, ensuring the integrity of the vitelline membrane. The freshness is estimated by measuring the height of the thick albumen, expressed in Haugh units, with the understanding that the number of Haugh units decreases with storage time (Sauveur, 1988). The freshness and the percentage of dirty or cracked eggs are essential parameters for the control of egg product contamination downstream.

14.3.2 Egg storage

The eggs are stored in air-conditioned rooms (usually at 15 °C) or in cold rooms at 4 °C, depending on the storage period. Good storage conditions, especially with regard to the control of temperatures, hygrometry and hygiene, are essential to limit the penetration, migration and multiplication of microorganisms in the eggs. The practice of egg washing is authorized by the European Union in the egg processing industry, though it is not authorized in the table egg industry.

14.3.3 Egg breakage

Egg breaking machines are designed to break eggs, remove eggshells and separate egg white from egg yolk. Contact of the egg's inner contents with eggshells and the surfaces of the machines leads to systematic contamination since, once broken, eggs lose their natural antimicrobial defences. Whole egg and egg yolk are indeed ideal environments for the development of microorganisms. Egg white limits microbial growth, provided that its defence mechanisms are not compromised by the presence of egg yolk (even traces have an impact), along with iron and nutrients (Baron *et al.*, 1997). The eggshells are quickly isolated in order to minimize their contact with the egg products. They are then crushed and centrifuged, an action that should be performed in a separate location from egg product manufacturing, in order to prevent cross-contamination. The egg products are filtered to make them more homogeneous and to remove eggshell debris and chalazae. The machine and filter cleaning and disinfecting procedures must be effective in order to avoid contamination and biofilm formation.

14.3.4 Stabilization of egg products

After filtration, the egg products undergo various separation, processing and/or stabilization steps, depending on their destination. These might include pasteurization, sugaring, salting, freezing, concentration or drying. These operations have a stabilizing effect, either by destruction or inhibition of the development of microorganisms.

Bacterial destruction

In the egg product industry, the destruction of microorganisms is mainly carried out by heat treatment. The treatments usually applied are temperatures of around 65–68 °C for 5–6 minutes for whole egg and egg yolk. The treatments are milder for egg white (around 55–57 °C for 2–5 minutes), owing to the higher thermal sensitivity of egg white proteins. These treatments are adequate for the destruction of vegetative microbiota, but are ineffective on the heat-resistant microbiota, including spore-forming bacteria. Many studies on heat resistance have been carried out on *S*. Enteritidis, because of its importance in the sector of egg production. More recently studies have been carried out on *Listeria*, as it is known to be more resistant than *S*. Enteritidis. The studies are hardly comparable because heat resistance depends, among other factors, on the strain, the growing conditions, the inoculation size and the equipment used for pasteurization. For *Salmonella*, the *D* values (decimal reduction time at a given temperature, i.e. time to reduce the population to a tenth of its initial value) described in the literature are between 0.22 and 0.44 min at 60 °C for whole egg, between 0.27 and 0.35 min at 63 °C for egg yolk, and from 1.7 to 2.1 min at 57 °C for egg white (Humphrey *et al*., 1990; Shah *et al*., 1991; Denis *et al*., 1995; Palumbo *et al*., 1995, 1996; Doyle and Mazzotta, 2000). For *Listeria*, the *D* values described in the literature are in the range of 1.3–2.1 min at 60 °C for whole egg, 0.7–2.3 min at 63 °C for egg yolk, and 4.35–20.9 min at 57 °C for egg white (Foegeding and Stanley, 1990; Bartlett and Hawke, 1995; Muriana *et al*., 1996; Knight *et al*., 1999; Michalski *et al*., 2000; Doyle *et al*., 2001). From these data and from the *z* values (increase in temperature necessary to divide the decimal reduction time by ten) provided by these authors, it is clear that the heat treatments as described above for whole egg and egg yolk are sufficiently effective against *Salmonella* and *Listeria*. However, they are not sufficiently effective to destroy these bacteria if they are present in egg white. It should be once more highlighted that egg white is not a favourable environment for bacterial development.

Other treatments have been studied or put into practice, particularly for egg white, for which the heat treatment is insufficient. In France, the regulations allow the irradiation of liquid, dried and frozen egg products, with doses of 3 kGy, but this treatment has had little or no take-up in the egg product industry, because of public perception of irradiated food. Processes such as pulsed electric fields have been developed recently but have only been reported in a small number of studies (Martin-Belloso *et al*., 1997; Jeantet *et al*., 1999).

Bacterial inhibition

Water activity (a_w) is an indirect measure of the amount of free water in a matrix. Bacteria cannot grow below an a_w of 0.91, whereas yeasts and moulds can grow up to an a_w of 0.65. No microbial development occurs below an a_w of 0.6. Decreasing water activity is hence used for egg product stabilization.

Concentration (reducing water content) and drying are technologies employed to remove water from egg products. Egg product concentration only slightly reduces the water activity and, therefore, has little influence on the growth of microorganisms. Drying, though, produces a powder with an a_w of between 0.2 and 0.3, inhibiting any bacterial development. The addition of sugar or salt (hydrophilic components) also limits the water available to microorganisms. The quantities of sugar and salt can reach 50% and 10% respectively, which lowers the water activity to values below 0.85. Sorbic and benzoic acids are the only bacterial inhibitors approved for addition to liquid egg products, at concentrations up to $5\,g\,kg^{-1}$. They have bactericidal and antifungal activities.

Freezing makes water unavailable to bacteria because of its solidification (i.e. ice formation), while the growth kinetics of microorganisms and the ability of pathogenic bacteria to produce toxins are reduced by refrigeration. Both strategies are commonly used in the food sector and the expansion of refrigeration practices has improved the quality and shelf-life of food products. However, refrigeration is also responsible for selection for psychrotrophic microbiota. Among the psychrotrophic microorganisms responsible for foodborne illness, the species *Listeria monocytogenes*, *Yersinia enterolitica*, *Bacillus cereus* and type E *Clostridium botulinum* are described. Concerning food spoilage, the genera *Pseudomonas* is predominant but *Shewanella*, *Alcaligenes*, *Acinetobacter*, *Aeromonas*, *Flavobacterium*, *Enterobacter*, *Serratia*, *Hafnia*, *Micrococcus* and *Brochothrix* are also described (Bornert, 2000). A temperature of 2 °C prevents the growth of these types of bacterium and hence represents an effective control. A low increase in temperature may, however, represent a risk, since metabolic activities can be observed at 4 °C, even if the growth rates are very low. The production of lipases and proteases by psychrotrophic bacteria may be responsible for food spoilage, especially since these enzymes are more heat resistant than the microorganisms that produce them. Toxin production is stopped at refrigeration temperatures for most microorganisms except some psychrotrophic bacteria. Baron *et al*. (2007) observed the production of enterotoxins by a strain of *Bacillus weihenstephanensis*, belonging to the *Bacillus cereus* group, incubated in liquid whole egg for 21 days at 6 °C and 7 days at 8 °C. It therefore appears that maintaining the refrigeration temperature below 4 °C throughout the production chain and until the distribution is essential for microbial control.

14.3.5 Egg product microbiota

The microbiota of egg products depends essentially on that of the raw material (egg) and is strongly influenced by the processes of transformation and stabilization that are employed, as already highlighted in the previous paragraphs. An exhaustive study of the contamination of stored raw and pasteurized liquid whole egg was conducted from 2001 to 2003 in western France (Protais *et al*., 2006). After pasteurization, the number of microorganisms decreased and the

samples met the regular microbiological criteria. Pasteurization significantly reduced the number of Enterobacteriaceae, *E. coli* and bacteria of the genera *Brochothrix*, *Pseudomonas* and *Campylobacter*. In this study, the authors showed that pasteurization reduced the number of positive samples, giving rise, after pasteurization, to 4.1% and 2.7% positive samples for *Salmonella* and *Listeria*, respectively. The values obtained for *Salmonella* are higher than those recorded nationally, where only 0.47% of whole eggs were positive (EFSA, 2005). Moore and Madden (1993) showed the presence of *Listeria* in raw egg product samples, but this pathogen was never detected after pasteurization (enrichment of 500 samples). Streptococci, Enterococci and *Bacillus* spores seem to be less affected by pasteurization. *Bacillus* spores are present at low levels, in both raw and pasteurized whole egg ($10^{0.63}$ and $10^{0.48}$ CFU/ml, respectively) but the level of contamination reached 58% for raw whole egg and 54% after pasteurization. The pasteurization processes are, as expected, ineffective on these spore-forming bacteria.

The presence of *Bacillus* in the egg products is of particular concern for the economy of the egg product industry since these bacteria cause enzymatic spoilage problems. Moreover, some species, and particularly those belonging to the *B. cereus* group, can produce toxins and be responsible for foodborne illness. These ubiquitous bacteria are difficult to eliminate because of their heat resistance (pasteurization is ineffective and can trigger spore germination), and their strong adhering capacities, which allow them to form biofilms in the pipelines or on the industrial surfaces. Finally, some psychrotrophic strains are able to grow at temperatures around 4–6 °C. Baron *et al.* (2007) isolated a psychrotrophic bacterium belonging to the *B. cereus* group from a sample of spoiled liquid whole egg. This study showed that the bacterium was able to grow in liquid whole egg and to produce toxins, even at refrigeration temperatures. Its ability to adhere to various materials also highlighted its presumed capacity to form biofilms.

14.3.6 Bacterial behaviour in egg products

Salmonella growth at temperatures between 4 and 10 °C in egg yolk (Gumadavelli *et al.*, 2007), whole egg (Abdel Karem and Mattar, 2001) or egg white (Clay and Board, 1991; Lock and Board, 1992; Ruzickova, 1994; Schoeni *et al.*, 1995; Chen *et al.*, 2005) seems not to occur or only to occur at very low levels. Between 20 and 30 °C, the lowest growth rates were observed in egg white, ranging from 1 to 4 logarithmic units per ml, depending on temperature and incubation time (Clay and Board, 1991; Humphrey and Whitehead, 1993; Schoeni *et al.*, 1995; Cogan *et al.*, 2001; Gast and Holt, 2001; Duboccage *et al.*, 2001; Messens *et al.*, 2006; Chen *et al.*, 2005; Murase *et al.*, 2005). In contrast, the highest growth rates were observed in egg yolk, from 7 to 8 logarithmic units per ml for 2 days of incubation (Ruzickova, 1994; Schoeni *et al.*, 1995; Gast and Holt, 2000, 2001; Gumadavelli *et al.*, 2007). The growth of *Listeria*, although slow, is

possible at refrigeration temperatures in the three types of egg products. In egg yolk and whole egg, the population increases by 2 to 12 logarithmic units per ml in 3 weeks at 4 °C, depending on the authors (Notermans *et al*., 1991; Leasor and Foegeding, 1989). In egg white, Notermans *et al*. (1991) observed a bacteriostatic effect at this temperature. For temperatures above 20 °C, growth is rapid in egg yolk with an increase of 3 logarithmic units per ml within 1 day at 22 °C. A growth of only 2 logarithmic units per ml was observed after 2 to 9 days in whole egg and no growth was observed in egg white.

Although it may be considered that *Salmonella* in egg products is now sufficiently controlled by surveillance, husbandry methods and effective pasteurization processes, manufacturers now have to deal with microorganisms selected for by the preservation processes used. These include the *B. cereus* group of spore-forming bacteria. These bacteria produce a diarrhoeal and emetic toxin that can cause illness in humans. Baron *et al*. (2007) studied the behaviour, in liquid whole egg, of a psychrotrophic *B. cereus* group strain isolated from a spoiled liquid whole egg product. The bacterial population increased by 2 logarithmic units per ml after 7 days at 8 °C and 14 days at 6 °C. A growth of 4.5 logarithmic units per ml was observed within less than 7 days at 10 °C. However, no growth was observed at 4 °C. This strain exhibited a cytotoxic activity on human enterocytes after 7 days at 8 °C of incubation in liquid whole egg, corresponding to a population of $10^{5.1}$ CFU/ml. More studies are needed on the behaviour of these microorganisms in liquid egg in order to better monitor it in the egg products sector.

The behaviour of microorganisms in an egg product depends on the nature of the egg product (liquid, dried, concentrated, added salt or sugar), its level of contamination and the initial temperature. Overall, we can say that growth in whole egg and the yolk can be rapid when the refrigeration temperature is not maintained, while the white is usually bactericidal or bacteriostatic. Control of the main pathogenic species, including *Salmonella*, is ensured by compliance with good hygiene and pasteurization processes. It may be noted that the conditions used in egg white pasteurization are, in absolute terms, not sufficient but contamination of egg white and the growth of microorganisms in egg white are very rare. The egg product industry must focus on controlling heat-resistant and/or spore-forming psychrotrophic species potentially selected for by the transformation and/or the stabilization processes. One of the best measures to manage these hazards is probably rigorous control of the cold chain, from production to consumption of the egg products.

14.4 References and further reading

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15

Alternative hen housing systems and egg quality

M. Rossi, Università degli Studi di Milano, Italy and K. De Reu, Institute for Agricultural and Fisheries Research (ILVO), Belgium

Abstract: According to European regulations conventional cages for egg production will be banned from 2012 in favour of alternative housing systems, or furnished cages systems. This chapter covers an overview of recent literature concerning the quality of eggs obtained from furnished cages and non-cage systems in comparison with conventional cages. The quality aspects considered include overall external egg quality, shell characteristics, interior egg quality, chemical composition, functional and sensory properties and bacterial contamination of shell and egg content.

Key words: housing system, external egg quality, interior egg quality, eggshell quality, hygienic quality, nutrients, functional properties, sensory properties.

15.1 Introduction

According to European regulations (Council Directive 1999/74/EC) conventional cages for egg production will be banned from 2012 in favour of alternative housing systems, or furnished cages systems. Since this directive was published, a number of such systems have been developed, especially in Europe, and improvements are ongoing. In particular, floor housing or barn, aviaries, free-range systems and organic production are currently implemented, but with a few notable exceptions, i.e. Sweden, France, the UK and Norway, European producers have not yet invested heavily in furnished cages.

While the conventional cage provided 450 cm^2 cage area for each hen which was increased to 550 cm^2 from 1 January 2003, furnished cages provide

at least 750 cm^2 per hen, a nestbox, a dust bath and 15 cm perch per bird. Non-cage systems provide 1111 cm^2 per hen, platforms with slatted floors at different heights (aviary) or at one height (floor housing), a litter area on the floor and nestboxes. Some systems also make use of perches at different heights, attached to an A-frame. When hens have also access to outdoor runs, the systems are called free-range systems (De Reu *et al.* 2008b).

Alternative and furnished cage hen housing systems are intended to improve the welfare of the hen as a response to consumers' ethical concerns about farming of layers in conventional cages. Moreover, consumers perceive the welfare of layers as a factor affecting positively the quality of eggs, thus the method by which hens are farmed has become one of the major factors for distributors to sell and consumers to purchase eggs. However, a clear relationship between the farming method and the egg quality is still to be demonstrated.

This chapter covers an overview of recent literature concerning the quality of eggs obtained from furnished cages and non-cage systems in comparison with conventional cages. The quality aspects considered include overall external egg quality, shell characteristics, interior egg quality, chemical composition, functional and sensory properties and bacterial contamination of shell and egg content. It must be kept in mind when reading this chapter that it is difficult to tease out the effect of housing system on single quality aspects as some quality aspects are related to each other and the individual housing system is a proxy of many production characteristics such as age of the building and equipment, nutrition, farm management, and so on.

15.2 External egg quality

15.2.1 Introduction

Egg quality has a genetic basis, but it is also affected by the age of the laying hens and by the hen's housing (Silversides *et al.*, 2006; Singh *et al.*, 2009). Eggs with obvious external defects, such as being broken, dirty or misshapen, are immediately segregated on the farm. After this first inspection at collection, the remaining eggs are candled to highlight previously undetected cracks, weak shells and internal defects. Data collected from different EU Member States on egg quality from different housing systems (pilot and commercial flock scale) (LayWel, 2006a) indicate that overall egg quality appears to be less good in non-cage systems (Table 15.1).

A trend is observable indicating that the percentage of first quality eggs is highest in conventional cages, followed by furnished cages and then non-cage systems. The opposite trend, although present, is less clear in the percentage of second category eggs. Still the overall egg quality seems slightly less good in non-cage systems.

Table 15.1 Overall egg quality in different housing systems (LayWel, 2006a)

System	First quality eggs (%)		All second quality eggs (%)	
	Mean	SD	Mean	SD
Conventional cage	93.29	6.95	6.50	8.43
Furnished cage	92.27	6.67	7.78	6.84
Non-cage system	91.50	7.92	7.81	7.96

15.2.2 Shape index and egg weight

Most eggs destined for the market have an oval shape that can be roughly expressed by the shape index given by breadth/length × 100 (Li-Chan *et al*., 1995). According to Romanoff and Romanoff (1949), the standard hen egg has a shape index of 74, with higher values indicating a rounder shape and lower values a longer shape. Except for Van den Brand *et al*. (2004) who reported longer eggs from caged layers than from outdoor-housed layers, most studies generally agree that housing systems have no effect on egg shape. These studies have compared the shape of conventional cage eggs with free-range eggs (Dukić-Stojčić *et al*., 2009; Petek *et al*., 2009; Wang *et al*., 2009), conventional cage eggs with various non-cage system eggs (Clerici *et al*., 2006), and conventional cage, free-range, barn and organic eggs acquired on the market (Hidalgo *et al*., 2008). No data are currently available on the shape of eggs produced in conventional versus furnished cage systems. Several studies have shown that housing systems do influence egg weight (Table 15.2).

Van den Brand *et al*. (2004) found no significant difference in the average weight of eggs produced by caged hens and outdoor-housed birds (free range). However, they observed that the free-range hens produced lighter eggs than caged hens at the start of the experiment, but laid heavier eggs after 37 weeks of age. According to Petek *et al*. (2009) and Dukić-Stojčić *et al*. (2009) eggs produced in conventional cages were heavier than those in free range, although only in the latter study this difference was statistically significant. Conventional cage eggs were on the other hand lighter than barn eggs according to Abrati (2006), Englmaierová and Tůmová (2009) and Singh *et al*. (2009). No significant difference in egg weight was observed between eggs produced in conventional and furnished cages (Wall and Tauson, 2007; Tactacan *et al*., 2009).

15.2.3 Dirt on the eggshell

Dirt on the eggshell can be considered to be a quality parameter but is also a hygiene indicator. Some types of dirt on the eggshell may support the growth of potentially harmful bacteria present on the shell, thus compromising consumer safety. Eggs can become soiled as a result of faecal contamination, contact with dirty equipment or broken eggs, dust, feathers and so on. In

Table 15.2 Average egg weight in different housing systems (the hen age experimental period is given in brackets)

Study	CC (g)	FC (g)	NC (g)	P
Pilot studies				
Van den Brand *et al.* (2004) (25–59 weeks)	56.4	n.i.	56.4[d]	n.s.
Wall and Tauson (2007) (22/24–77/78 weeks)	65.4	65.0[a]	n.i.	n.s.
Dukić-Stojčić *et al.* (2009) (one year)	66.7	n.i.	65.2[d]	*
Englmaierová and Tůmová (2009) (20–60 weeks)	63.3	n.i.	64.5[c]	***
Singh *et al.* (2009) (20–50 weeks)	54.3	n.i.	58.6[c]	*
Petek *et al.* (2009) (24–36 weeks)	61.9	n.i.	60.3[d]	n.s.
Tactacan *et al.* (2009) (21–61 weeks)	59.7[b]	59.4[b]	n.i.	n.s.
Commercial flock scale (on farm)				
Abrati (2006) (27–68 weeks)	62.8	n.i.	66.0[c]	*

CC = conventional cage, FC = furnished cage, NC = non-cage.
n.i. = not included in the study.
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; n.s. = non-significant ($P > 0.05$).
[a]Mean weight calculated from the values reported in the original paper for three different models of furnished cage; [b]mean weight calculated from the values reported in the original paper for the different hen ages; [c]barn system; [d]free-range system.

Table 15.3 the occurrence of dirty eggs in different housing systems, found by different research groups, is summarized.

In the on farm comparison of De Reu *et al.* (2009b) and in the pilot study of Tauson *et al.* (1999) the frequency of dirty eggs in nests of non-cage systems was not any higher than observed in cage systems. This was also confirmed by De Reu *et al.* (2009a) who compared eggs for sale in retail outlets and showed that only 4.4% of the eggs from non-cage systems were dirty compared with 17.2% of the eggs from cages. The LayWel database (2006a), in contrast, showed more dirty eggs (8.1%) in non-cage systems as compared with both cage systems. This conflicting result, however, was apparently due to the inclusion of floor eggs in the latter case.

Wall and Tauson (2007; Wall *et al.*, 2008) did not find differences in the proportion of dirty eggs between conventional and furnished cages. On the other hand, Mallet *et al.* (2006) and Tactacan *et al.* (2009) indicated that furnished cage systems can give rise to more dirty eggs if the cage design is less than optimal. The study of Mallet *et al.* (2006) for example showed that a less optimal furnished cage design increased the number of eggs laid outside nest and increased the percentage of dirty eggs from 3.0% to 7.1%. Comparing both conventional cage designs with both furnished cage designs, Mallet *et al.* (2006) found on average the two types of housing systems contained a comparable amount of dirty eggs (4.9% versus 5.0%).

An important aspect to remember is that 85–98% of the floor eggs laid in non-cage systems will have dirty eggshells (Abrahamsson and Tauson, 1998; De Reu, 2006). Abrahamsson and Tauson (1998) found that the proportion of dirty eggs in an aviary system is highly dependent on the proportion of

Table 15.3 Occurrence of dirty eggs (in % of eggs) in different housing systems

Study	CC (%)	FC (%)	NC (%)	P
Pilot studies				
Tauson *et al.* (1999)	6.5[a] (5.3–7.6)[b]	n.i.	5.7 (4.0–8.8)[c]	**[d]
Mallet *et al.* (2006)	4.9 (4.9–4.9)	5.0 (3.0–7.1)	n.i.	***[d]
Wall and Tauson (2007)	6.4 (6.3–6.5)	7.1	n.i.	n.s.
Wall *et al.* (2008)	5.4	4.2	n.i.	n.s.
Tactacan *et al.* (2009)	4.2 (2.4–5.3)	12.1 (7.2–15.5)	n.i.	***[d]
Pilot + commercial flock scale (on farm)				
LayWel, (2006a)	4.9	5.9 (4.7–8.2)	8.1 (7.7–8.4)	–
Commercial flock scale (on farm)				
De Reu *et al.* (2009b)	n.i.	22[c]	24[c]	n.s. [e]
On shop comparisons				
De Reu *et al.* (2009a)	17.2	n.i.	4.4	–

CC = conventional cage, FC = furnished cage, NC = non-cage.
n.i. = not included in the study.
*$P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; n.s. = non-significant ($P > 0.05$).
[a]Average of the study; [b]range of individual results; [c]only nest eggs; [d]based on individual results; [e]based on averages of the study.

eggs laid outside the nest, since most misplaced eggs will be contaminated with faeces. In one of their trials 12.9% of the nest eggs were dirty, compared with 85.5% for the floor eggs, and on average a total of 18.5% eggs (nest + floor eggs) contained dirtiness.

15.2.4 Shell quality

Eggshell strength and shell thickness

Eggshell strength is a very important economic factor because broken eggs are discarded and result in financial loss for the producers. Shell mechanical properties are also crucial for egg safety, as eggshell cracks represent an easy way for bacteria to penetrate the shell and contaminate the egg content (see also Section 15.3.2). Table 15.4 summarizes the data relating to eggshell breaking strength and shell thickness (often used as an indicator of strength) in cage and non-cage systems by various authors.

According to Table 15.4, most studies found no differences in shell breaking strength between eggs from conventional cages and non-cage systems, including barn and free range. However, Abrati (2006) reported significant differences in shell strength, although the difference is small, for caged eggs compared with barn eggs, and the highest shell strength in eggs from organic production. Van den Brand *et al.* (2004) found no difference in shell thickness for caged as compared with free range eggs, this was also confirmed by Dukić-Stojčić *et al.* (2009) and Wang *et al.* (2009). Differences in shell thickness between cage and barn eggs were reported by Englmaierová and Tůmová (2009) and by Abrati (2006). Interestingly the former authors

Table 15.4 Average shell breaking strength and shell thickness in different housing systems (the hen age experimental period is given in brackets)

Study	Shell breaking strength (N)			Shell thickness (mm)		
	CC	NC	P	CC	NC	P
Pilot studies						
Van den Brand *et al.* (2004) (25–59 weeks)	n.i.	n.i.	–	0.321	0.321[f]	n.s.
Wang *et al.* (2009) (26–50 weeks)[b]	34.92[c]	34.43[cf]	n.s.	0.33	0.33[f]	n.s.
Petek *et al.* (2009) (24–36 weeks)	37.21	37.39[f]	n.s.	0.336	0,343[f]	*
Dukić-Stojčić *et al.* (2009) (one year)	2.48[a]	2.52[af]	n.s.	0.374	0.371[f]	n.s.
Englmaierová and Tůmová (2009) (20–60 weeks)	n.i.	n.i.	–	0.365	0.370[e]	**
Commercial flock scale (on farm)						
Abrati (2006) (27–68 weeks)	37.79	35.06[e]	*	0.463[d]	0.456[de]	*
		40.05 [g]	*		0.477[dg]	*

CC = conventional cage, NC = non-cage.
n.i. = not included in the study.
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; n.s. = non-significant ($P > 0.05$).
[a]Values expressed as breaking stress (kg/cm^2); [b]blue-shelled eggs; [c]original values multiplied by 9.81 to convert kgf to N; [d]shell membranes not removed; [e]barn system; [f]free-range system; [g]organic production.

reported thicker shells for barn eggs and the latter author reported exactly the opposite effect. However, both studies reported only slightly different values between the two systems. A small difference, although significant, was also reported by Petek *et al.* (2009) between eggshell thickness of cage eggs and free-range eggs. According to values of Abrati (2006), the difference becomes considerable when cage and organic eggs are compared, the latter being significantly thicker. These two last studies agree with the findings of Leyendecker *et al.* (2001), who reported thicker shells in free range eggs than in conventional cage eggs.

Data concerning the effect of furnished cage on shell quality are rare, except for Leyendecker *et al.* (2005) who reported a significantly thinner and weaker shell in furnished cages than conventional cages and outdoor run eggs, with the outdoor eggs (aviary + outdoor run) having the thickest shells. On the other hand, Wall and Tauson (2002) found no difference between shell breaking strength of eggs produced in furnished cages equipped with different devices.

Eggshell cracks

The requirement to downgrade eggs due to cracks and broken eggshells is also an important quality parameter as here both shell strength and the strength of the insult to which the egg is exposed during routine collection and handling are taken into account. Cracked eggshells provide the opportunity

to bacteria to penetrate the eggshell and hence contaminate the egg content. Table 15.5 summarizes the results of different research groups who studied the influence of housing systems on eggshell cracks are.

Furnished cages result in the highest number of cracked eggs (Guesdon *et al*., 2006; Mertens *et al*. 2006; Wall and Tauson, 2007; De Reu *et al*. 2009b). Guesdon *et al*. (2006) found in pilot studies levels as high as 15.4–19.6% in furnished cages compared with only 8.1–12.2% in conventional cages. The high level of cracks in furnished cages here was attributed to the design of the nest, the lack of an egg saver and the low frequency of manual egg collection. The study of De Reu *et al*. (2009b) documented variations of cracked eggs from 0 up to 24% for individual farms with furnished cages. Again the highest percentage (24%) of cracks was attributed to the poor adjustment of the egg saver and an accumulation of eggs next to the nestbox on a short part of the egg collection belt. In the study of Tauson *et al*. (1999) cracks varied from 2.2 to 7.0%, with no significant difference on average between the housing systems: 5.0% compared with 4.6% for conventional and floor housing (barn), respectively.

In a market study on the quality characteristics of eggs from different housing systems, Hidalgo *et al*. (2008) found no significant difference in occurrence of cracked eggs between cage (14%), free-range (10%), barn (11%) and organic (5%) eggs. At the retail level De Reu *et al*. (2009a) also showed that non-cage eggs do not contain more cracks than cage eggs (5.6 versus 7.8%). Of course, the eggs in these market studies had already been candled and sorted at the packaging station. Finally, Abrahamsson and

Table 15.5 Occurrence of cracked eggs (in % of eggs) in different housing systems

Study	CC (%)	FC (%)	NC (%)	P
Pilot studies				
Tauson *et al*. (1999)	5.0[a] (4.3–5.6)[b]	n.i.	4.6 (2.2–7.0)[c]	** [e]
Guesdon *et al*. (2006)	10.2 (8.1–12.2)	17.5 (15.4–19.6)	n.i.	*** [e]
Wall and Tauson (2007)	2.2	3.3 (3.2–3.6)	n.i.	*** [e]
Tactacan *et al*. (2009)	0.7	0.6	n.i.	n.s. [f]
Pilot + commercial flock scale				
Laywel (2006a)	2.6	1.7–2.0	1.1–3.2	–
Commercial flock scale				
Mertens *et al*. (2006)	6.7	10.7	2[d]	–
De Reu *et al*. (2009b)	n.i.	7.8%[c]	4.1[c]	* [f]
On shop comparisons				
Hidalgo *et al*. (2008)	14	n.i.	8.7 (5–11)	n.s. [e]
De Reu *et al*. (2009a)	7.8	n.i.	5.6	–

CC = conventional cage, FC = furnished cage, NC = non-cage.
n.i. = not included in the study.
*$P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; n.s. = non-significant ($P > 0.05$).
[a]Average of the study; [b]range of individual results; [c]only nest eggs; [d]sampled at egg collecting area; [e]based on individual results; [f]based on averages of the study.

Tauson (1998) found that even aviary floor eggs did not have more cracks (1.6–3.4%) than nest eggs (3.3–3.5%).

15.2.5 Conclusions

To conclude, most studies generally agree that housing system has no effect on egg shape, while a general conclusion on the influence on egg weight cannot be drawn owing to inconsistencies of results from the different authors. The same reason hinders a general conclusion on the effect of the different housing systems on shell strength and shell thickness, especially for thickness. As suggested by Van den Brand *et al*. (2004), ambiguities or contrasts between the different studies might be explained by differences in the amount of dietary Ca and available P, which can strongly affect shell quality.

Concerning the presence of dirt on eggshell, the available research results indicate that with the correct design of furnished cages and nestboxes, and with a good nestbox use and maintenance, dirty eggs should not be a problem in furnished cages and non-cage systems compared with conventional cages.

In summary the different research results indicate that nest eggs of non-cage systems are in normal circumstances not more susceptible to cracks than those of cage eggs. In addition, results indicate that with a good design of furnished cages and a good egg collection (reduce roll out speed, timing of running the egg belt), cracked eggs should also be no problem in furnished cages than in conventional cages and non-cage housing systems.

15.3 Interior egg quality

15.3.1 Introduction

Interior egg quality relates to many factors of albumen and yolk characteristics as well as the presence of defects, such as meat and blood spots. Albumen in particular has a major influence on overall interior egg quality of fresh eggs through the measurements of albumen height, Haugh unit (HU) and pH. The most evident yolk property is colour, but this factor depends on the hen's diet; moreover, colour preferences also vary among countries. The proportion of yolk is also a quality factor for both table eggs and eggs destined to the production of egg products. A large but prominent yolk (not flattened) is in fact appreciated by the consumers when opening shell eggs, moreover industrial liquid yolk has larger economic value than albumen product.

Egg is a staple food containing high digestible and available nutrients and well-balanced essential constituents (Nys, 2001). Besides the incomparable nutritional value, eggs are appreciated in culinary for their multi-faceted functional properties that contribute to the texture and sensory characteristics of foods (Rossi *et al*., 2010). Thus the knowledge of the possible effects

of alternative farming systems on the egg nutritional value, taste and other functional properties is of outstanding importance. However, when considering such possible effects, one must always keep in mind the major influence on egg composition of other factors, such as the hen age and diet that are known to affect the macro- and micronutrients of the hen egg.

15.3.2 Percentage of fractions, albumen and yolk quality

Tables 15.6 and 15.7 summarize the results of recent studies reporting the effects of housing system on some interior egg quality factors. With the exception of eggs sourced at retail, all data concern freshly laid eggs, avoiding the well-known influence of egg storage on albumen characteristics (Karoui *et al*., 2006).

The housing system appears to have no effect on the proportions of the different egg fractions (Van den Brand *et al*., 2004; Abrati, 2006; Englmaierová and Tůmová, 2009; Samman *et al*., 2009), although Abrati (2006) reported a higher proportion of yolk in organic eggs than in conventional cage and barn eggs (Table 15.6). Contrasting results are reported for albumen height (Table 15.7). Albumen height was not found to be different between cage and non-cage eggs in the study of Van den Brand *et al*. (2004) and in the on market investigation of Hidalgo *et al*. (2008). However, some authors observed lower albumen height in cage versus non-cage eggs (Abrati, 2006; Dukić-Stojčić *et al*., 2009) or just the opposite, as found by Singh *et al*. (2009). Less incongruous data were reported for HU values either showing no differences between cage and non-cage eggs (Englmaierová and Tůmová, 2009; Petek *et al*., 2009) or higher values in non-cage eggs than in cage eggs, according to Abrati (2006) and Dukić-Stojčić *et al*. (2009). Eggs acquired on the Italian market and analysed by Hidalgo *et al*. (2008) showed no HU differences among the housing systems, except that organic eggs had much lower HU, suggesting these eggs were less fresh. Patterson *et al*. (2001) also observed lower HU and albumen height in organic versus cage eggs, probably as a consequence of a slower retail turnover of organic eggs in the US market. Albumen pH was not found to be different between cage and non-cage eggs (Van den Brand *et al*., 2004; Englmaierová and Tůmová, 2009), although Petek *et al*. (2009) and Abrati (2006) reported significant lower pH values in non-cage eggs. By averaging observations on albumen pH and HU, a tendency to higher albumen quality in non-cage eggs can be argued. Data on yolk colour reported in Table 15.7 are deeply variable among the different studies because yolk colour is a factor mainly depending on hen diet. It is interesting to note the paler colour reported by the British Columbian researchers (Singh *et al*., 2009), reflecting the different colour preferences between Europe and Western Canada, where much paler yolks are readily accepted (ISA, 2008).

Few data have been published about the effect of furnished cage on the interior egg quality. Valkonen *et al*. (2008) observed no HU difference

Table 15.6 Average values of albumen, yolk, and shell percentages in eggs from different housing systems (the hen age experimental period is given in brackets)

Study	Albumen (%)			Yolk (%)			Shell (%)		
	CC	NC	P	CC	NC	P	CC	NC	P
Pilot studies									
Van den Brand *et al.* (2004) (25–59 weeks)	58,79	59.05[b]	n.s.	32.74	32.40[b]	n.s.	12.59	12.64[b]	n.s.
Englmaierová and Tůmová (2009) (20–60 weeks)	60.6	61.7[a]	n.s.	27.2	26.7[a]	n.s.	12.2	11.7[a]	n.s.
Commercial flock scale (on farm)									
Abrati (2006) (27–68 weeks)	65.59	65.57[a] 64.38[c]	n.s. *	23.53	23.45[a] 24.44[c]	n.s. *	10.88	10.97[a] 11.10[c]	n.s. *
On shop comparison									
Samman *et al.* (2009)	62.1	62.6[c]	n.s.	26.07	26.56[c]	n.s.	11.37	11.03[c]	n.s.

CC = conventional cage, NC = non-cage.
* $P \leq 0.05$; n.s. = non-significant ($P > 0.05$).
[a] Barn system; [b]free-range system; [c]organic production.

Table 15.7 Average values of albumen quality factors and yolk colour in eggs from different housing systems (the hen age experimental period is given in brackets)

Study	Albumen height (mm)			Haugh unit			Albumen pH			Yolk colour (DSM yolk colour fan)		
	CC	NC	P	CC	NC	P	CC	NC	P	CC	NC	P
Pilot studies												
Van den Brand *et al.* (2004) (25–59 weeks)	5.88	6.04[b]	n.s.	n.i.	n.i.	–	8.14	8.16[b]	n.s.	9.3	11.0[b]	***
Dukić-Stojčić *et al.* (2009) (one year)	7.96	8.57[b]	*	87.08	91.25[b]	*	n.i.	n.i	–	12.08	11.76[b]	n.s.
Englmaierová and Tůmová (2009) (20–60 weeks)	n.i.	n.i.	–	85.0	87.2[a]	n.s.	8.34	8.32[a]	n.s.	n.i.	n.i.	–
Petek *et al.* (2009) (24–36 weeks)	n.i.	n.i.	–	85.95	85.89[b]	n.s.	8.96	8.88[b]	*	9.89	10.13[b]	*
Singh *et al.* (2009) (20–50 weeks)	8.58	8.45[a]	*	n.i.	n.i.	–	n.i.	n.i.	–	5.05	6.11[a]	**
Commercial flock scale (on farm)												
Abrati (2006) (27–68 weeks)	7.7	8.0[a]	*	86.0	87.5[a]	*	8.78	8.72[a]	*	10.5	9.4[a]	*
On shop comparison												
Patterson *et al.* (2001)	5.0	4.1[c]	***	67.5	57.6[c]	***	n.i.	n.i.	–	n.i.	n.i.	–
Hidalgo *et al.* (2008)	5.3	5.1[a]	n.s.	69.2	67.6[a]	n.s.	n.i.	n.i.	–	10.5	9.7[a]	**
		5.2[b]	n.s.		66.2[b]	n.s.		n.i.	–		10.0[b]	n.s.
		4.7[c]	n.s.		61.0[c]	**		n.i.	–		9.4[c]	**

CC = conventional cage, NC = non-cage.
n.i. = not included in the study.
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; n.s. = non-significant ($P > 0.05$).
[a]Barn system; [b]free-range system; [c]organic production.

between conventional and furnished cage eggs for hens offered either low- or high-energy diet, and Vits *et al*. (2005) reported no effect of three different furnished cage systems on HU.

15.3.3 Nutrients

Data reported in Table 15.8 show contrasting results on the effect of the housing system on egg macronutrients (proteins and lipids). These macronutrients are mainly modified by the hen's age, and by the genetic origin of the hen to a lesser extent (Nys, 2001). A tendency to higher cholesterol levels in non-cage eggs is shown for both barn systems and organic production (Table 15.8). This tendency is further confirmed by the lowest cholesterol level (1.25 g/100 g yolk) found in furnished cage eggs (Zemková *et al*. 2007). In a previous review, Sauveur (1991) also reported a tendency, although rarely significant, to higher cholesterol in non-cage eggs.

Fatty acid composition of cage and non-cage eggs is reported in Table 15.9. Both Hidalgo *et al*. (2008) and Samman *et al*. (2009), analysing eggs purchased from supermarkets, found higher levels of saturated fatty acids (SFA) on average in organic eggs than in cage eggs. However, this was not found for supermarket eggs from barn and free-range systems (Hidalgo *et al*., 2008). Also Abrati (2006) reported no effect of barn system on SFA, while Pignoli *et al*. (2009) observed lower SFA in free-range eggs than in cage eggs, partly justifying the difference as a consequence of the free-range hens' access to grassland area with available grass, insects and worms. The last authors also reported differences between cage and non-cage eggs for mono- (MUFA) and polyunsaturated fatty acids (PUFA), in contrast with the findings of Abrati (2006) who found an opposite trend for MUFA and non-significant difference for PUFA. The studies of Hidalgo *et al*. (2008) and Samman *et al*. (2009) showed instead no differences in the levels of both MUFA and PUFA of market eggs from different housing systems. Considering the ratios PUFA/SFA and n-6/n-3 fatty acids, no substantial differences between cage and non-cage systems can be argued from the studies of Hidalgo *et al*. (2008) and Pignoli *et al*. (2009). Although some statistically significant differences are reported for these ratios by Abrati (2006) and Samman *et al*. (2009), the values so close to each other (PUFA/SFA, 0.49 vs 0.50; n-6/n-3, 11.03 vs 11.19) hardly have a nutritional significance. An exception is the particularly low n-6/n-3 value calculated from the data of Matt *et al*. (2009) for cage eggs in comparison to organic eggs, but in this case the difference was attributed to a difference in the diet received by the hens at each location.

In general, either no differences or contrasting trends can be found in the literature for the influence of the housing systems on fatty acid composition. This is explained by the fact that it is relatively easy to manipulate the fatty acid profile of eggs through the hen diet. Thus, the possible effect of the housing system is frequently overcome by the effect of diets administered

Table 15.8 Average protein, lipid and cholesterol content of eggs from different housing systems (the hen age experimental period is given in brackets). If otherwise not indicated, results are on whole eggs

Study	Protein (g/100g)			Lipid (g/100g)			Cholesterol (mg/100g)		
	CC	NC	P	CC	NC	P	CC	NC	P
Pilot studies									
Pištěková *et al.* (2006) (9 months)	n.i.	n.i.	–	n.i.	n.i.	–	1.12	1.17[ab]	**
Zemková *et al.* (2007) (39–75 weeks)	n.i.	n.i.	–	n.i.	n.i.	–	1.33[a] 1.33[a]	1.41[ab] 1.34[ac]	n.c. n.c.
Pignoli *et al.* (2009) (54–56 weeks)	n.i.	n.i.	–	58.7[d]	61.5[de]	*	3.61[d]	3.42[cd]	n.s.
Commercial flock scale (on farm)									
Abrati (2006) (27–68 weeks)	12.20	12.43[b]	*	9.13	9.08[b]	n.s.	4.09	4.24[b]	*
Minelli *et al.* (2007) (28–73 weeks)	n.i.	n.i.	–	n.i.	n.i.	–	1.21[a]	1.26[ae]	**
Matt *et al.* (2009) (35–37 weeks)	12.35	11.90[e]	n.c.	8.88	7.94[e]	n.c.	3.41	4.89[e]	n.c.
On shop comparison									
Hidalgo *et al.* (2008)	12.1	12.6[b] 12.5[c] 12.5[e]	* * *	9.5	9.5[b] 9.4[c] 10.1[e]	n.s. n.s. n.s.	n.i.	n.i.	–

CC = conventional cage, NC = non-cage.
n.i. = not included in the study; n.c. = not calculated.
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; n.s. = non-significant ($P > 0.05$).
[a]As g/100 g yolk, [b]barn system; [c]free-range system; [d]as g/100 g lipid; [e]organic production.

to hens, according to management options made by the different producers (Rossi, 2007). Table 15.10 highlights the major influence of the producers on the fatty acid profile of eggs from different housing systems.

Vitamins also can be modulated in the hen egg through the addition of vitamin supplements (Nys, 2001). Table 15.11 compares the total tocopherols and retinol levels in eggs from cage and non-cage systems. Some of these results, however, cannot simply be attributable to differences in diet. Stressors such as heat could explain the lower value of total tocopherol reported by Abrati (2006) in barn eggs compared with cage eggs, receiving the same diet.

15.3.4 Functional and sensory properties

Few studies have been published on the influence of hen housing systems on egg functional and sensory properties. Mizumoto *et al.* (2008) found no

Table 15.9 Fatty acid composition, PUFA/SFA and n-6/n-3 of eggs from different housing systems (the hen age experimental period is given in brackets)

Study	SFA (%)			MUFA (%)			PUFA (%)			PUFA/SFA			n-6/n-3		
	CC	NC	P	CC	NC	P	CC	NC	P	CC	NC	P	CC	NC	P
Pilot studies															
Pignoli *et al.* (2009) (54–56 weeks)	34.55	33.71[c]	*	34.96	37.91[c]	*	30.49	28.37[c]	**	0.88	0.84[c]	n.c.	12.42	12.76[c]	n.s.
Commercial flock scale (on farm)															
Abrati (2006) (27–68 weeks)	33.92	33.55[b]	n.s.	49.56	47.00[b]	*	16.51	16.75[b]	n.s.	0.49	0.50[b]	*	10.15	10.12[b]	n.s.
Matt *et al.* (2009) (35–37 weeks)	n.i.	n.i.	–	n.i.	n.i.	–	n.i.	n.i.	–	0.64[a]	0.48[ad]	n.c.	7.00[a]	10.00[ad]	n.c.
On shop comparison															
Hidalgo *et al.* (2008)	33.9	35.3[b]	n.s.	40.5	41.7[b]	n.s.	22.9	21.3[b]	n.s.	0.8	0.7[b]	n.s.	11.2	12.0[b]	n.s.
		34.4[c]	n.s.		43.6[c]	n.s.		22.0[c]	n.s.		0.6[c]	n.s.		11.1[c]	n.s.
		36,4[d]	*		39.6[d]	n.s.		24.0[d]	n.s.		0.7[d]	n.s.		11.5[d]	n.s.
Samman *et al.* (2009)	33.8	34.6[d]	*	50.0	49.0[d]	n.s.	16.3	16.4[d]	n.s.	n.i.	n.i.	–	11.03[a]	11.19[ad]	*

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
CC = conventional cage, NC = non-cage.
n.i. = not included in the study; n.c. = not calculated.
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; n.s. = non-significant ($P > 0.05$).
[a]Calculated from the original data; [b]barn system; [c]free-range system; [d]organic production.

Table 15.10 Effects of farming methods and producers on the fatty acid composition of eggs. Data concern eggs from four different producers, each managing conventional cage, barn, free-range systems and organic production (modified from Rossi, 2007)

Fatty acids	Housing systems					Producers				
	Cage $n = 4$	Barn $n = 4$	Free range $n = 4$	Organic $n = 4$	P	A $n = 4$	B $n = 4$	C $n = 4$	D $n = 4$	P
SFA (%)	34.1[a]	35.1[b]	34.5[ab]	35.0[b]	*	34.5[a]	34.7[a]	34.0[a]	35.6[b]	**
MUFA (%)	43.7	43.4	42.9	42.7	n.s.	44.4[b]	43.2[b]	39.9[a]	43.8[b]	*
PUFA (%)	22.9	21.3	22.5	22.1	n.s.	21.0[a]	22.1[a]	25.9[b]	20.6[a]	*
n-6/n-3	10.8	11.2	11.7	11.3	n.s.	11.6[b]	11.1[b]	9.4[a]	11.2[b]	***

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[a–b]Different letters in the same row indicate significantly different values.
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; n.s. = non-significant ($P > 0.05$).

Table 15.11 Total tocopherol and retinol contents of eggs from different housing systems (the hen age experimental period is given in brackets)

Study	Total tocopherols (mg/100 g yolk)			Retinol (mg/100 g yolk)		
	CC	NC	P	CC	NC	P
Pilot studies						
Pignoli *et al*. (2009) (54–56 weeks)	23.0[a]	25.2[ac]	*	n.i.	n.i.	–
Commercial flock scale (on farm)						
Abrati (2006) (27–68 weeks)	13.39	9.86[b]	*	0.63	0.62[b]	n.s.
Matt *et al*. (2009) (35–37 weeks)	15.77[de]	6.78[de]	n.c.	0.57[d]	0.46[d]	***

CC = conventional cage, NC = non-cage.
n.i. = not included in the study; n.c. = not calculated.
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; n.s. non-significant ($P > 0.05$).
[a] As mg/kg lipids; [b]barn system; [c]free-range system; [d]organic production; [e]calculated as sum of the original data on different tocopherol homologues.

significant sensorial differences between hard-boiled eggs from cage, semi-organic and organic systems. Likewise Rossi (2007) found no difference among overall taste acceptability of hard-boiled eggs from cage, litter and organic systems. However, in Rossi's study, the overall appearance of raw broken-out eggs was judged to be different, with the cage eggs' appearance being less appreciated. In this study the panellists' judgement was first based on albumen appearance and then on yolk colour.

Concerning the eggs' functional properties, Hidalgo *et al*. (2008) reported significant differences for whole egg foam consistency and overrun of eggs sold in supermarkets. These authors found better foaming properties in organic and free-range eggs than in cage and barn eggs. Moreover, on average cage eggs performed the least well.

Rossi (2007) carried out an on farm study on the functional properties of eggs from different systems and found barn eggs to be poorer in terms of yolk emulsifying capacity and albumen foam overrun compared with those from cage and organic eggs.

15.3.5 Conclusions

As a conclusion on the influence of housing system on interior egg quality, it is possible to tell that the majority of research studies agree in finding no influence of the housing system on the proportions of the different egg fractions (albumen, yolk, shell). Instead, the albumen quality, expressed as pH and Haugh unit shows a tendency to higher quality in non-cage eggs. For yolk colour and egg nutrients, results are generally contrasting, due to the ability to modulate pigments, fatty acids and vitamins by the addition of supplements in the hen diet. However, a tendency toward higher cholesterol in non-cage eggs can be argued. The few studies concerning the

egg taste acceptability report no difference due to the housing system. A general conclusion cannot be drawn for the effect of housing systems on egg functional properties, owing to the rarity of the studies on this subject. However, the differences observed are probably dependent on variations in the egg composition, as reported by Rossi *et al*. (2008), who found for whole egg foaming properties direct correlations between foam consistency and albumen protein, and between foam instability and fat content.

15.4 Hygienic quality

15.4.1 Introduction

The eggshell can become bacterially contaminated when passing through the vent, but many researchers suggest that the main bacterial contamination occurs within a short period after laying due to contact with a dirty environment and dirty surfaces (Quarles *et al*., 1970; Gentry and Quarles, 1972). Messens *et al*. (2005) and De Reu *et al*. (2006a, 2006c) reported that increasing numbers of microorganisms on the eggshell consequently increase the risk of microbial eggshell penetration and egg content contamination through a horizontal transmission route. As a result most research on the influence of housing systems on the hygienic quality of eggs was focused on eggshell contamination. Besides the horizontal route of bacterial infection of eggs, egg content contamination also occurs through the vertical or transovarian route.

Early studies compared bacterial eggshell contamination in litter and wire floor houses. Quarles *et al*. (1970) reported that litter floor houses had on average nine times more bacteria in the air, and 20 to 30 times more aerobic bacteria on the shell than wire floor houses. Harry (1963) reported that the shells of eggs from deep litter systems had 15 times more bacteria and a higher proportion of potential spoilage organisms than eggs from battery cage systems. The Council Directive 1999/74/EC, introducing alternative housing systems for laying hens in the EU, has driven recent attention again towards the effect of housing system on the bacterial eggshell contamination of table eggs.

15.4.2 Eggshell contamination

Conventional cages as compared with furnished cages

De Reu *et al*. (2005b) compared the bacterial eggshell contamination of eggs laid in conventional cages with eggs laid in the nestboxes of furnished cages (Fig. 15.1). No systematic difference in shell contamination with total counts of aerobic bacteria was found between these systems (ranging from 4.0 to 4.5 log CFU/eggshell). Further, for Gram-negative bacteria no difference was detected (both means ca. 3.0 log CFU/eggshell). The type of nest-flooring material in the nestboxes of the furnished cages did not

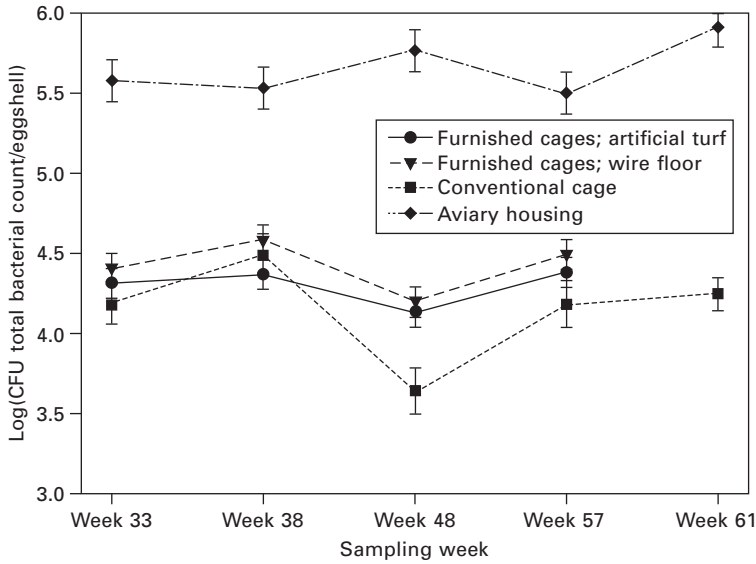

Fig. 15.1 Average eggshell contamination (n = 40 eggs) with total count of aerobic bacteria on different times for four compared designs including three different housing systems (De Reu *et al.*, 2005b).

systematically influence the bacterial eggshell contamination. Cepero *et al.* (2000) also found no differences in counts of aerobic bacteria but reported a higher prevalence of coliforms on shells of eggs laid in furnished cages. Mallet *et al.* (2006) studied the hygienic aspects of eggs laid at different locations in furnished cages. A significant difference in total count of aerobic bacteria was observed on the eggshell of eggs collected from furnished cages (4.83 log CFU/eggshell) compared with conventional cages (4.56 log CFU/eggshell). This was mainly attributed to eggs laid outside the nest in the litter area (4.96 log CFU/eggshell) or in the cage (4.94 log CFU/eggshell). The bacterial load on eggs laid in the nests was similar to those collected from the conventional cages. Similar conclusions were obtained for *Enterococcus*. Wall *et al.* (2008) also found a higher bacterial load on eggs from furnished cages than for conventional cages. The bacterial counts were significantly higher in the furnished cages as compared with the conventional cages as regards Enterobacteriaceae, *Enterococcus* and total count of aerobic bacteria. All above mentioned studies were performed in experimental units.

In an on farm comparison, Hunau-Salaün *et al.* (2010) also found a higher eggshell contamination on eggs from furnished cages (5.09 log CFU/egg) compared with conventional cages (4.40 log CFU/egg).

Cage as compared with non-cage systems

Further experimental studies show that eggs from aviaries were contaminated with higher numbers of aerobic bacteria than eggs from cage systems (Protais

et al., 2003; De Reu *et al*., 2005b). The results of De Reu *et al*. (2005b) are shown in Fig. 15.1.

The difference was more than 1 log unit (up to 5.1–6.0 log CFU/eggshell for eggs from aviaries), with much higher counts on those eggs laid on the floor of the aviaries (up to 7 log CFU/eggshell). For Gram-negative bacteria no systematic differences were found between cage and non-cage housing systems (De Reu *et al*., 2005b).

De Reu *et al*. (2005a, 2006b) evaluated whether the differences in eggshell contamination, found in the experimental housing systems, were also applicable to commercial conventional cage and non-cage housing systems. Two conventional cage systems, one organic aviary system and one floor housing system (barn), were included. On average, a significantly higher initial eggshell contamination with total count of aerobic bacteria was found for eggs from non-cage systems compared with conventional cage systems; 5.46 versus 5.08 log CFU/eggshell, respectively. However, the contamination with total count of Gram-negative bacteria on the eggshells was significantly lower in the non-cage systems; 3.31 versus 3.85 log CFU/eggshell. The studies showed that the major differences in eggshell contamination with total count of aerobic bacteria, found between conventional and non-cage systems in the experimental studies (>1 log) were less pronounced in the sampled commercial housing systems. The even lower initial contamination with Gram-negative bacteria in the commercial non-cage systems was remarkable. Schwarz *et al*. (1999) also found fewer Gram-negative bacteria on free-range eggs than on cage eggs.

A second and larger on farm comparison by De Reu *et al*. (2009b) compared six flocks of laying hens in furnished cages and seven flocks in non-cage systems (three aviaries and four floor systems). On average, eggshells from furnished cages were slightly, but significantly, less contaminated with total count of aerobic bacteria compared with non-cage eggshells (4.75 versus 4.98 log CFU/eggshell) (Fig. 15.2).

In the non-cage systems, no difference in average contamination between aviary and floor (barn) systems was found; 4.95 and 5.00 log CFU/eggshell respectively. Major differences (>1 log) in eggshell contamination with total count of aerobic bacteria were found between farms, both within the groups of furnished cage- and non-cage systems. Differences in farm construction and management can possibly explain this. For Enterobacteriaceae no significant difference in average eggshell contamination was found between furnished and non-cage systems: 88 and 94% of eggshells contained < 10 CFU Enterobacteriaceae/eggshell, respectively. Hunau-Salaün *et al*. (2010) also found a higher eggshell contamination for eggs from non-cage systems (4.82 CFU/eggshell) compared with conventional cages (4.40 CFU/eggshell). On the other hand, no difference between furnished cages and non-cage systems was found. Schwarz *et al*. (1999), sampling at the shell egg processing plant, found 10 times more bacteria present on free-range eggs (3.20–4.30 log CFU/cm^2) as compared with cage eggs (2.30–3.90 log CFU/cm^2). Finally Singh

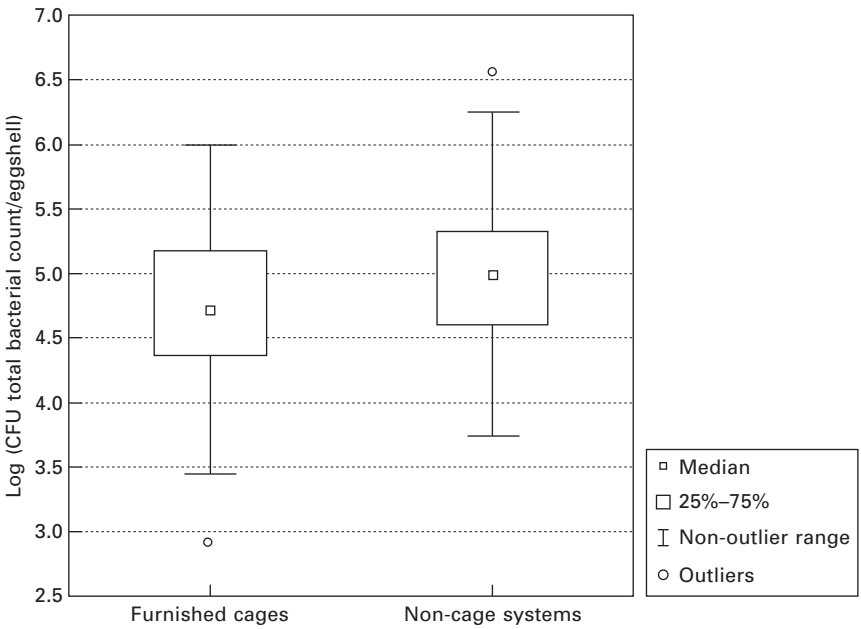

Fig. 15.2 Eggshell contamination with total count of aerobic bacteria in furnished cages (seven flocks) compared to non-cage systems (six flocks) (De Reu *et al*., 2009b).

et al. (2009) found higher counts of coliforms and *Escherichia coli* on nest eggs from floor systems than on cage eggs.

Bacterial air contamination and its relationship to eggshell contamination
In some studies the total count of aerobic bacteria in the air of poultry houses was proven to be positively correlated with the bacterial eggshell contamination at the henhouse (Protais *et al*., 2003; De Reu *et al*., 2005b). Averages of 4 log CFU/m^3 air for the conventional and furnished cages were found compared with a 100 times higher average (> 6 log CFU/m^3) for aviary housing systems.

15.4.3 Egg content contamination

Currently, it is not known whether the housing systems and the differences in bacterial counts on the shell of eggs produced in different housing systems have an impact on the global bacterial contamination of egg contents. Very little literature is available on the subject. Most of the studies focus on egg content contamination with *Salmonella* and not on the general bacterial egg content contamination. Harry (1963), De Reu *et al*. (2006a, 2006c), Messens *et al*. (2005) and many other researchers found a correlation between bacterial eggshell contamination and egg infection or egg content contamination. The

higher prevalence of coliforms on the shells of eggs laid in furnished cages was not correlated with signs of coliform contamination in egg yolk or albumen (Cepero *et al.*, 2000). In a preliminary study of De Reu *et al.* (2007, 2008a,b), egg content contamination of nest eggs was 1.9% for furnished cages as compared with 2.3% for non-cage systems.

15.4.4 Conclusions

It is clear that eggshell contamination with aerobic bacteria is significantly higher on average for nest eggs from non-cage systems as compared with nest eggs from furnished cages or eggs from conventional cages. The major differences found in experimental studies between cage and non-cage systems are less pronounced under commercial circumstances. In addition to the housing system, farm management also seems to play an important role in the bacterial eggshell contamination. The scarce information available on the influence of the housing systems on the egg content contamination indicates no major differences in egg content contamination between cage eggs and non-cage eggs (ignoring outside nest and floor eggs).

15.5 Conclusion

This present review illustrates the difficulties experienced when trying to determine the effects of housing systems on egg quality from the confounding effects of differences in hen diet, farm construction, general management, etc. A complete description of the wide variety of housing systems for laying hens, including various facilities and equipments used, has been reported in LayWel (2006b). The laying hens themselves also contribute to the variability of egg quality within the same category of housing system. Notwithstanding these difficulties, certain conclusions can be drawn concerning the effect of the housing system on the quality of egg. Consistency under different housing systems of some of the main quality attributes (i.e. shape, proportions of albumen, yolk and shell) are generally recognized. In contrast, yolk colour, fatty acid and vitamin compositions are more likely to be affected by the hen diet, although some stress due to unfavourable ambient conditions (i.e. temperature) may also affect vitamin deposition in eggs. Farm construction and management also seem to play an important role in the bacterial eggshell contamination when taken together with the housing system. Overall there is evidence that the total count of aerobic bacteria on eggshells is higher in non-cage than in cage systems. Dirty and cracked eggs are more likely to occur in furnished cages and to a lesser extent in non-cage systems (especially floor eggs). However, the available research also indicates that in this respect the problem in furnished cages can be overcome with the correct design of furnished cages and the nestboxes and good egg collection procedures. Finally there is clear evidence that shows that as long as the system is well

managed, it is possible to provide top quality eggs in any of the cage and non-cage systems currently available.

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16

Avian diseases which affect egg production and quality

J. R. Roberts, University of New England, Australia, R. Souillard, Agence nationale de sécurité sanitaire de l'environnement et du travail (Anses), France and J. Bertin, Coopérative Le Gouessant, France

Abstract: This chapter addresses diseases and syndromes which have been shown, or are reported, to have adverse effects on egg production and quality. However, any disease of poultry can adversely affect egg production and quality indirectly, by affecting the health of the bird. The main topics are the effect on egg production and quality of bacteria (*Salmonella*, *Mycoplasma*, *Escherichia coli*, infectious coryza, *Ornithobacterium*, *Gallibacterium*, spirochaetosis), viruses (infectious bronchitis virus, egg drop syndrome, swollen head syndrome, avian encephalomyelitis, influenza, Newcastle disease, laryngotracheitis), syndromes (fatty liver haemorrhagic syndrome, cage layer osteoporosis) and toxic agents. A short section on clinical perspectives reports on information obtained from practising poultry veterinarians as such observations can provide valuable starting points for future research.

Key words: egg quality, avian disease, virus, bacteria.

16.1 Introduction

Any disease of poultry can adversely affect egg production and quality either directly, by having effects on the reproductive system, or indirectly, by affecting the health of the bird. Respiratory infections which result in air sacculitis may, in turn, infect the ovary and oviduct. In addition, some diseases infect the oviduct and ovary by ascending infection. It is frequently difficult to diagnose the cause or causes of reduced egg quality because it is often a combination of factors that leads to poor egg quality. Management,

nutrition and disease may, in combination, result in a reduction in egg internal quality and/or egg shell quality. The most recent comprehensive review of diseases affecting egg quality is that of Spackman (1987) who, although focusing on egg shell quality, also discussed the effects of disease on egg internal quality. This chapter addresses diseases and syndromes which have been shown, or are reported to, have adverse effects on egg production and quality. Emphasis will be on developments since Spackman's 1987 review, except where earlier information is fundamental to the discussion. The main experimental developments that have occurred since Spackman's review are new information about the effect of Australian strains of infectious bronchitis virus on the oviduct and egg quality and the reporting of egg apex abnormalities caused by *Mycoplasma synoviae* (particularly in combination with infectious bronchitis virus).

16.2 Effects of bacteria on egg production and quality

16.2.1 *Salmonella*

Aetiology

The genus *Salmonella* (family Enterobacteriaceae) consists of more than 2500 serologically distinguishable variants and can be divided into non-motile and motile serotypes (Gast, 2008). All salmonellae that are associated with poultry are members of a single genetically defined species, *Salmonella enterica*.

Epidemiology

The non-motile serotypes, *Salmonella enterica* serovar Gallinarum (fowl typhoid) and *Salmonella enterica* serovar Pullorum (pullorum disease), can cause septicaemic disease in poultry. The motile serotypes are often referred to as paratyphoid (PT) salmonellae and include *S. enterica* serotype Enteriditis and *S. enterica* serotype Typhimurium (usually abbreviated as *S.* Enteritidis and *S.* Typhimurium). The paratyphoid salmonellae are often asymptomatic in avian species, but can cause food-borne illness in humans (Gast, 2008). Age and genetic strain influence the resistance of birds to avian enteric salmonellosis (Beal *et al.*, 2005; Berchieri *et al.*, 2001).

Clinical signs in layers: egg production and quality

In general, young birds are more susceptible to *Salmonella* infections than mature birds. *S.* Gallinarum and *S.* Pullorum cause septicaemic disease resulting in decreased egg production and hatchability, as well as morbidity and mortality (Shivaprasad, 2000). Transovarian infection can occur (Berchieri *et al.*, 2001). Experimental infection of birds with *Salmonella* Enteritidis increased the incidence of hairline cracks in eggs, leading to an increased risk of contamination (Guard-Bouldin and Buhr, 2006).

16.2.2 Mycoplasma

Aetiology

Mycoplasmas are eubacteria devoid of cell walls and are members of the class Mollicutes, Order I Mycoplasmatales. Genus I, *Mycoplasma*, has more than 100 species of which 25 infect avian species, and 10 of which infect chickens (Kleven, 2008).

Epidemiology

Mycoplasma gallisepticum causes chronic respiratory disease (CRD) in chickens as well as infectious sinusitis in turkeys and often co-occurs with a respiratory virus infection (Ley, 2008). Oviduct infection can occur owing to proximity to infected air sacs (Nunoya *et al*., 1997). *M. synoviae* also causes respiratory disease but can become systemic, causing disease primarily in joints and tendon sheaths in chickens and turkeys (Kleven, 2008). *M. gallisepticum* and *M. synoviae* also infect a range of other avian species.

Clinical signs in layers: egg production and quality

Spackman (1987) states 'The role of Mycoplasmata in egg production and quality is somewhat controversial'. At that time, it was not clear to what extent *Mycoplasma synoviae* (MS) or *M. gallisepticum* (MG), alone, were involved in abnormal eggshells and production losses and what was caused by interactions with other infectious agents such as infectious bronchitis virus (Giambrone *et al*., 1977). MG had been implicated in production losses and, in the case of breeder flocks, decreased hatchability. Until about 2000, the main concern in flocks of laying hens was *Mycoplasma gallisepticum* (Kleven 2008; Kleven *et al*., 1990; Stipkovits and Kempf, 1996; Yoder 1986) although there were reports of MS in layer flocks (Branton *et al*., 1989; Mohammed *et al*., 1986, 1987a,b; Morrow *et al*., 1990; Opitz, 1983; Reece *et al*., 1986a). MS was regarded as being a problem predominantly in broiler and turkey flocks and has also been reported from other poultry species such as ducks (Bencina *et al*., 1988a; Yamada and Matsuo, 1983), geese (Bencina *et al*., 1988b) and pigeons (Reece *et al*., 1986b). The production of eggs with abnormal shells has been reported for geese infected with *Mycoplasma* sp strain 1220 (Dobos-Kovacs *et al*., 2009). The vertical transmission of MS was recognized relatively early (Carnaghan, 1961; MacOwan *et al*., 1984; Roberts and McDaniel, 1967). Both MG and MS have the potential to cause salpingitis in laying birds (Domermuth *et al*., 1967). Opitz (1983) reported MS infection on 48% of farms surveyed in Maine, US. Mohammed *et al*. (1986) reported prevalence of MG as 73% in southern California and 3% in central California and MS as 91% in southern California and 32% in central California. An economic analysis indicated that there was no association between MS infection and egg production but that MG resulted in an estimated 127 million eggs lost in southern California (Mohammed *et al*., 1987b).

A recent study by Feberwee *et al*. (2009a) has shown that MS, isolated from the oviduct of birds producing abnormal eggs, can induce eggshell apex abnormalities (EAA). These abnormalities are characterized by changes in the colour and texture of the shell in the region of the air sac. When this region of the egg shell is observed under the scanning electron microscope, the mammillary layer and the lower part of the palisade layer are missing. These EAAs ceased after an injection of long-acting oxytetracycline but reoccurred 12 days later. The incidence of EEAs was higher in the presence of infectious bronchitis virus. The presence of EAAs was accompanied by shell thinning, increased translucency and reduced shell breaking strength. A later experiment with SPF white layers (Feberwee *et al*., 2009b) investigated the ability of an MS vaccine to protect against egg shell abnormalities in the presence of an infectious bronchitis challenge. EAAs were produced only in birds that were challenged with *M. synoviae*, whether or not they had been previously vaccinated for MS. Again, this eggshell abnormality was associated with reduced shell strength. Vaccination reduced the incidence and delayed the appearance of EAAs in eggs from birds challenged with MS but did not completely prevent the occurrence of EAAs.

16.2.3 *Escherichia coli*

Aetiology

Escherichia is the type genus of the family Enterobacteriaciae and *E. coli* is the type species of the genus *Escherichia*. *E. coli* causes colibacillosis in most avian species, with younger birds being most susceptible (Barnes *et al*., 2008).

Epidemiology

E. coli is commonly found in the intestines of poultry and is transmitted to eggs primarily by faecal contamination of the shell surface followed by entry into the egg.

Clinical signs in layers: egg production and quality

Escherichia coli affects production and, potentially egg quality, in laying hens by causing colibacillosis associated with salpingitis (Trampel *et al*., 2007). Other avian species such as ducks may also be affected (Bisgaard, 1995). Many serotypes of *E. coli* are found in poultry but it is only the avian pathogenic *E. coli* (APEC) which possess specific virulence factors and are capable of causing salpingitis and peritonitis (Landman and Cornelissen, 2006). Salpingitis may be caused by either a systemic infection or by ascending infection of the oviduct from the cloaca. The study of Ozaki and Murase (2009) reported postmortem findings of fibrinous exudates in the vagina, caseous exudates in the upper oviduct, degenerated ovaries and a thickened and oedematous oviduct mucosa. The stress associated with the onset of lay may act as a precipitating factor in colibacillosis outbreaks (Zanella *et al*.,

2000). Virulent *Mycoplasma synoviae* can act as a complicating factor in *E. coli* peritonitis syndrome (Raviv *et al.*, 2007). However, Vandekerchove *et al.* (2004) concluded that colibacillosis outbreaks are not necessarily associated with other respiratory pathogens.

16.2.4 Ornithobacterium

Aetiology

Ornithobacterium rhinotracheale is a Gram-negative, non-motile, pleomorphic, rod-shaped, non-sporulating bacterium (Chin *et al.*, 2008).

Epidemiology

O. rhinotracheale causes respiratory disease in chickens and a wide range of other avian species (van Empel and Hafez, 1999). A high seroprevalence of *O. rhinotracheale* was found in a study in the north central region of the United States (Heeder *et al.*, 2001). The organism is transmitted vertically (van Empel and Hafez, 1999).

Clinical signs in layers: egg production and quality

Clinical signs are respiratory disease and the severity of the disease is worsened when birds have coexisting infections with other respiratory disease agents (Thachil *et al.*, 2009). It can also develop into peritonitis. In commercial laying flocks, *O. rhinotracheale* results in production drops, decreased egg size, misshapen eggs and increased mortality (Sprenger *et al.*, 2000). Similar symptoms have been reported for flocks of broiler breeders (Chin *et al.*, 2008).

16.2.5 Gallibacterium

Aetiology

The genus *Gallibacterium* is in the family Pasteurellaceae and contains avian bacteria formerly known as *Pasteurella haemolytica*, *Actinobacillus salpingitidis* or *Pasteurella anatis*. It includes the species *G. anatis* and *G. genomospecies* 1 and 2. *G. anatis* comprises two biovars, a haemolytic biovar haemolytica and a non-haemolytic biovar anatis (Barnes *et al.*, 2008; Christensen *et al.*, 2003; Neubauer *et al.*, 2009).

Epidemiology

Gallibacterium spp can be isolated from a wide variety of birds.

Clinical signs in layers: egg production and quality

Gallibacterium anatis, biovar haemolytica, has been suggested as causing peritonitis and salpingitis in chickens (Christensen *et al.*, 2003; Barnes and Nolan, 2008) and other species (Bisgaard, 1995) and has been isolated from laying birds suffering from reproductive disorders (Neubauer *et al.*,

2009). Similar symptoms have been induced experimentally (Bojesen *et al*., 2004).

16.2.6 Spirochaetosis

Aetiology

Spirochaetes are classified in the Order Spirochaetales which contains three families, Spirochaetaceae, Brachyspiraceae and Leptospiraceae, and a total of nine genera (Hampson and Swayne, 2008). However, spirochaetes which cause avian intestinal spirochaetosis (AIS) are all of the family Brachyspiraceae, genus *Brachyspira*.

Epidemiology

AIS occurs primarily in flocks of layers and broiler breeder hens so is a disease of birds that are producing eggs. A survey conducted in eastern Australia reported that birds in 43% of broiler breeder and 68% of layer flocks were infected with intestinal spirochaetes but no broiler flocks were infected (Stephens and Hampson, 1999). This study identified *Serpulina pilosicoli* (now *B. pilosicoli*) and *S. intermedia* (now *B. intermedia*) among the flocks but could not identify other species found. Colonization can be enhanced by a wheat-based diet (Phillips *et al*., 2004) and this could not be consistently and significantly reduced by the use of endogenous feed enzymes. A recent paper (Ivanics *et al*., 2009) reported problems in Hungary in layer flocks.

Clinical signs in layers: egg production and quality

Intestinal spirochaetes colonize the caecum and/or rectum and can cause diarrhoea (Hampson and Swayne, 2008; Ivanics *et al*., 2009). The disease also results in reduced egg production and hatchability and eggshell quality deteriorates (Ivanics *et al*., 2009).

16.3 Effects of viruses on egg production and quality

16.3.1 Infectious bronchitis virus

Aetiology

Infectious bronchitis (IB) is a poultry viral disease caused by a coronavirus, an enveloped RNA virus. The coronavirus belongs to the Coronaviridae family. The essential coronavirus characteristic is antigenic plasticity, because of the variable amino acid sequences of spicules on the surface. Many serotypes exist and the most frequent is the Massachussetts strain, and new variants like the 'Qx' strain.

Epidemiology

IB affects *Gallus* of all ages, but is more severe in young poultry. The virus spreads in aerosol and is transmitted by the respiratory route. Droppings and

nasal discharge are the virulent matter. Transmission is horizontal either directly from bird to bird or indirectly via personnel or material (Cavanagh and Gelb, 2008).

Clinical signs in layers: egg production and quality

With both respiratory and genital tropism, IB infection involves respiratory signs in broilers, drops in egg production and deterioration in egg quality in layers. Some strains are also nephropathogenic. Sevoian and Levine observed internal and external egg quality alteration in laying hens experimentally infected with Massachussetts IB strain (Sevoian and Levine, 1957).

In layers, the disease causes drops in egg production, commonly by from 5 to 10%, and by up to 50%. A commercial chicken flock from which IB virus was recovered showed a drop in egg production of 15%. The drop lasted for approximately 4 weeks and production did not return to the pre-infection level (Cook, 1984). The severity of the production drop may vary with the period of lay and with the causative virus strain. An Arkansas strain experimental infection in White Leghorn layers studied the effects of the virus on egg production (Muneer *et al*., 1986). Infected hens laid fewer eggs and the shell quality and internal quality were inferior. Egg production was 62% in infected birds and 77% in controls 15 days post-infection. IB-infected hens laid eggs with a watery albumen consistency and yolk size was smaller.

Chousalkar and Roberts studied the effect of Australian IB strains on egg production and egg quality. The classic 'IB egg', an egg that is wrinkled and corrugated, as described by Dhinakar Raj and Jones (1997), was not observed. Although there was no decline in egg production, there was a deterioration of egg internal quality (albumen) and a loss of egg shell colour (Chousalkar and Roberts, 2009). In addition, egg shape changed, with IB causing eggs to become more elongate. Studies of the histopathology of the oviduct of White Leghorn (Chousalkar *et al*., 2007a), Hy-Line Gray (Chousalkar *et al*., 2007b) and Isa Brown hens (Chousalkar *et al*., 2009a) as well as ultrastructural investigations (Chousalkar and Roberts, 2007a,b; Chousalkar *et al*., 2009a) confirmed that IB induces pathology in various regions of the oviduct of laying hens. Virus was also isolated from the oviduct of previously challenged laying hens (Chousalkar *et al*., 2009b).

Coronavirus infection in the shell gland cells causes declines in egg shell quality: thin, soft, misshapen or pale unpigmented shells. The thin and watery albumen occurs when the coronavirus affects the cells of the magnum. Haugh unit values are reduced. A day-old coronavirus infection can also create false layers. Layers are then unable to lay due to the oviduct damage. This situation has been described in France in breeders and layers associated with a new variant strain, Qx (Robineau and Moalic, 2009).

16.3.2 Egg drop syndrome (EDS)

Aetiology

Egg drop syndrome (EDS) virus is caused by duck adenovirus A and was first described in laying hens in 1976. The adenovirus is a member of the genus *Atadenovirus* and family *Adenoviridae*.

Epidemiology

Egg drop syndrome affects chickens and quail. Ducks and geese seem to be natural hosts of the virus. The virus is transmitted vertically by eggs. Droppings also contain the virus that can be horizontally transmitted by the oral route. Infected wild birds can be a contamination source for poultry (Adair and Smyth, 2008).

Clinical signs in layers: egg production and quality

The disease is characterized by drops in egg production and increased incidence of abnormal eggs. Birds remain generally healthy. Egg production is usually reduced by from 10% to 40%. The fall in production can be rapid, and the drop in egg production usually lasts from 4 to 10 weeks (McFerran and Smyth, 2000). The first sign is loss of shell pigments. This loss of colour is followed by thin, soft, rough and granular shell and shell-less eggs. Higashihara and coworkers observed few clinical signs except production of abnormal eggs in hens infected orally with EDS virus (Higashihara *et al*., 1987). Egg production was about 20% lower and aberrant eggs were shell-less, soft-shelled, thin shelled and pale coloured. In this study, internal quality was not altered. In EDS 76 field cases reported by Van Eck, watery and thin albumen was reported (Van Eck *et al*., 1976).

16.3.3 Swollen head syndrome

Aetiology

Swollen head syndrome is an infectious disease caused by an avian pneumovirus from the Paramyxoviridae family. The virus is enveloped. It is a single-stranded, RNA virus 80–200 nm.

Epidemiology

The swollen head syndrome affects chickens and guinea fowl. Respiratory signs occur in young birds and the adults are affected by drops in egg production. The transmission of the virus is lateral by aerosol through the respiratory route. It is spread by both airborne and mechanical (feed, water and equipment) routes (Gough and Jones, 2008).

Clinical signs in layers: egg production and quality

Respiratory signs occur in young birds and adults are affected by drops in egg production usually by from 5% to 30%. The disease lasts from 2 to 3 weeks. An avian pneumovirus infection study using laying hens was conducted

by Cook. Intravenous inoculation caused a drop in egg production of up to 25% and a high incidence of soft and thin-shelled eggs. Some respiratory signs were also observed (Cook *et al*., 2000).

16.3.4 Avian encephalomyelitis

Aetiology

Avian encephalomyelitis is a poultry viral disease caused by a picornavirus from the Picornaviridae family. It is an RNA virus, not wrapped and icosahedric.

Epidemiology

Avian encephalomyelitis affects chickens, pheasants, quails and turkeys. The disease occurs in young birds, usually less than three weeks of age and also in layers. Vertically transmission in eggs is the main route of contamination. The virus is also excreted in faeces by the horizontal route from bird to bird (Calnek, 2008).

Clinical signs in layers: egg production and quality

The disease is characterized by neurological signs in young birds with uncoordination, ataxia and tremors. Affected laying hens show a temporary drop in egg production (5–10%), but not neurological signs. Meroz reported a severe drop in egg production of from 75% to 51% associated with encephalomyelitis in a commercial laying flock aged 43 weeks (Meroz *et al*., 1990). Production returned to its previous level within two weeks. No clinical signs and no abnormal eggs were observed in the flock.

16.3.5 Influenza

Aetiology

Avian influenza is a viral infection caused by influenza A virus, which is a member of the family Orthomyxoviridae. These are RNA viruses classed in three types A, B and C. Influenza A viruses are isolated in birds, swine, horse and human. Types C and D are isolated in humans. The glycoproteins haemagglutinin (H) and neuraminidase (N) characterize the virus subtypes. 16 H antigens and 9N antigens have been described by Swayne and Halvorson (2008).

Epidemiology

Influenza virus has been recovered from domestic and wild avian species all over the world. Migratory waterfowl are a wild reservoir of influenza virus. Turkeys and chickens are highly susceptible. Avian influenza virus transmission is horizontal and includes direct and indirect contact (Swayne and Halvorson, 2008).

Clinical signs in layers: egg production and quality
Clinical signs vary depending on species infected, bird age and the virulence of the viral subtype. Mortality of up to 90%, without any clinical signs of illness, can be observed in a highly pathogenic influenza outbreak. In a low pathogenic outbreak, some birds may also develop general symptoms with cough, diarrhoea, depression, anorexia and drops in egg production. In the United States, influenza A virus was isolated from commercial laying hens 55 weeks old with up to 69% mortality and severe decrease in egg production. Egg production dropped from as high as 80% to 13% (Johnson and Maxfield, 1976). With low pathogenic influenza A virus, egg drop production is less severe. In 1997 and 1998, in Pennsylvania, H7N2 (nonpathogenic) influenza A virus was diagnosed in commercial leghorn laying hens (Ziegler *et al.*, 1999). Mortality was less than 4% and egg production declined by from 2% to 4%.

16.3.6 Newcastle disease

Aetiology
Newcastle disease is an infectious disease affecting domestic and wild birds and is caused by a paramyxovirus, of the family Paramyxoviridae and genus *Rubulavirus*. The paramyxoviruses are RNA viruses. They are enveloped with two glycoproteins haemagglutinin-neuraminidase (HN) and glycoproteins F, for virus attachment and fusion.

Epidemiology
Newcastle disease affects all poultry species, but the pathogenicity varies with host. Chickens are highly susceptible, but ducks and geese are less susceptible. The virus spreads through horizontal transmission, in bird secretions (nasal discharge) and droppings. Birds are contaminated by inhalation or ingestion directly from bird to bird or indirectly by mechanical means (personal, material). Vertical transmission is controversial. The virus can survive for several weeks in the environment in contaminated manure or material (Alexander and Senne, 2008).

Clinical signs in layers: egg production and quality
Clinical signs are variable depending on host species and virus strain. Newcastle virus is classed into pathotypes velogenic, mesogenic and lentogenic. The viscerotropic velogenic strains cause high mortality and enteritic lesions. Neurotropic velogenic strains cause also high mortality with respiratory and nervous signs. With mesogenic strains, clinical signs are respiratory and nervous with low mortality level. Lentogenic stains cause respiratory disorders particularly in young birds. In adult birds, egg production is affected by mesogenic strains. Layers are depressed and anorexic. A partial to complete drop in egg production is observed. Egg quality is affected with thin-shelled eggs.

16.3.7 Laryngotracheitis

Aetiology

Laryngotracheitis (LT), also known as infectious laryngotracheitis (ILT), is caused by a member of the family Herpesviridae, subfamily Alphaherpesviridae, genus *Iltovirus*. It is taxonomically identified as *Gallid herpesvirus* (Guy and Garcia, 2008).

Epidemiology

Laryngotracheitis is a respiratory disease of chickens that can result in production losses due to mortality, morbidity and decreased egg production (Jordan, 1966; Bagust *et al*., 1986; Guy and Garcia, 2008). The chicken is the primary natural host and, although the disease may affect chickens of all ages, it is mostly observed in adult birds. However, the onset of lay can affect the rate of shedding of carrier birds (Hughes *et al*., 1989).

Clinical signs in layers: egg production and quality

Clinical signs involve primarily the respiratory system with nasal discharge, respiratory depression, mucoid tracheitis, sinusitis, conjunctivitis, gasping and expectoration of blood mucus. Decreased egg production and failure to thrive appear to be secondary to the effects on other body systems. There is no direct evidence for effects of ILT on egg quality.

16.4 Effects of syndromes on egg production and quality

16.4.1 Fatty liver haemorrhagic syndrome (FLHS)

Fatty liver haemorrhagic syndrome (FLHS) is a metabolic disease affecting caged hens in high production. It is characterized by fat infiltration into the liver, haemorrhage and mortality. The disease is associated with a high energy ration and probably induced by mycotoxins, hot weather and dietary lipids. High levels of plasma oestradiol also increase the FLHS risk (Haghighi-Rad and Polin, 1981). Clinical signs are essentially mortality in full production and sudden drops in egg production. The disease can cause up to 5% mortality during the laying cycle (Julian, 2005). The liver is friable, enlarged, yellow, engorged with fat and haemorrhagic. Restricted feeding may prevent the disease. Fatty liver and hepatic steatosis is frequently confused with fatty liver syndrome. Hepatic steatosis is associated with a low protein and high energy level in the ration and causes little mortality and egg production drops (Julian, 2005).

16.4.2 Cage layer osteoporosis

Osteoporosis is a metabolic disorder affecting bone structure and reduction in bone mass. The bones are more fragile and susceptible to fractures. The disease may occur with calcium (Ca) depletion. This may occur because

of inadequate dietary Ca, vitamin D3 and phosphorus (P) (Julian, 2005). If Ca is insufficient in the diet, in order to produce eggs, hens withdraw the mineral from cortical bone. Phosphorus deficiency causes osteoporosis, often called cage layer fatigue. Phosphorus is essential to the medullary bone structure. Vitamin D3 deficiency results also in osteoporosis by affecting Ca metabolism. Adequate nutrition helps to reduce this disorder. More exercise results also in better bone quality but may not decrease the fracture incidence. Lines resistant to osteoporosis have been selected (Whitehead and Fleming, 2000).

16.5 Effects of toxic agents on production and egg quality

Various toxic agents and contaminants can produce disease in poultry. Contamination of feed with mycotoxins has the potential to reduce production and egg quality (Garaleviciene *et al*., 2001; Chowdhury and Smith, 2004, 2005; Chowdhury *et al*., 2005; Pandey and Chauhan, 2007). Chowdhury and coworkers showed that mycotoxins result in increased plasma uric acid concentrations, most likely due to effects on the liver, and also cause immunosuppression. Some of these effects are mediated via a reduction in feed intake of the contaminated feed (Suksupath *et al*., 1989). Egg production in broiler breeders has been shown to be reduced by a mycotoxin but only at relatively high levels (Brake *et al*., 2002).

Ingestion of crude oil resulted in lower Haugh units in poultry (Ekweozor *et al*., 2002). Vanadium has also been shown to reduce albumen quality by reducing the amount of crude ovomucin per millilitre of thick egg albumen (Toussant and Latshaw, 1999). The ovomucin content of the thin albumen was not affected by vanadium supplementation of the diet.

16.6 Clinical perspectives

Opinions were sought from poultry veterinarians via organizations such as the American Association of Avian Pathologists, the World Veterinary Poultry Association and the Australian Veterinary Poultry Association. A number of poultry veterinarians offered an opinion from their own clinical experience. A veterinarian from New Zealand commented on the problems associated with rearing birds to point of lay in cages and then placing them in floor systems (barn, free range). This often results in problems with parasites such as worms and coccidia and producers are not always clearly aware of what treatments are required. This veterinarian also mentioned problems with nutrition that are blamed on disease. He is of the opinion that infectious bronchitis tends to be blamed for shell and albumen quality problems when the cause may be something else (or a combination of other factors).

A veterinarian from the UK, Dr David Burch from Octagon Services Ltd, offered the opinion that egg drops are not so much a syndrome as a production failure. Dr Burch maintains a website on which information about disease and egg drops is provided (Burch, 2010). A veterinarian from Australia offered the following list of diseases and other factors which affect egg production and quality: *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, Egg Drop Syndrome-76, avian influenza (high and low pathogenicity), Newcastle disease virus, infectious bronchitis virus, turkey rhinotracheitis, avian herpesvirus and avian encephalomyelitis virus. Rarely, paramyxoviruses and other viruses can cause problems. This veterinarian also stressed the importance of management factors in achieving good egg quality: feed, water, heat, cold, electricity and light.

16.7 References and further reading

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17

Parasitism in egg production systems: the role of the red mite (*Dermanyssus gallinae*)

O. A. E. Sparagano, Northumbria University, UK and A. Giangaspero, University of Foggia, Italy

Abstract: Parasites are a problem wherever poultry are raised, either in large poultry infrastructures or small backyard flocks, and are responsible for economic losses that can be significant. This chapter will discuss the most economically important parasites, all having a negative impact on egg production and egg quality. However, it will mostly focus on the red mite, *Dermanyssus gallinae*, because of its outstanding negative impact on animal health, welfare and production. The authors will review different aspects including biology of red mites and new insights of its control.

Key words: endo and ectoparasites, *Dermanyssus gallinae*, poultry diseases, control methods.

17.1 Major parasites in poultry production

Ecto- and endoparasites can have a serious impact on poultry production, depending on the rearing system, because transmission depends on environmental factors and management practices. In general, parasites with short life cycles and direct transmission are more successfully maintained in a closely confined situation, while parasites requiring intermediate host/s prefer free-range poultry. Newly introduced housing systems that give the animals some outdoor access may increase the risk of parasitism. This section will touch briefly on the more economically important parasites, which all have a negative impact on egg production and egg quality.

17.1.1 Endoparasites

Eimeria is the most studied and a well-known protozoan agent responsible for coccidiosis in poultry birds because of its worldwide prevalence and the economic losses that are induced. Seven species of *Eimeria* (*E. necatrix, E. tenella, E. brunetti, E. acervulina, E. maxima*, *E. mitis, E. praecox*) are recognized in poultry birds; most of them have a specific localization in the digestive tract and produce specific pathologies. Each species causes a problem only if present in large enough numbers, but some species (*E. brunetti* and *E. necatrix*) are more pathogenic than others. Several factors affect the appearance and development of coccidiosis on a poultry farm. Density, size, bio-safety practices, litter quality, general hygiene, ventilation and the presence of animals of different ages are the most important factors, together with rearing methods, chicken breed sensitivity, health conditions, immunity status and interference from other diseases. Consequently, each farm will present a specific and unique disease status.

Another important, thus less well known, coccidian parasite is *Cryptosporidium*. This genus is responsible for avian cryptosporidiosis in domesticated, caged and wild birds. Only three valid *Cryptosporidium* species affecting birds are recognized: *Cryptosporidium meleagridis, Cryptosporidium bayleyi* and *Cryptosporidium galli*. All species infect the small and large intestine and the bursa of Fabricius. However, *Cryptosporidium bayleyi* is more frequently associated with respiratory cryptosporidiosis – the most common form in broiler chickens – while clinical diseases and high mortality are associated only with *C. galli* infections (Ryan, 2010). More focus is now given to *C. meleagridis* due to its wide host range and its status as an emerging pathogen in humans. This species is the third most common *Cryptosporidium* parasite in humans (Fayer, 2010). It affects immunocompromised and healthy people and has already been shown to be transmitted from an infected patient with diarrhea to chickens and other animals (Akiyoshi *et al*., 2003). Humans and free-ranging chickens in some deprived conditions can share *C. meleagridis* (D'Alfonso *et al*., 2009). No treatments are effective against *Cryptosporidium*; only good hygiene and management can prevent cryptosporidiosis.

Regarding nematodes, *Ascaridia galli* is re-emerging in chickens. The immature forms produce the most severe damages because these round worms live in the intestinal mucosa. Chicks (1–2 months of age) are more susceptible and heavily infected chicks present anemia, droopiness, emaciation and diarrhea. The primary damage consists of reduced feed conversion, but death has been observed in severe infections (Gauly *et al*., 2005). Even subclinical infections can increase serum testosterone concentrations and have effects on hen behavior (Gauly *et al*., 2007). *A. galli* is the only parasite found inside the hen's egg, but this has only been an occasional finding (Reid and McDougald, 1997). The parasite can only be controlled by strict sanitation measures: cleaning the poultry house before a new group of birds arrives and segregating birds by age groups is one of the most important preventive measures to take.

Species of the genera *Heterakis, Capillaria* and *Syngamus* are very common and well-known nematodes encountered in commercial poultry systems, and sometimes they have reached high prevalence levels. Cestodes (tapeworms) are commonly encountered in free-range or backyard poultry farms due to the availability of intermediate hosts. Most cestode species seem not to cause disease of economic importance, especially with infections of only a few hundred worms. The exceptions are *Davainea proglottina* and *Raillietina tetragona*.

17.1.2 Ectoparasites

Lice and mites are the most common poultry pests in Europe.

Only chewing lice of the Mallophaga order infect birds, and they may be extremely pathogenic in very young birds. The lice cause irritation, so that the birds do not feed or sleep well, and they damage their feathers by pecking or scratching irritated body areas. As a consequence, body weight and egg production may drop. *Menacanthus* is the most common and destructive louse of domestic chickens; it prefers the skin and featherless parts of the body. A large population is particularly common in caged layers. *Menopon*, *Goniocotes* and *Lipeurus* can stay on the body feather shafts of chickens. While most lice are not highly pathogenic to mature chickens, they may be fatal to chicks.

Poultry birds are susceptible to mites, some of which are bloodsuckers and others burrow into the skin. Among the haematophagous mites, *Dermanyssus gallinae* (see details in Section 17.2) and *Ornithonyssus sylviarum* are of primary concern due to their prevalence and to the seriousness of their effects on the poultry industry. *O. sylviarum* remains on the bird and does more damage than any other mite species. Unlike *D. gallinae*, the mite does not leave the host bird, and it can be observed on birds in large numbers during daylight hours. It prefers the feathers below the cloaca and around the tail, but can be found on any part of the body. It has been shown to cause economic damages such as anemia, reduced egg production and weight gain, and ultimately death. The mites will also bite humans, causing itching and irritation to the skin. Treatment is necessary to destroy the mites, because they remain on the birds most of the time. *Knemidocoptes* (or 'scaly leg mite') affects the skin beneath the leg scales and occasionally the neck and comb. Infections may remain latent until conditions of stress occur. It is more common in birds which have access to the ground, and therefore tends to be more prevalent in barnyard and deep-litter systems rather than in caged production facilities.

Muscids are not a parasite of poultry, but are a major concern to poultry producers. *Musca domestica, Fannia canicularis* and *Ophyra aenescens* are the most common. They are not true ectoparasites of poultry but they cause serious irritation to both animals and personnel working in poultry farms. *F. canicularis* is called the 'little house fly' because it is smaller than *M.*

domestica. *O. aenescens*, also called 'dump flies' or 'black garbage flies', can be distinguished from houseflies because they are shiny, black, slender and about half the size. Poultry manure, grass clippings and garbage are all excellent breeding mediums for all these species. While *M. domestica* and *O. aenescens* can fly everywhere around the farm, *F. canicularis* (Linnaeus) often flies slowly in circles in the chicken shed. These species can spread to nearby areas, disturbing people living close to poultry sheds, and all may harbor human and avian disease microorganisms mechanically transmitted by regurgitation, fecal spots and body hairs, thus presenting potential for the transmission of pathogens onto the freshly laid egg. Only a serious 'integrated system' of fly control can reduce muscid populations in poultry farms.

17.2 *Dermanyssus gallinae*: biology and behavior

17.2.1 Morphology, biology and life cycle

Dermanyssus gallinae (De Geer, 1778) (Acarina: Dermanyssidae), also called 'red mite' or improperly 'red louse', is one of the most important problems in poultry farms because it has both direct and indirect pathogenic effects. *D. gallinae* adults (0.75–1 mm long) have long legs and usually a grayish-white body, which becomes reddish-brown when engorged. They have a single dorsal shield, which tapers at the back, and is truncated at its back edge. The anal shield is relatively large and is at least as wide as the genitor-ventral plate. Three setae are present on the anal plate, in both sexes (Baker, 1999) (Fig. 17.1). *D. gallinae* is an obligatory but temporary blood feeder which that affects chickens, but also turkeys, ducks and wild birds. It can also be found on dogs and rodents (Abd El-Halim *et al.*, 2009).

This mite spends much of its life cycle off its host, which it visits to feed mainly between sunset and the first hours of sunrise with most activity 5–11 h after darkness (at 12/12 h light/darkness). During daytime the mites live concealed in all possible crevices, for example in walls or floors, hosts' nests, under the cribs and roosts, on dried litter, egg conveyor belts, cardboard boxes and transportation cages (Fig. 17.2). They can be found in clusters formed by thigmokinesis: the larval stages usually stay in the center with the females on the outside, and the males on the top of the group (Entrekin and Oliver, 1982).

Five life stages are recognized for this species: egg, larva, protonymph, deutonymph, and adult. Mating occurs off the host and requires from 14 minutes to 1 hour, during which the male inserts the penis into the female spermatic orifice, and transfers the spermatophor sack containing about 200 spermatozoa. Males can mate up to four times in four days, and the longest fertile period of females lasts three weeks (Hutcheson and Oliver, 1988).

After mating, the female lays eggs (4–8 per day), and takes a blood meal between each batch for 3 days consecutively. A total of 30 eggs are produced in a lifetime, and most are produced after the third, fourth and fifth blood

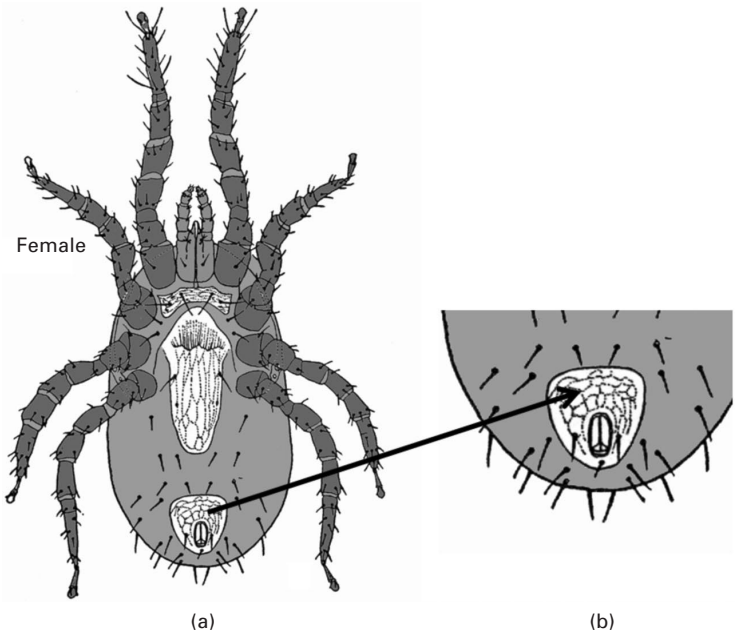

Fig. 17.1 Chetotaxis of *Dermanyssus gallinae* (a): details of the anal plate (*arrow*) with three anal setae (b) (modified from Baker, 1999).

Fig. 17.2 Cluster of *Dermanyssus gallinae* (*arrow*) (A. Giangaspero).

meal. The highest numbers of eggs are laid at temperatures of 20–25 °C and 70% relative humidity. At 5 °C, eggs do not hatch but they are still alive, and at 45 °C they dehydrate in a short time (Maurer and Baumgartner, 1992; Nordenfors *et al*., 1999). Eggs mature into hexapod larvae after 13–51 hours, and the newly hatched larvae moult into octopod protonymphs after about 24 hours (Tucci and Guimarães, 1998) without feeding. The protonymphs take a blood meal before moulting into deutonymphs, which then need a blood meal before changing into adults. The sex ratio is 1:1. The life cycle can be as short as 5.5 to 7 days at 25–37 °C and as long as 17 days at 20 °C (Maurer and Baumgartner, 1992) (Fig. 17.3).

Red mites are poikilothermic because temperature and humidity can influence the population ecology. Temperatures between 25 and 30 °C are optimal for survival and reproduction; however, the life cycle can take place even at 5 °C although less efficiently. Temperatures of –20 °C and 45 °C are fatal for red mites. The ideal relative humidity level is 80%, and higher or lower levels stop the development cycle (Nordenfors *et al*., 1999).

D. gallinae is widespread also in winter, but more common between May and late October when the temperatures are ideal for development of the immature forms. In poultry farms, the number of mites rises progressively for 4–6 months until it reaches a plateau (Nordenfors and Hoglund, 2000). In layer sheds, the density of red mites can be 25–50 000 per chicken; this number can treble in the case of massive infestations. A poultry shed remains infested for four to five months after birds are removed. Mites are transmitted by dispersion between farms (on crates, egg trays or even humans) or by direct contact between birds.

D. gallinae is distributed worldwide, and high percentages of infested birds are reported in Italy, Serbia, Poland, the UK, Morocco, Japan, Montenegro and

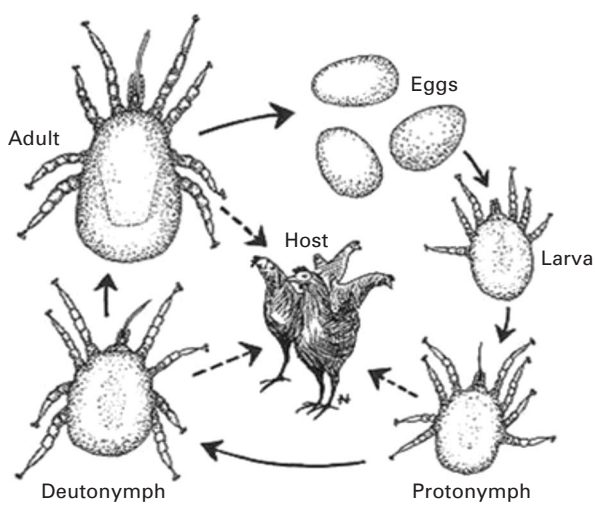

Fig. 17.3 *Dermanyssus gallinae* life cycle (Maurer, 1993).

the Netherlands (Sparagano *et al.*, 2009). In Europe, *D. gallinae* is one of the major problems in caged laying hens because of their longer production cycle, but is less of a problem in broiler industries (Chauve, 1998). Less intensive farming systems, such as barns, free-range and organic farming often have higher infestation rates because *D. gallinae* can find more hiding places and can more easily avoid chemical control methods in these environments.

17.2.2 Host–parasite relationship

The red mite bites mainly on the neck, back and shoulders and where veins are superficial, and does this by using chelicerae in the form of a stylet. One female can engorge about 200 µg of blood while males only suck blood occasionally. The color, vibrations and CO_2 produced by the host attract the red mites. However, stimuli captured by the mite vary according to its nutritional status; if two or more days have passed since the last meal, the search for a host becomes more rapid and induces *D. gallinae* to feed more frequently. The search slows down if the starvation period lasts for more than 15–20 days. This behavior is justified by metabolic saving, which stops if a constant and prolonged thermal stimulus appears (Kilpinen and Mullens, 2004). Koenraadt and Dicke (2010) have demonstrated that both mite-related cues (aggregation pheromones) and host-related cues (kairomones) mediate the behavior of the poultry mite. While fed mites respond strongly to host-related cues (including CO_2 eliminated by the host), and to volatile cues from conspecific mites, as they search for a place to hide and aggregate with conspecifics after a blood meal, unfed mites do not respond strongly to conspecific mites as, in contrast to fed mites, they are more motivated to locate a blood meal than a hiding place with conspecifics. Understanding the parasite behavior may have important implications for *D. gallinae* control.

17.3 Pathologies associated with *Dermanyssus gallinae*

17.3.1 Direct effects

The most evident direct signs of the dermanyssosis appear to be the dermatitis which is more intense and severe if the parasite burden is high. In caged layers, the mite is responsible for itching and chickens appear nervous and irritable. The birds tend to peck both themselves and their companions, causing wounds and increasing the likelihood of cannibalism within the flock. A marked anemia is evident mainly in young subjects with pale or pinkish combs and wattles; in extreme cases the blood sucking behavior of the mites can cause mortality.

Case reports of red mite dermatitis are becoming more and more common also in humans. In Morocco, poultry farm workers showed increased levels of dermatitis following egg collection (Sahibi *et al.*, 2008) while in Italy dermatitis was observed in the general public and hospital workers, usually

associated with feral pigeons nesting under the infrastructure roofs or near bedroom windows (Cafiero *et al*., 2008a,b, 2009).

17.3.2 Vector capacity and pathogen transmission

According to the definition given by the World Health Organization (WHO, 2008), different criteria are needed to consider an arthropod as a vector of a pathogen. A competent arthropod vector is able to (1) infect from a septicemic host or an artificial substrate and (2) transmit the pathogenic agent to a new host during a blood meal. If ingested pathogenic agents are able to disseminate and multiply in their body, the arthropod vector can be qualified as a biological vector. Moreover, natural infected mites have to be isolated from field samples. The last criterion is the existence of a high co-association with a specific host. Owing to these specific conditions, only very few studies have clearly demonstrated the role of *D. gallinae* in the transmission of pathogens. Several authors have underlined the role of the mite in the transmission of bacteria and viruses, responsible for both animal infections and zoonoses. For some of them, only the isolation of pathogens from field samples has been reported. The simple detection of a vector-borne bacterial agent in an ectoparasite does not demonstrate vector competency, but is a preliminary step in incriminating the particular ectoparasite in the transmission of the agent. It is the case for the bacteria *Erysipelothrix rhusiopathiae*, *Salmonella gallinarum*, *Listeria monocytogenes* and the virus of Newcastle disease.

Erysipelas is a bacterial disease caused by *Erysipelothrix rhusiopathiae*, which may infect swine as well as several other species of mammals and birds, including domestic fowl. Chirico *et al*. (2003) isolated *Erysipelothrix rhusiopathiae* from the integument as well as from the interior of *D. gallinae* collected from poultry production facilities. However, a recent study failed to show, under experimental conditions, that this arthropod could transmit *E. rhusiopathiae* (Brännström *et al*., 2009). Serotypes 1a and 1b of *E. rhusiopathiae* were found in mites isolated from diseased birds. Chirico *et al*. (2003) concluded that in cases of hen erysipelas where the transmission of bacteria cannot be related to swine, the occurrence of blood-feeding mites should be considered as a potential source of infection. This result was recently confirmed by Valiente Moro *et al*. (2009), which also isolated the pathogen from *D. gallinae* originating from French poultry farms which were clinically healthy. Similar results were reported for the bacterium *Salmonella* Gallinarum and the avian paramyxovirus, which were isolated from *D. gallinae,* respectively by Zeman *et al*. (1982) and Arzey (1990). The mites could carry the bacteria in their bodies for at least four months. Zeman *et al*. (1982) suggested that the spontaneous infection of the *D. gallinae* population with *S.* Gallinarum showed the importance of effective disinfection in poultry breeding farms for a successful liquidation of this pathogen. Finally, concerning *Listeria monocytogenes*, Grebenyuk *et al*.

(1972) discussed the role of wild animals and blood-sucking arthropods in their transmission even when only a single isolation from *D. gallinae* was observed.

For other pathogens associated with *D. gallinae*, experimental transmissions under laboratory conditions have been carried out. Some assays remained fruitless as *D. gallinae* was unable to infect or transmit the pathogens. This is the case for viruses responsible for tick-borne encephalitis and Saint-Louis encephalitis (Chamberlain *et al.*, 1957). Laboratory studies were conducted in attempts to infect *D. gallinae* with the virus of Saint-Louis encephalitis. Detectable virus persisted less than 2 days in the mites and no transmissions by bites were shown, supporting the relative unimportance of this mite in the transmission of active St Louis encephalitis infection. Similar results were obtained with tick-borne encephalitis viruses (Wegner, 1976). Zemskaya and Pchelkina (1962) fed *D. gallinae* on mice or fowl which had been artificially infected with the tick-borne encephalitis virus and suspensions prepared from them causing infection when injected into mice. Mites from the samples in which infection was demonstrated did not preserve the virus.

Several studies have also concerned experimental transmissions with equine encephalitis viruses (EEV). Laboratory studies were undertaken to test further the ability of *D. gallinae* to serve as vectors or hosts of equine encephalitis viruses. Chamberlain and Sikes (1955) and Durden *et al.* (1993) showed that *D. gallinae* which engorged on chicks infected with Eastern equine encephalitis virus remained carriers for at least a month without viral replication and without transmitting the virus to their offspring, although they were able to transmit the virus to other chicks by bites when taking a blood meal. Cockburn *et al.* (1957) obtained an infestation from *D. gallinae* which had fed on EEV-infected chickens, but were not able to demonstrate either transmission to healthy birds or transovarian transmission in the acarian. Interesting results were also obtained by Shirinov *et al.* (1972), where samples of *D. gallinae* collected from poultry farms known to have birds infected with fowl pox virus were also found to harbor the virus. When naturally infected mites were kept in the laboratory, the virus survived inside them up to 300 days. Transovarian transmission was demonstrated and the disease was transmitted to healthy fowl by the bite of infected mites. Microbiologic studies and biological experiments revealed that *Pasteurella multocida*, a bacterium causing pasteurellosis, persisted in the body of *D. gallinae* mites after they engorged on blood from infected birds. The carrier status was shown to last from 42 to 64 days depending on the temperature (Petrov, 1975). Concerning *Coxiella burnetii*, a bacterium responsible for Q fever, *D. gallinae* could acquire infection while feeding on infected animals. The ricketssiae survived in the mites, which subsequently fed on healthy animals, for about sixth months, and for about one year in dead mites. *D. gallinae* transmitted rickettsiae from guinea pigs to birds and from birds to guinea pigs by feeding. The role of the poultry red mite was also studied in the transmission of spirochaetosis; the bacterium *Borrelia anserina* infects

chickens, turkeys, geese, ducks, pheasants, grouse and canaries with morbidity and mortality up to 100%. It is usually transmitted by *Argas persicus* arthropods and occasionally by infected faeces. Transmission experiments were carried out using cages containing hens heavily infested with *D. gallinae* and where one compartment had been inoculated with *Borrelia anserina* (Ciolca *et al*., 1968). The authors observed that spirochetes were regularly transmitted to healthy hens, if the mites became infected and fed on the healthy hens within 48 h of the infective meal. The spirochetes were usually eliminated in the excreta shortly after ingestion suggesting that the mite was only an occasional vector of them. With the species *Spirochaeta gallinarum*, Reshetnikov (1967) observed similar results except that the interval between blood meals should not exceed 48 h in order to reproduce the disease in the host.

The role of the poultry red mite as a mechanical vector (a vector that simply carries a microorganism without replication in the mite) has been clearly shown for some pathogens even if its precise role in the epidemiology of the associated pathology remains to be determined. Most of the studies are incomplete for conclusions to be made about the precise role of *D. gallinae* in the transmission of pathogens, as different criteria remain to be proven. This is a surprising observation given that all the conditions are present for making *D. gallinae* highly suitable vectors for maintaining an area of endemic disease: zoonotic reservoirs (rodents, birds), acarian parasites (Dermanyssoidea) and possible hosts (man, domestic animals, pests).

17.4 Acaricide treatments and consequences

Dermanyssus gallinae (see Section 17.2) and *Ornithonyssus sylviarum* are the haematophagous mites of primary concern for the poultry industry all over the world. In the text below, focus will be on *D. gallinae* because of its greater prevalence, the seriousness of its effects on the chicken industry in Europe, and the number of publications available.

17.4.1 Acaricides and their efficacy

Several acaricide molecules are available, and organophosphates, carbamates, amidines, pyrethroids are the most commonly used. Organophosphates (OPs) and the carbamates are cholinergic pesticides, and these are acetylcholinesterase inhibitors restraining the metabolism of acetylcholine in arthropods. Amidines have synergic effects with other pesticides and act as neurotoxins and antagonists of $\alpha 2$-adrenoreceptors, and they also cause inhibition of the enzyme monoaminooxidase. Pyrethroids interact with the Na sodium channel in the cell membranes leading to repolarization and paralysis in arthropods. These molecules have been tested for their efficacy against red mites. Some carbamates, such as carbaryl (Zeman and Zebzny,

1985), have been very effective, while propoxur requires a very high dosage to be effective (Hamscher *et al.*, 2003). Of the pyrethroids, flumethrin proved to be highly effective and safe even in a concentration of up to 120 ppm (Cooper and Cobb, 1987), and cyalothrin was more effective than permethrin (Nordenfors *et al.*, 2001). Both ivermectin and moxidectin were effective in subcutaneous or intraperitoneal injection, but only for a short period and at high dosages (5.4 mg/kg and 8 mg/kg) (Zeman, 1987; Ash and Oliver, 1989; Reynaud *et al.*, 1997) close to toxicity. Metriphonate embedded in traps at 2% proved effective against *D. gallinae,* giving a reduction of up to 95% in eight weeks of treatment (Chirico and Tauson, 2002).

Some acaricides are more efficient in theory than in practice, because mites in cracks and crevices can escape acaricide treatments due to the difficulty of distributing molecules in all the concealed places in poultry housing infrastructures. The new housing systems that will be in use after the ban of conventional cages will not overcome red mite infestation and will probably not guarantee a better efficiency. The efficacy of the molecule or formulations also depends on the duration of their efficacy and on the kind of surfaces where they are distributed. Wood, galvanized metal, cardboard and plastic retain chemicals differently and acaricides are more efficient on clean surfaces than on dirty surfaces.

In Europe, very few products are licensed for use against *D. gallinae*, and except for a recently approved phoxim-based product, they can be used only when the poultry house is empty, i.e between two productive cycles.

Despite the existence of precise EU legislation on the correct use of pesticides, farmers treat repeatedly – even twice weekly – continuously and indiscriminately against red mite infestation. In many cases, though mainly in the past, farmers treat even using molecules and formulations not specifically labeled for treating red mite infestation.

17.4.2 Consequences

The improper use of acaricides by farmers to control *D. gallinae* infestation can lead to the following main consequences:

- ineffectiveness of molecules employed and appearance of suspected resistant red mite populations;
- accumulation of residues of pesticides in organs or tissues of poultry, or in eggs, and consequent negative effects on food quality, with risks for human health.

Acaricide ineffectiveness and the possibility of resistance

Poultry farmers worldwide report a reduced efficacy of these molecules. Mite resistance to treatments can be attributed to acquired potential resistance or to inadequate or injudicious acaricide application (wrong concentration, treatment not properly scheduled, etc.). Resistance can be accelerated when a lower dosage is applied; however, if the treatment is ineffective, farmers

tend to increase the recommended dose. This incorrect behavior is not only dangerous for human and animal health, but also causes mortality of sensitive individual red mites and the survival of some resistant individuals. In this way, new generations of resistant individuals develop. Strains tolerant to pyrethroid-based formulations (cypermethrin and alfa-cypermethrin) have been detected in red mite populations from the UK (Fiddes *et al*., 2005; Thind and Ford, 2007), Sweden (Nordenfors *et al*., 2001) and France (Beugnet *et al*., 1997). Resistance to carbamates and pyrethroids has been suspected in Germany (Liebish and Liebish, 2001) and more recently in Italy (Marangi *et al*., 2009), where also phoxim did not always show to be very efficient (Camarda *et al*., 2010). These investigations document the existence of only potentially *D. gallinae*-resistant strains; for this reason there is a need for pharmaco-genomic studies, as they will help to detect the gene/s responsible for acaricide resistance in red mite populations which are not susceptible to acaricides.

Residues

It is well known that animals intended for human food may absorb pesticides from residues in their feed and water or during direct/indirect exposure during pest control. When the chemical treatment pressure is high, acaricides can easily accumulate in muscle, liver, kidney, fat, skin and also in eggs because of their highly lipophilic structure and their stability. Residues in poultry tissues and in eggs have been detected even recently, and not always in remote countries (Matsumoto *et al*., 2006; Van Overmeire *et al*., 2006; Tao *et al*., 2009; Achmad *et al*., 2010). EU legislation regulates the detection of pesticides also in poultry meat and eggs and identifies a limit of residues for each molecule. However, owing to the methodology used (random sampling; the limited classes of acaricides investigated, low number of animals tested; and the kind of technology used) it is possible that some specific/restricted situations of misuse or abuse of chemicals will not be detected. For this reason, the detection of *D. gallinae* strains less susceptible or completely not susceptible to acaricides – probably due to the repeated and frequent treatments – is an important signal indicating possible accumulation of residues in organs or tissues of chickens for human consumption at the end of their productive cycle.

17.4.3 Conclusion

The ideal pesticide should be able to penetrate into cracks and crevices harboring red mites, be long-lasting, selective, and should not induce resistance, be also safe for the host, easy to apply and quick-acting, should not corrode farm equipment, with a short withdrawal period and be cheap. At present, none of the molecules used against dermanyssosis has all these features. The exclusive use of acaricides is still a reality worldwide. However, the ineffectiveness of some acaricides is increasingly widespread in Europe.

The scenario is worrying; reduced effectiveness of acaricides can confirm their extensive and improper use by farmers. It is possible that poultry farmers have little choice and often use acaricides licensed for crops and/or livestock pest control because of the lack of molecules licensed for use against *D. gallinae*, and for the prophylaxis and control of this poultry pest. Carbamates are now banned by the EU, but it is well known that they were extensively used in the past, and continued illegal use cannot be ruled out in some areas of Europe. To ensure a correct integrated control strategy until better alternative control methods have been developed, farmers should use acaricides appropriately, i.e. use only licensed products, first testing their efficacy, rotate the molecules used, and apply the correct concentrations and formulations. In the meantime, health authorities in Europe should guarantee the registration of new safe and effective molecules, and the national health authorities should ensure that the appropriate chemicals are used by farmers and are correctly applied. These measures are vital for farmers and consumers to reduce the risk that resistant red mite populations will proliferate, and to ensure that meat and egg products are without residues thus safe to eat.

17.5 New methods to control dermanyssosis

17.5.1 Management issues

Monitoring

Various methods for monitoring *Dermanyssus gallinae* infestation within a free-range egg production unit have been compared in a recent study. The study was carried out in five egg-producing free-range poultry buildings infested with *D. gallinae*. Each farm was divided into six zones (each zone including nestboxes, perches and duckboard) for placing two different types of traps (corrugated cardboard and thick card traps) or examining dried droppings for presence of mites. Placing traps in the nestboxes is a less reliable indicator than placing them on the perches. It appears that the most coherent method for evaluating the *D. gallinae* population within a free-range flock is to place thick card traps throughout the building, on perches favoured by birds (Zenner *et al.*, 2009).

Hazard Analysis and Critical Control Point (HACCP)

Infestations are noticed by the majority of the poultry farmers because workers are being bitten by poultry red mites, or they find fecal (mite) spots on feeders and other equipment, clumps of mites on the belt and feeders, or blood spots on eggs. When these signs are evident, the infestation is already heavy and widespread. Preventing the establishment of poultry red mite populations is therefore important. Using Hazard Analysis and Critical Control Point (HACCP) method, hazards and associated risks of the introduction and spread of poultry red mites in poultry facilities can be reduced (Mul and Koenraadt, 2009). To achieve insight in the hazards,

all farm processes were compiled in a schedule and divided into 13 hazard categories. The hazard analyses were conducted by a panel of experts due to a scarcity of quantitative information on epidemiological risk factors. The highest average risks were calculated for the hazard category 'Poultry farmer/employee', 'manure aeration', 'cadavers' and 'growing hens'. The lowest risks were calculated for the categories 'feed' and 'ventilation' (Mul and Koenraadt, 2009). With knowledge of possible points of introduction and spread of poultry red mites within the poultry facility, corrective actions can be taken to prevent a population to establish. According to the HACCP method corrective actions can be taken preventively but also when mites are present. One of the requirements for a good monitoring tool is the ability to detect small numbers of poultry red mites in poultry facilities and the place of introduction in the house. Known monitoring tools are the ADAS© trap (ADAS, UK), the tube trap (with paper, corrugated cardboard, cloth or a wooden stick), use of manure (Zenner *et al.*, 2009), folded board (Levot, 1991), Nordenfors trap (with/without acaricide; Nordenfors and Chirico, 2001), the Trap perch (Kirkwood, 1963), the BT trap (with lure or with lure and lipid fraction) (Thind, 2005). Only the Trap perch meets the above-mentioned requirements but is, unfortunately, not easily applicable in different kinds of housing systems. Also, checking the trap is labor intensive. Thus although HACCP may be a good method to prevent the introduction and spread of poultry red mites, in order to fulfill all the requirement of the HACCP, a good monitoring tool still has to be developed.

17.5.2 Biological approach

Predators

Biological control by the introduction of predatory mites is one of the various options to control poultry red mite populations. Recently it has been attempted to identify potential predators by surveying the mite fauna of starling nests, by assessing their ability to feed on poultry red mites and by testing for their inability to extract blood from bird hosts, i.e. young starlings and chickens. At least two genuine predators of poultry red mites were revealed in this way: *Hypoaspis aculeifer* (Acarina: Hypoaspididae) and *Androlaelaps casalis* (Acarina: Laelapidae). A review of the literature showed that some authors suspected the latter species to parasitize on the blood of birds and mammals, but they did not provide experimental evidence for these feeding habits and/or overlooked published evidence showing the reverse. We advocate careful analysis of the trophic structure of arthropods inhabiting bird nests as a basis for identifying candidate predators for control of poultry red mites (Lesna *et al.*, 2009).

Plant products

Recent research considering plant-derived products as acaricides for *D. gallinae* has produced some promising results (George *et al.*, 2008a). Results

showed some variability according to the country of collection, the time of collection or the plant parts used to produce lavender essential oils (George *et al.*, 2008b). Several pesticides based on plant constituents are already used widely in certain areas of pest management, including against pests of veterinary significance. In work by George *et al.* (2009, 2010), 50 plant essential oils were assessed for their toxic effect on *D. gallinae*. Twenty of the essential oils chosen gave greater than 80% mite mortality over 24 hours when used at a concentration of 0.14 mg/cm^3, with 20% of essential oils used, including thyme, tea tree and garlic, giving 100% mortality. In a recent study, side effects such as egg tainting were not observed when using thyme or pennyroyal essential oils (Smith *et al.*, 2009).

Fungi

Entomopathogenic fungi are currently used worldwide for control of a wide range of arthropod pests, particularly pests in protected crops or field crops. A survey of naturally occurring fungal pathogens in *D. gallinae* populations from 30 locations in Denmark documented that this mite species is not infected by entomopathogenic fungi in egg production facilities. In contrast, in a recently completed study funded by the EU, *D. gallinae* was found to be susceptible to infection by two of the most widely used fungus species, *Beauveria bassiana* and *Metarhizium anisopliae*, originally isolated from other arthropod species. When treated with high doses of dry fungal conidia in laboratory tests, very high mortalities were recorded. The fungus is easily transmitted from mite to mite by contact, and although time to kill at 25 °C is fairly long (approximately 5–6 days), thus allowing blood fed females to oviposit, these fungi are likely to have considerable *D. gallinae* control potential (Steenberg *et al.*, 2006). Field tests so far have shown less promising results when conidia were applied to artificial mite harborages, particularly when *D. gallinae* populations were large.

The pathogenicity of three strains of the entomopathogenic fungus *Metarhizium anisopliae* on different life stages of *Dermanyssus gallinae* was evaluated under laboratory investigation. All the strains tested were virulent to *D. gallinae* but the pathogenicity varied among the strains. Strain V245 induced the highest mortality rate using different concentrations than other two strains. It was concluded that the pathogenicity of the entomopathogenic fungus *M. anisopliae* on different life stages of *D. gallinae* was concentration- and time-dependent (Tavassoli *et al.*, 2008).

Vaccine development

The development of arthropod vaccines is notoriously difficult due to the limiting step of identification and characterization of new protective antigens, and this has restricted the availability of commercial vaccines against arthropod ectoparasites. Immunization of birds with somatic *D. gallinae* antigens has been performed with variable success (Arkle *et al.*, 2008). However, Harrington *et al.* (2009b) reported a significant 50.6% increase in

in vitro mite mortality when fed blood spiked with egg-extracted antibodies from *D. gallinae* immunized birds. This and similar recent research on *D. gallinae* antigens has demonstrated that there is potential to develop a vaccine to control the poultry red mite. However, further research is still required before a commercial vaccine becomes available. Recently the use of two recombinant proteins (Bm86 and Subolesin) resulted in significant mortality rates (Harrington *et al*., 2009a).

17.5.3 Physical treatments

Heat

Temperatures above 45 °C are considered to be lethal for *Dermanyssus gallinae* (Nordenfors *et al*., 1999). In Scandinavian countries, steam treatment is used and vacuum cleaning the poultry houses or washing between flocks has been recommended (Chauve, 1998).

Dust

Control has often relied on chemical pesticides, but inert dusts, which are thought to kill target hosts primarily by desiccation, have become one of the most commonly applied alternative control methods for poultry red mites in Europe. This development has occurred despite a lack of knowledge of the efficacy of the different types of inert dusts and how this is affected by, for example, the high relative humidity found in poultry houses. In a laboratory study the efficacy of different commercial inert dust products against *D. gallinae* was compared (Kilpinen and Steenberg, 2009). All tested compounds killed mites, but there was a clear ranking of efficacy (measured as weight loss after 24 h and as time until 50% mortality), particularly at 75% relative humidity (RH). At 85% RH the efficacy was significantly lower for all tested compounds ($p < 0.001$). Results showed that 24 h exposure to surfaces treated with doses much lower than those recommended by the producers is sufficient to kill mites as fast as when they were dusted with massive doses. These data emphasize the need for thorough treatment of all surfaces in a poultry house in order to combat *D. gallinae* (Kilpinen and Steenberg, 2009).

Lighting

Lighting conditions may have an impact on the proliferation of *Dermanyssus gallinae,* which mainly attacks birds at night. However, national legislation for the bird welfare does not very often authorize such changes in the lighting pattern (Stafford *et al*., 2006). Stafford *et al*. found that short-cycle intermittent lighting programs (four periods of 3–5 hours of light (L), two periods of 2 hours of darkness (D) or 24 periods of 0.25L, 0.75D) numerically controlled mite populations. However, such a lighting pattern is not allowed on European poultry farms.

17.6 Conclusion

Although a lot of ecto- and endoparasites can potentially affect laying hens and egg production systems only a few are a major issue such as *Dermanyssus gallinae*. It seems that every time the industry controls one parasite, another one takes over the top spot. Current control methods could in the long run become inefficient due to some resistance becoming more widespread. Therefore, a more integrated approach is recommended, using a broad range of control methods (management, biological, physical and chemical) to prevent parasite proliferation and protect poultry meat and egg production systems. Parasites are constantly adapting to new control methods and therefore the scientific community and the industry need to be pro-active and constantly developing research programs on novel control methods and treatment products.

17.7 References and further reading

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18

Health risks for workers in egg production systems and methods of control

S. Le Bouquin and A. Huneau-Salaün, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (Anses), France and B. Eniafe-Eveillard, CHU A. Morvan, France

Abstract: Work-related diseases in poultry worker populations are important and represent a significant proportion of occupational diseases in agriculture. This chapter proposes a review on occupational exposures of workers on egg and poultry farms to potential hazards with a special emphasis on exposures to aerosol, gas, bioaerosol and chemical products. Their potential effects on worker health are presented based on results of epidemiological studies. Preventive and remedial measures based on modification of husbandry practices and use of personal protective equipment are discussed.

Key words: health, occupational exposure, prevention, respiratory diseases, epidemiological studies.

18.1 Introduction

Work-related diseases in poultry worker populations represent a significant proportion of occupational diseases in agriculture. Though often underestimated, these diseases can be serious, sometimes fatal and should therefore not be neglected. The dangers to which professionals in poultry and animal productions are exposed may be diverse, as shown in Table 18.1 (Kouimintzis *et al.*, 2007). In the poultry sector the number of health hazards is very important but exposure to these hazards may be rare and is not constant: each actor in the chain will be subjected to a different risk in his or her activity. This

Table 18.1 Hazards in the livestock agricultural environment by health risk

Health risk	Health outcomes	Hazards
Injuries and accidents	Disabilities, amputations, death due to suffocation, slipping and crushing	Animal handling Machinery repairing Noise, heavy machinery Heat, cold
Malignancies	Haematopoietic cancers, skin cancer, glioma	Sunlight, disinfectants Insecticides, fungicides Animal contact
Non-malignant respiratory disorders	Asthma, chronic bronchitis, organic dust toxic syndrome, hypersensitivity pneumonitis	Dust (inorganic, organic) Ammonia, H_2S Animal dander, pollen/allergens Moulds, endotoxins Fungi, zoonotic infections
Musculoskeletal disorders	Knee and hip osteoarthritis, low back pain, neck/upper extremities	Animal handling Heavy machinery
Neurological	Acute neurological effects, Parkinson's disease, encephalopathy	Insecticides, fungicides, solvents
Psychiatric disorders	Stress, depression, suicides	Financial, intergenerational problems, isolation, bureaucracy, substance abuse
Skin disorders	Dermatitis, mycoses, infections	Pesticides, fuels, fungi, cow dander and epithelium, mites, parasites, metals
Sensory organs	Hearing loss	Noise

Source: (Adapted from Kouimintzis *et al.*, 2007).

review is mainly devoted to health risks experienced by workers on poultry farms and in egg production in particular. Other worker categories in poultry productions are also concerned, such as poultry catchers or workers in slaughtering plants, but these populations are not specifically addressed in this review focused on production at farm level. In the first part of this chapter the harmful exposures for poultry workers are reviewed with a special emphasis on aerial exposures which are well described in poultry buildings. The consequences of these exposures on workers' health are subsequently described and some preventive and protective measures are proposed.

18.2 Professional exposures in egg farming

18.2.1 Exposure to gases

Ammonia is recognised as one of the most predominant air pollutants in layer houses (Wathes *et al.*, 1997). Ammonia results from the microbiological

breakdown of uric acid and undigested proteins contained in bird faeces (Groot Koerkamp *et al*., 1998). The concentration of ammonia in the ambiant air is generally higher in aviaries and in floor housing than in caged houses (Table 18.2). It is also greatly influenced by ventilation rate, manure handling practices (Garner *et al*., 2008; Groot Koerkamp *et al*., 1998; Martensson, 1995; Whyte, 2002) and by hen activity and litter management in alternative systems (Groot Koerkamp and Bleijenberg, 1998; Oehm and Petersen, 1999). The measures of worker exposure with a continuous measurement apparatus have shown that the average exposure for a working day ranges from 7 to 13 ppm with a significant higher exposure in barn system than in cage system (Kirychuck *et al*., 2006; Whyte, 2002). Those exposures are inferior to the European Occupational Exposure Standard (OES, 20 ppm on 8 h of exposure) but are in some cases superior to the concentration of 12 ppm above which decrements in pulmonary functions of poultry workers may occur (Donham *et al*., 2000). In addition peak values in stockmen exposure (50 ppm and above) can be encountered during tasks relative to manure handling and the European short-term limit exposure of 50 ppm for 15 minutes may be exceeded (Whyte, 2002).

Gaseous pollutants other than ammonia are not frequently studied in layer houses. The concentrations of carbon dioxyde in the ambient air vary from 600 to 3000 ppm in a Swiss study (Radon *et al*., 2002) and range from 500 to 2500 ppm in both cage and perchery buildings in a British study (Wathes *et al*., 1997). The average concentrations are 1800 ± 300 ppm in an on-floor building, 1900 ± 270 ppm in an aviary and 2500 ± 300 in a house with furnished cages in a Swedish study (Nimmermark *et al*., 2009). Those concentrations are more influenced by the ventilation rate than by the housing system. They appear to be lower than the value of 5000 ppm proposed as a limit value for occupational exposure in Europe (EEC, 1991). Therefore the exposure to carbon dioxide is not likely to be a major risk for stockmen working in layer houses (Wathes *et al*., 1997). Concentrations of methan and nitrous oxide are also low in the air of henhouses, less than 10 ppm and 5 ppm, respectively, in both cage and perchery buildings in the study of Wathes *et al*. (1997). Hydrogen sulphide is generally not a hazard in poultry houses although some high levels might occur in buildings with deep pit storage systems if the accumulated droppings become too wet (Whyte, 1993).

18.2.2 Exposure to dust

In henhouses dust consists of a complex mixture of fine particles in suspension in the air or settled. The sources of dust include animals (skin scales, faeces and feathers), feed, litter material, microorganisms, insects and mites (Ellen *et al*., 2000). High levels of dust are observed in the ambient air of henhouses compared with those reported in cattle houses and in pig buildings (Bakutis *et al*., 2004; Takai *et al*., 1998). The data reported in Table 18.3 should be

Table 18.2 Ammonia concentrations in poultry houses and worker exposure

Country	Production	Time (h)	Housing system	*N*	Ammonia concentration ppm mean ± std or range	Remark	Reference
Ambient air							
Sweden	Laying hen	nd	Cage Aviary On-floor	3 1 1	2–28 7–19 61 ± 7	Continuous measures	Martensson (1995)
Sweden	Pullet	24	Cage Aviary	19 19	2.1 ± 0.6 2.0 ± 0.4	Experimental trial. 1 measure/hour	Martensson and Pehrson (1997)
UK	Laying hen	12	Perchery Cage	48 48	12.3 (0–45) 13.5 (0–40)	Measures: 60 min intervals	Wathes *et al.* (1997)
UK	Laying hen	24	Perchery Cage	40 40	8.3 ± 2.5 11.9 ± 3.4	Continuous measures	Groot Koerkamp *et al.* (1998)
Netherlands			Perchery Cage	40 40	29.6 ± 7.7 5.9 ± 1.8		
Denmark			Perchery Cage	40 40	25.2 ± 7.0 6.1 ± 2.4		
Germany			Cage	40	1.6 ± 0.4		
Germany	Laying hen	24	Cage	8	2.7 ± 2.3	Continuous measures	Seedorf and Hartung (1999)
Sweden	Laying hen	nd	nd	36	< 5.0–40.0	1 measure/day	Radon *et al.* (2002)
Ireland	Laying hen	24	Cage On-floor	5 5	2.3 ± 0.07 7.6 ± 0.08	Measures: 5 min intervals	Hayes *et al.* (2006)
France	Laying hen	nd	Cage Aviary	13 13	4.2 ± 1.5 14.1 ± 9.2	Experimental trial. 1 measure/day	Huonnic *et al.* (2009)
France	Laying hen	nd	Cage On-floor	59 65	0.0–35.0 2.0–50.0	1 measure/day	

Norway	Laying hen	nd	Fur. cage Aviary On-floor	4 4 4	2.5 ± 0.4 38.0 ± 13.0 57.0 ± 10.0	1 measure/day	Nimmermark *et al.* (2009)
		24	Fur. cage Aviary On-floor	10 10 10	5.2 ± 4.1 32.0 ± 6.5 85.0 ± 17.0	Measures: 10 min intervals	
Personal exposure							
Sweden	Laying hen	nd	Cage Aviary Aviary	6 5 5	3.6 ± 0.4 14.2 ± 1.7 11.3 ± 1.2	Experimental trial. 1 measure/day	Larsson *et al.* (1999)
US	Laying hen Turkey, broiler	nd	nd	174	18.4 ± 17.5	1 measure/day	Donham *et al.* (2000)
UK	Laying hen	8	On-floor Cage	12 9	11 7	Measures: 10 s intervals	Whyte (2002)
US	Laying hen	3	Cage	31	10.5 ± 11.2	Measures: 60 s intervals	Kirychuck *et al.* (2006)

nd, not determined.

Table 18.3 Dust concentrations in poultry houses and worker exposure

Country	Production	Time (h)	Housing system	*N*	Dust concentrations (mg/m^3) Mean ± std or range	Remark	Reference
Ambient air							
US	Laying hen	1	Cage	5	Tot.[1]: 1.13–3.68		Clark *et al.* (1983)
Finland	Laying hen	0.5–1.5	Cage	11	Tot.: 2.7–13.1	During the feeding passage of animal sheds	Louhelainen *et al.* (1987)
Sweden	Laying hen	8	Cage Aviary On-floor	3 1 1	Tot.: 1.3–1.5/Resp[2].: 0.08–0.36 Tot.: 4.1 ± 2.7/Resp.: 1.13 ± 0.65 Tot.: 2.6 ± 0.6/Resp.: 0.08 ± 0.01		Martensson (1995)
UK	Laying hen	12	Perchery Cage	16 16	Inh.[3]: 2.8/Resp.: 0.40 Inh.: 1.7/Resp.:0.27		Wathes *et al.* (1997)
Sweden	Pullet	24	Cage Aviary	13 16	Tot.: 1.0–3.6/Resp.: 0.3–1.3 Tot.: 2.8–8.1/Resp.: 0.5–2.2	Experimental trial	Martensson and Pehrson (1997)
UK Netherlands Denmark Germany	Laying hen, broiler	12	Cage Perchery On-floor Cage On-floor	48 50 32 32	Inh.: 3.31/Resp; 0.51 Inh.: 4.58/Resp; 0.58 Inh.: 4.52/Resp; 0.64 Inh.: 2.22/Resp; 0.19		Takai *et al.* (1998)
Spain	Laying hen broiler	2	nd	14	Tot. median: 2.6	Interquartile range: 1.8–4.9	Borghetti *et al.* (2002)
France	Laying hen	2.5	Cage Aviary	3 6	Tot.: 0.1–1.4 Tot.: 7.7–24.0	Experimental trial	Protais *et al.* (2003)
South Africa	Laying hen	nd	Cage	18	Tot.: 0.0–0.25	9 sampling locations in 2 buildings. Measures with aerosol monitor	Venter *et al.* (2004)

Germany	Laying hen	24	Fur. cage Cage Aviary	12 12 12	Inh.: 0.4–1.3/Resp; 0.2–0.6 Inh.: 0.2–2.0/Resp; 0.2–1.1 Inh.: 1.2–9.5/Resp.:0.4–4.4	Experimental trial	Saleh *et al.* (2004)
France	Laying hen	5	Cage Aviary	15 15	Tot.: 0.0–3.0/Resp.:0.0–0.8 Tot.: 8.1–26.4/Resp.: 0–6.4	Experimental trial. 2 measures/month during the laying period	Michel *et al.* (2004)
France	Laying hen	8	Cage Aviary	13 13	Resp.: 0.15 ± 0.18 Resp.: 2.12 ± 0.75	Experimental trial. 2 measures/month during the laying period	Michel *et al.* (2007)
France	Laying hen	6–8	Cage On-floor	54 63	Resp.: 0.0–0.3 Resp.: 0.0–1.3		Huonnic *et al.* (2009)
Norway	Laying hen	24	Fur. Cage Aviary On-floor	3 8 3	Tot.: 2.0–2.5 Tot.: 0.7–2.4 Tot.: 6.8–17.6		Nimmermark *et al.* (2009)
Personal exposure							
Sweden	Laying hen	nd	Cage	11 6	Tot. unloading cages : 23.0 ± 11.0 Tot. loading cages: 28.1 ± 7.9	Exposition of poultry handlers during loading/ unloading of hens	Thelin *et al.* (1984)
Finland	Laying hen broiler	0.5–1.5	Cage On-floor	13 11	Tot.: 5.7–37.6 Tot.: 0.5–14.7		Louhelainen *et al.* (1987)
US	Laying hen	8	Cage	4	Tot.: 0.3–84.5	Measures taken during a safety and health inspection on a large egg farm.	Eberts and Wilson (1999)
Sweden	Laying hen	3	Cage Aviary	12 24	Inh.: 1.9–2.7 Inh.: 3.3–5.2	Experimental trial	Larsson *et al.* (1999)
US	Laying hen, turkey, broiler	2–4	nd	238/210	Tot.: 6.5 ± 7.8/Resp.: 0.63 ± 0.98		Donham *et al.* (2000)

Continued

Table 18.3 Continued

Country	Production	Time (h)	Housing system	*N*	Dust concentrations (mg/m^3) Mean ± std or range	Remark	Reference
Switzerland	Laying hen	0.4–2.2	nd	36	Inh.: 7.01 (0.42–21.75)		Radon *et al.* (2002)
UK	Laying hen	8	On-floor Cage	12 9	Inh.: 9.5 Inh.: 4.4		Whyte (2002)
France	Laying hen	5	Cage Aviary	15 15	Tot.: 0.0–14.9/Resp.: 0.0–3.6 Tot.: 9.2–34.8/Resp.: 1.4–5.8	Experimental trial. 2 measures/month during the laying period	Michel *et al.* (2004)
US	Laying hen broiler	0.5–1.0	Cage On-floor	31 80	Tot.: 7.57 ± 8.99 Tot.: 9.56 ± 7.95		Kirychuck *et al.* (2006)
France	Laying hen	6	Cage Aviary	13 13	Resp.: 0.21 ± 0.22 Resp.: 1.12 ± 0.50	Experimental trial. 2 measures/month during the laying period	Michel *et al.* (2007)
France	Laying hen	1.5–10.0	Cage On-floor	32 43	Resp.: 0.02–1.12 Resp.: 0.03–0.86		Pedrono *et al.* (2009)

[1]Total, [2]respirable, [3]inhalable, nd, not determined.

compared with care due to different measurement techniques and time of sampling. From an occupational health point of view, dust is classified by aerodynamic size into three categories: the inhalable fraction with a diameter lower than 100 µm, the thoracic fraction with a diameter of less than 10 µm and the respirable fraction with a diameter under 5 µm. About one-half of particles of the respirable fraction entering the respiratory system will reach the alveoli (Just *et al.*, 2009). The ratio of the concentrations of respirable dust to inhalable dust is equal to 10–20% in poultry houses but varies from 5% in cage buildings to 35% in aviaries (Martensson and Pehrson, 1997; Takai *et al.*, 1998). The housing system greatly influences the airborne dust concentration with higher levels of dust in alternative systems. In Sweden the maximum allowed concentration of dust in poultry houses is 10 mg/m^3 and this threshold appears to be frequently exceeded in aviaries and on-floor houses. The effect of furnishing cages is less clear: low levels of dust have been observed in experimental buildings with furnished cages (Nimmermark *et al.*, 2009; Saleh *et al.*, 2004) but high dust concentrations have been reported in French farms with large furnished cages (Huonnic *et al.*, 2009). The degradation of the air quality in alternative systems is due to providing hens with litter and to high activity of birds. Independently of the housing system bird activity also increases the aerial dust concentration during daytime in contrast to nighttime (Takai *et al.*, 1998; Wathes, *et al.*, 1997). In addition the ventilation system and litter management have an impact on dust concentration (Takai *et al.*, 1998; Larsson *et al.*, 1999; Radon *et al.*, 2001b; Venter *et al.*, 2004).

In European countries, the OES for 8 hours' work varies from 10 to 15 mg/m^3 for total dust and from 1.5 to 5 mg/m^3 for respirable dust. The exposure concentration associated with significant pulmonary function decrements in poultry workers is 2.4 mg/m^3 for total dust and 0.16 mg/m^3 for respirable dust according to Donham *et al.* (2000). The mean exposures reported in Table 18.3 are frequently higher than these thresholds, mostly in alternative systems. The most exposing tasks for workers are hen handling, litter and manure handling and cleaning, whereas the least exposing is sorting and conditioning the eggs (Pedrono *et al.*, 2009; Whyte, 2002).

18.2.3 Exposure to bioaerosols

Bioaerosols or organic dust are usually defined as aerosols of microbial, plant or animal origin (Douwes *et al.*, 2003). They comprise airborne bacteria, fungi, viruses and their by-products, endotoxins and mycotoxins. The organic dust represents 39% ± 22 of the dust mass in the poultry working environment (Skogstad *et al.*, 1999). The specific OES for organic dust is 3 mg/m^3 in Denmark and 5 mg/m^3 in Scandinavia but these limits have not been fixed for dust originated from poultry houses in particular.

For the measure of exposure to microorganisms in poultry working environment, traditional culture-based methods considerably underestimate

the exposure in comparison with non-culture dependent methods such as epifluorescence and quantitative polymerase chain reaction (q-PCR) (Rinsoz *et al.*, 2008). Using the latter methods the concentration of microorganisms in the air of henhouses exceeds 1×10^7 bacteria per m^3 and the highest levels are reached in alternative systems (Borghetti *et al.*, 2002; Nimmermark *et al.*, 2009; Radon *et al.*, 2002; Rinsoz *et al.*, 2008). The personal exposure also ranges from 2.7×10^7 to 4.2×10^{10} cells/m^3 for bacteria and from less than 3.0×10^3 to 1.1×10^9 for fungi in Swiss henhouses (Radon *et al.*, 2002). The qualitative composition of airborne bacteria is characterised mainly by Gram-positive bacteria such as *Staphylococcus* and *Bacillus* with a relative abundance of more than 95% (Rinsoz *et al.*, 2008; Sauter *et al.*, 1981); the most frequent Gram-negative families are Enterobacteriaceae and Pseudomonadaceae (Zucker *et al.*, 2000). Concerning fungi, *Penicillium*, *Aspergillus* and to a lesser extent *Eurotium* and *Cladosporium* are the most frequent genera identified (Lee *et al.*, 2006; Michel *et al.*, 2007; Radon *et al.*, 2002). According to Brooks *et al.* (2010), bacteria isolated from aerosols in broiler houses are rarely resistant to more than four antibiotic classes. None of the species of bacteria or fungi commonly isolated from poultry dust is a strict pathogen for human by respiratory route (Guillam *et al.*, 2007).

Endotoxins, derived from the outer membrane of Gram-negative bacteria, constitute a major component of organic dust (Rylander, 2002). Levels of endotoxins in henhouses are higher than in cattle and pig buildings (Seedorf *et al.*, 1998). The concentrations of airborne endotoxins are greater in alternative systems than in caged systems (Table 18.4) and may also be influenced by season, ventilation rate and temperature (Schrierl *et al.*, 2007). The stockmen exposure in both caged and alternative systems is often higher than the threshold of 50 UE/m^3 over 8 hours proposed by the Dutch Expert Committee on Occupational Standards (Douwes *et al.*, 2003). The American ICOH (Schenker *et al.*, 1998) fixed the limit concentration, with no effect on workers' health for a short-term exposure, at less than 10 ng/m^3 while a concentration above 10 ng/m^3 was associated with inflammatory symptoms of the airways, a concentration above 100 ng/m^3 with systemic effects on health and a concentration higher than 200 ng/m^3 with Organic Dust Toxic Syndrome (ODTS).

Mycotoxins or fungal toxins are biomolecules produced by fungi that are toxic for both animals and humans (Douwes *et al.*, 2003). Trichotecenes B and zearalenone have been detected in dust from henhouses under experimental conditions (Michel *et al.*, 2007) but little is known about occupational airborne exposure to mycotoxins and respiratory health effects. (1 → 3)-β-D-glucans are other biomolecules originated from fungi but also from some bacteria and plants (Douwes *et al.*, 2003). Their concentration in the air of poultry houses varies from 4 to 870 ng/m^3 for the non-water soluble fraction and from 0.01 to 70 ng/m^3 for the water soluble fraction in the study of Rylander and Carvalheiro (2006). Health effects of (1 → 3)-β-D-glucans exposure in

Table 18.4 Endotoxin concentrations in poultry houses and worker exposures

Country	Production	Time (h)	Housing system	*N*	Endotoxins concentration (ng/m^3) average ± std or range	Remark	Reference
Ambient air							
US	Laying hen	1	Cage	5	Tot.[1]: 40–280		Clark *et al.* (1983)
Sweden	Laying hen	8	Cage Aviary On-floor	3 1 1	Tot.: 28–75/Resp.[2]: < 10 Tot.: 504 ± 614/Resp.: 158 ± 142 Tot.: 46/Resp.: 10		Martensson (1995)
Sweden	Pullet	24	Cage Aviary	12 16	Tot.: 5.3–43.0/Resp.: 3.4–11.0 Tot.: 14.0–110.0/Resp.: 2.6–16.0	Experimental trial	Martensson and Pehrson (1997)
UK	Laying hen	12	Perchery Cage	16 16	Inh.[3]: 170/Resp.: 16 Inh.: 100/Resp.:4		Wathes *et al.* (1997)
UK	Laying hen	12	Perchery Cage	10 6	Inh.: 2,816/Resp.: 172 Inh.: 549/Resp.: 68		Seedorf *et al.* (1998)
Denmark			Perchery Cage	6 2	Inh.: 265/Resp.: 27 Inh.: 116/Resp.: 14		
Germany			Cage	4	Inh.: 31/Resp.: 3		
Netherlands			Perchery Cage	8	Inh.: 431/Resp.: 20 Inh.: 21/Resp.: 2		
Spain	Laying hen broiler	2	nd	14	Tot.: 137.1 (median)	Interquartile range: 58.6–243.9 ng/m^3	Borghetti *et al.* (2002)
Sweden	Laying hen broiler	0.5–1	nd	39	Tot.: 10–1,003		Rylander and Carvalheiro (2006)
France	Laying hen	8	Cage Aviary	7 7	Inh.: 11.0 ± 11.2 Inh.: 40.0 ± 21.9	Experimental trial	Michel *et al.* (2007)
Germany	Laying hen	1	nd	18	Inh.: 11–2,085/Resp.: 0.2–1,116	During the feeding period	Schrierl *et al.* (2007)

Continued

Table 18.4 Continued

Country	Production	Time (h)	Housing system	*N*	Endotoxins concentration (ng/m^3) average± std or range	Remark	Reference
France	Laying hen	8	Cage On-floor Aviary	15 18 3	Inh.: 6.5–48.0 Inh.: 39.3–185.6 Inh.: 27.3–90.8		Huonnic *et al.* (2009)
Personal exposure							
Switzerland	Laying hen	0.4–2.2	nd	36	Inh.: 19–1.635		Radon *et al.* (2002)
US	Laying hen	0.5–1	Cage	31	Tot.: 129 ± 135		Kirychuck *et al.* (2006)
US	Laying hen Turkey broiler	2–4	nd	236	Tot.: 2–391,670/Resp.: 3–6940		Donham *et al.* (2000)
France	Laying hen	6	Cage Aviary	3 3	Inh.: 5.4 ± 0.3 Inh.: 25.5 ± 14.3	Experimental trial	Michel *et al.* (2007)
Sweden	Laying hen	3	Cage Aviary	12 24	Inh.: 96–108 Inh.: 83–175	Experimental trial	Larsson *et al.* (1999)
Sweden	Laying hen	nd	Cage	11 6	Tot. unloading cage : 1090 ± 840 Tot. loading cage: 150 ± 60	Exposition of poultry handlers during loading/unloading of cage	Thelin *et al.* (1984)

[1]Total, [2]respirable, [3]inhalable, nd, not determined.

the occupational environment are plausible but no limit exposure value has yet been fixed.

18.2.4 Chemical exposure

Chemical hazards arise when workers are exposed to chemical products such as fuels, pesticides or cleaning agents. Spilling such chemicals on the skin can result in poisoning, irritation or allergic reactions. Certain chemicals are rapidly absorbed through the skin and may cause systemic poisoning. Workers in egg farms are more particularly exposed to disinfectant products such as formaldehyde and quaternary ammonium compounds (QAC). A French study comparing the use of disinfectants in poultry and pig productions showed that workers in egg farms used more often formaldehyde than pig producers (Gérault *et al.*, 2003). To the best of our knowledge there are no studies reporting worker exposure to disinfectants in poultry production. In pig production strong associations between disinfecting procedures and chronic respiratory symptoms and lung function decrease were observed (Preller *et al.*, 1995). In addition atopic sensitisation was more frequent in pig farmers exposed to QACs (Preller *et al.*, 1996). Poultry farmers are also particularly exposed to acaricid products widely used against poultry red mites.

18.3 Health effects and epidemiological features in poultry worker populations

18.3.1 Non-malignant respiratory diseases

Health problems resulting from the respiratory exposure precedently described may be chronic (lasting a long time or relapsing) or acute (severe but short in duration). Acute problems are often recognisable since they may be dramatic, but chronic problems may be mistaken for or be aggravated by other health conditions such as flu, allergies, or cough and bronchitis due to smoking.

Studies of the health of poultry workers in broiler and egg productions in the United States and in the United Kingdom have shown that the primary adverse health effects due to respiratory exposures were breathlessness, chest tightness and airway inflammation, often manifested as bronchitis (Morris *et al.*, 1991; Reynolds *et al.*, 1993). Workers who smoke and those who have worked with poultry for more than 5 years were at greatest risk for such ailments. Although smoking can aggravate or cause respiratory problems, not all poultry workers with respiratory symptoms and reduced lung function were smokers. In a Swiss study on poultry farm workers experienced the highest rates of respiratory symptoms in comparison to other animal farmers or crop farmers (Danuser *et al.*, 2001); poultry farming was established as a risk factor for reporting nasal irritation at work (Odds Ratio 5.3 [1.6–18.0]). A dose–response relationship could also be established between wheezing and nasal irritation at work and the number of hours spent daily inside the

poutry house: the risk was twice as high for farmers spending more than 1 hour per day in the building (Radon *et al.*, 2001a). Among farmers keeping only poultry, no elevated risk of work-related symptoms was found, probably due to the low numbers of these farmers in the survey. A Canadian study confirmed that poultry workers reported greater prevalences of current and chronic respiratory symptoms than pig and grain workers (Kirychuck *et al.*, 2003). In particular workers in caged hen buildings reported higher prevalences of current cough and wheeze than workers in broiler houses and presented lower lung capacities.

An ailment known as organic dust toxicity syndrome (ODTS) has occurred in workers exposed to dust in swine operations and grain handling and storage facilities. ODTS is characterised by flu-like symptoms such as headaches, muscle aches, and malaise occurring 2 to 6 hours after exposure. The occurence of ODTS is closely related to the exposure to endotoxins (Rylander, 2002). In a study on health of workers exposed to organic dust, the highest prevalence of ODTS was observed in poultry handlers (5.9%) and exposures to dust and endotoxins were found to be predictive of respiratory symptoms (Simpson *et al.*, 1998).

Other respiratory problems that can occur in poultry workers include allergic reactions, asthma and hypersensitivity pneumonitis. Hypersensitivity pneumonitis, often called 'farmer's lung', is characterised by an interstitial lymphocytic pneumonitis. In a study in New Zealand, an increased prevalence of wheezing, but not of hayfever, was seen among poultry farmers (Kimbell-Dunn *et al.*, 1999); half of the poultry farmers complaining about asthma and/or work-related wheezing also reported nasal allergies. Müller *et al.* (1986) described a high prevalence of sensitisation and respiratory symptoms in a group of 339 poultry farmers. Thus poultry farmers might be at higher risk for the development of asthma, but it is still unknown whether this disease is caused by an allergic or inflammatory response. In a Croatian study a significantly higher prevalence of work-related nose, asthma, eye and skin symptoms, and slight decline in ventilatory lung function was found in poultry workers in comparison to control subjects (Rimac *et al.*, 2010). Poultry workers had significantly higher prevalence of IgG antibodies to moulds compared with controls (63 vs 36%, $P = 0.01$) especially to *Alternaria* and *Aspergillus* species.

18.3.2 Infectious and parasitic diseases

The risk of infectious disease by respiratory route exposure is mainly related to zoonotic agents as no respiratory pathogen specific to human has been described in poultry building atmospheres. *Chlamydophila psittaci* is a zoonotic agent which could infect both *Gallus gallus* birds and humans but cases of psittacosis are more often related to close contacts with turkeys or ducks than with poultry (Harkinzhad *et al.*, 2009). Food-borne *Salmonella* and *Campylobacter* infections in humans became major public health concerns

in industrial countries during the 1980s. Outbreaks of salmonellosis and campylobacteriosis are mainly related to the consumption of contaminated eggs or poultry meats. Occupational infections by those pathogens are less documented than foodborne infections although poultry workers spend their working days in environments potentially contaminated by *Salmonella* and *Campylobacter*. Between 1996 and 2003, 496 occupational campylobacteriosis were reported in the United Kingdom, poultry dressers being the most affected group (Wilson, 2004). Workers in poultry slaughterhouses appear to be at elevated risk of *Campylobacter* exposure (Price *et al.*, 2007) but they might acquire protective immunity from long-term exposure to *Campylobacter* (Cawthraw *et al.*, 2000). No epidemiological study has been carried out on poultry workers at farm level but breeding hens or chickens was a risk factor for campylobacteriosis in a Swedish case-control study (Studahl and Andersson, 2000).

For many years the 'bird flu' or highly pathogenic avian influenza (HPAI) did not seem to present a danger to humans and, thus, was not classified as a zoonosis despite some exceptional cases and benign transmission reported in the literature typically with mild to moderate symptoms of conjunctivitis or influenza-like illness. The emergence of the highly publicised 'bird flu' caused by an influenza virus H5N1 in Hong Kong in 1997, affecting 18 people and killing 6 of them, revealed a clear risk of contamination from chicken to human for the first time. Following this first outbreak an epizootic of H5N1 began in 2003 in South Korea and rapidly spread to Asia, Europe and Africa. To date, human cases have been reported in six countries since January 2004, most of which are in Asia. All human cases have coincided with outbreaks of highly pathogenic H5N1 avian influenza in poultry. All evidence to date indicates that close contact with dead or sick birds is the principal source of human infection with the H5N1 virus. A new element is the ability of the subtype H5N1 to infect people severely even if these cases are rare: some 190 cases of the disease and 91 deaths have been recorded since 2004. In terms of public health, avian influenza H5N1 is a proven occupational disease but the risk is very low and quantitatively linked to close contact with infected animals. HPAI viruses other than H5N1 could infect humans as reported during the outbreak of HPAI H7N7 in the Netherlands in 2003. An epidemiological survey on workers handling infected poultry concluded that 83 persons were infected by A/H7 virus over 583 exposed to poultry and with health complaints. The main symptom related to the infection was conjunctivitis (Koopmans *et al.*, 2004). LPAI may also infect humans as in Italy during the LPAI H7N7 outbreak: 3.8% of workers exposed to infected poultry developed an antibody response (Puzelli *et al.*, 2003).

Dermanyssus gallinae is the most important ectoparasite affecting laying hens in several countries. Recent surveys have confirmed its endemicity in poultry farming worldwide (Sparagano *et al.*, 2009). Infestation rates can reach 80–90% in poultry, as observed in UK, Italy, Serbia, and the Netherlands. Alternative housing systems often exhibit higher prevalence rates due to the

greater potential of *Dermanyssus gallinae* to hide in litter, cracks or crevices and avoidance of chemical control methods (Sparagano *et al*., 2009). Infested birds show decreased production, irritation and, in severe infestation, anaemia leading to death. The poultry red mite is an obligatory blood-feeder parasite found mainly on poultry. However, it is also a common infestant of other avian species and occasionally bites mammals including dogs, cats, rodents, horses and humans (Brockis, 1980). Thus poultry red mites can constitute a problem to personnel working in infested poultry premises. This parasite is a cause of pseudoscabies among persons working in the poultry industry, especially in layer houses using battery cage systems. The result is a nonspecific dermatitis that presents as an erythematous, papular eruption associated with intense pruritus. It can easily be misdiagnosed as atopy, prurigo or pediculosis (Cafiero *et al*., 2008). Several reports suggest it could have a vector role for viral and bacterial human and animal diseases (Sparagano *et al*., 2009). As an example, De Luna *et al*. (2008) demonstrated the presence of *Mycobacterium* spp. within *D. gallinae*. Chemical control methods are often used to control poultry red mites infestation in henhouses: organophosphates, pyretrinoids and carbamates (Chauve, 1998). The removal of acaricide products from national markets due to the increase in acaricide resistance, environmental or welfare concerns and safety aspect has a tremendous impact on the proliferation of such pest. The reduction in number and efficacy of many acaricide products has increased the prevalence rates.

18.3.3 Malignancies

In general, farmers have significantly elevated risks for several cancers including lip, stomach, pancreas, nasal sinus, prostate, leukaemia, non-Hodgkin's lymphoma, Hodgkin's disease, and multiple myeloma (Burmeister *et al*., 1983). Analysis of deaths from leukaemia-lymphoma group cancers and occupation as stated on death certificates revealed a statistically significant association between farming occupations and death from leukaemia and multiple myeloma (Burmeister *et al*., 1982; Milham, 1994). Poultry farmers in particular showed the highest proportionate case excess of leukaemia in the study of Milham (1994). A Finnish study based on cancer recordings over the period 1979–1993 described an excess risk for multiple myeloma among pig and poultry farmers in contrast to other types of farmers (Pukkala and Notkola, 1997). In a recent case-control study based on death certificate data, Svec *et al*. (2005) also observed a slightly increased risk of mortality for most haematopoietic cancers in farmers involved in the livestock industry in comparison with farmers involved in crop industry. These findings are consistent with the hypothesis that agricultural environments contain agents which may cause leukaemia and various haematopoietic cancers (Descatha *et al*., 2005). Statistical associations between animal breeders and various cancer types have been reported – mainly chronic lymphocytic leukaemia (CLL), non-Hodgkin's lymphoma (NHL) and T-cell leukaemia, speculating

that animal retroviruses or chemicals involved in animal breeding could be the aetiological factors (Amadori *et al.*, 1995; McDuffie, *et al.*, 2002). As an example Choudat *et al.* (1996) observed a higher prevalence of antibodies against Marek's disease herpes virus (MDV) in workers exposed to poultry than in workers in other animal production and white-collar workers. This finding suggests the presence of the MDV in humans but the involvement of this animal oncogenic virus as an aetiological agent in human cancer has still to be proven.

18.3.4 Musculoskeletal disorders

Musculoskeletal disorders (MSDs) cover a broad range of health problems. The main groups are back pain and injuries, and work-related upper limb disorders, commonly known as 'repetitive strain injuries' (RSI). Lower limbs can also be affected. Farmers are at great risk for developing musculoskeletal disorders, due to the physical demands of their job, their interaction with machinery and animals and the repetitive tasks each specific job requires. Almost 61% of the sick leave claims filed by farmers in the Netherlands were due to a musculoskeletal injury or disease (Hartman *et al.*, 2003). In France these disorders represent 93% of the cases of compensable occupational diseases of the agricultural sector and one third of the poultry sector (Bernard and Tourne, 2007). Walker-Bone and Palmer (2002) reviewed the latest epidemiological studies in order to estimate the risk for each type of disorder in conjunction with farming in general or specific risk factors. Hip osteoarthritis has emerged as a serious problem, as the relative risk for the disease among farmers was 2 to 3. Knee osteoarthritis is also suggestively associated with farming practices although fewer studies exist to support this. Low back pain among farmers is as common as other heavy-duty professions. Although low back pain was the most prevalent symptom among the general farming population, working with animals seems to be a significant risk factor for shoulder pain as suggested in a recent study (Rosecrance *et al.*, 2006). Still, there is some evidence that livestock farmers are at reduced risk for lumbar spine disorders compared with crop farmers (Manninen *et al.*, 1995). There are few studies examining the association between specific types of animal farming and musculoskeletal disorders, suggesting a potentially increased risk and identifying significant risk factors. A survey carried out in Dutch agriculture showed that musculoskeletal symptoms or psychological symptoms related to workload were reported by farmers as frequently in poultry production as in other agricultural sectors (Hildebrandt, 1995). In a subsequent survey focused on poultry workers (377 workers) the 12 month prevalence of low back, neck/shoulder, upper limbs and lower limbs complaints was 44%, 29%, 15% and 25%, respectively. The percentage of complaints that was attributed to the labour conditions varied from 41% for the lower limbs to 56% for the upper limbs (Drost and Dooren, 2000).

These physical conditions are secondary to intense physical effort involved

in raising poultry. They may also be related to psychosocial factors and factors related to work organisation: they may have indirect effects on the level of exposure to biomechanical factors (work environment and quality of relationships with colleagues or supervisors, working hours, remuneration, and insufficient breaks during the work shift, quality standards, etc.). In addition individual factors such as age and sex influence the occurrence of MSDs. These diseases are often referred to as 'over-solicitation syndrome'. The pain caused is directly related to achievement of a difficult task in a short time. The upper limb is mainly affected, with a predominance of the wrist, shoulder and elbow. The joint and bone structures of the upper extremity are the most stressed whatever the activity within the sector. The lesions may be purely musculoskeletal (tendinitis, elongation, strain) or nervous (carpal tunnel syndrome) (Roquelaure *et al.*, 2005). This syndrome is extremely common because it is secondary to heavy load manipulation and repetitive wrist movements. It corresponds to a compression of the median radial nerve in the wrist, causing tingling and hypoesthesia in some areas of the hand, especially at night. More rarely, this syndrome may cause a loss of palmar sensitivity, decreased muscle strength and vascular spasm. Associated with these afflictions of the upper extremity is an increase of back pain, secondary to repeated malposition putting the lower back in extension. It seems obvious that these conditions are directly related to the occupation. Indeed, repeated gestures requiring a large muscle or a position requiring extreme joint at a sustained pace are often responsible for the syndrome of over solicitation: for example sexing, debeaking, declawing, vaccination, semen collection, knowing that the animal's weight is important and that the time allowed for carrying out these activities is short (French Institute for Public Health Surveillance) (INVS, 2005). However, factors other than professional can intervene in the development of these disorders as environmental conditions (cold) or extra-occupational activities (sports, crafts, and gardening).

The recent evolution of hen housing systems in Europe represents a new challenge in terms of working conditions and ergonomic problems. An ergonomic study carried out in an aviary indicated that collecting eggs on floor level, manual cleaning of equipment and bird collection before slaughtering result in considerable work load, whereas collecting eggs from egg conveyors or handling of manure system were less troublesome (Lundqvist, 1995). A study on working practices and work-related postures in a perchery system also concluded that manual collection of floor eggs and reaching into the middle of the perches from the litter side put the most strain on workers (Scott and Lambe, 1996).

Independently of housing systems there are very general conditions found in all occupations related to poultry. These traumatic disorders are generally not specific, such as falls when the ground is level or rolling. Poor visibility also increases the risk of falling, causing wounds and even fractures. Falls from height are also identified: they usually cause more severe injuries of the back of the head and limbs. In a Californian study cited by Lundquist

(1995) these non-specific traumatic disorders contributed to occupational disorders in poultry farming at the same level as overexertion of body parts or back injuries. The most common accidents identified were being struck by a falling or flying object, pressing injuries from feed barrows, doors, etc. and slipping and falling accidents caused by tripping over objects in the alleys or falling from high levels. Various skin lesions (scratches, abrasions) result from animal and equipment handling. They can be a source of infection if they are not protected and disinfected immediately.

18.3.5 Accidents and injuries

Other safety and health hazards in poultry production which could provoke accidents and injuries include mechanical, electrical and fire hazards, and heat and cold stress. Often these potential hazards become a higher risk when workers compromise safety precautions by taking guards from equipment or disregard safety policies. Machinery such as feeding equipment or fans must be equipped with safety guards, which should always be replaced after servicing. Improperly guarded or handled chains, sprockets, winches, belts and pulleys may act as pinch points. If a person's extremity happens to encounter such a pinch point, lacerations (cuts), avulsions (tearing off of tissue) or crushing injuries may occur. Electrical hazards arise when electrical equipment is not properly grounded or is not corrosion resistant, or when insulation around electrical wires is in poor repair. In such cases, electrocution or nonfatal electrical shocks may occur. Fires may also develop from damaged electrical wires. In addition, lockout systems for breaker boxes should be used while servicing electrical equipment to prevent machinery from being turned on accidentally. Working in extremely hot or cold weather also constitutes a health hazard. Frequent work breaks and fluid replacement are needed in hot weather to prevent heat exhaustion. Adequate dry clothing, including gloves, is needed in cold weather to prevent frostbite.

18.4 Prevention and control of health risks

18.4.1 Prevent and control of exposure to gas and aerosol

Many sources of gases, dust and bioaerosols are intrinsic to egg production and are therefore difficult to control. In addition the implementation of reduction measures to minimise the concentrations of airborne pollutants must not impair bird productivity and has to comply with new requirements on animal welfare and environmental protection. Despite these difficulties, both preventive and control methods to limit worker exposures could be implemented in henhouses. Preventive measures aim to reduce the generation of pollutants by modifying building design or herd management whereas remedial measures target reducing existing airborne pollution problems. Examples of preventive and remedial measures to control ammonia and

dust levels are given in Table 18.5. These measures generally rely on modifications of husbandry practices and have proven their effectiveness, such as regular evacuation of manure. High ventilation rates dilute gas and dust concentrations inside the building but lower inside ambient temperature and provoke discharge of gaseous pollutants in the environment.

Most of the suggested measures are derived from experiences in pig production and are not always directly applicable to poultry production. As an example, reducing water content in pig feed is effective at reducing ammonia release. Poultry feed, however, is already very dry and its water content cannot be modified to a significant extent. Various methods of air treatment to reduce dust concentration in the air of the building (physical and electrical filtration) or ammonia and dust in the exhaust air (bio-filters, air-scrapers) have been tested with success under experimental conditions. However, their application in commercial conditions appears to be difficult, taking into account that large volumes of air are to be treated. In contrast spraying oil or fogging water droplets is an inexpensive and effective method that is relatively easy to use in commercial poultry houses. A reduction of 50% of aerial dust concentration in an aviary was achieved by water fogging without degradation of feather condition of hens (Gustafsson and von Wachenfelt, 2006). As concentrations of bioaerosols are closely correlated to those of dust, measures taken to reduce dust exposure should also reduce the bioaerosol exposures (Donham *et al.*, 2000). However, the correlation between

Table 18.5 Control strategies to reduce ammonia and dust concentrations in hen poultry houses

	Ammonia	Dust
Preventive measures	Reduce water content in manure: manure drying systems (Fabbri *et al.*, 2007), drinker design (Garner *et al.*, 2008) Manure management: regular evacuation from poultry house (Hayes *et al.*, 2006; Nicholson *et al.*, 2004; Whyte 2002) Appropriate litter material: sawdust (Oehm and Petersen, 1999)	Effective cleaning between batches of hens Minimising hen agitation: constant inspection and egg collection patterns (Whyte 2002), lighting cycles, Appropriate litter material: peat or clay pellets (Gustafsson and von Wachenfelt 2006) Spraying oil on litter (Ellen *et al.*, 2010; Gustafsson and von Wachenfelt, 2006; von Wachenfelt, 1993) Feed: pelleted food, fat or oil addition
Remedial measures	High ventilation rate (Wathes *et al.*, 1997) Bio-filters, air-scrapers (Ellen *et al.*, 2010)	High ventilation rate (Gustafsson and von Wachenfelt, 2006) Fogging water droplets (Gustafsson and von Wachenfelt, 2006; von Wachenfelt, 1993) Negative air ionisation (Lyngtveit and Eduard, 1997; Mitchell *et al.*, 2000) Bio-filters, air-scrappers (Ellen *et al.*, 2010)

ammonia and dust concentrations is not verified and control measures have to be implemented separately but are sometimes discrepant. For example dry litter is a key point in controlling ammonia production but it is aerosolised more easily by the hens, increasing the dust burden.

18.4.2 Personal protective equipment

When worker exposure to aerial pollutants cannot be avoided, use of a respiratory protection is recommended. The effectiveness of respirators against aerosol and gas is generally lower and more variable in field conditions than in laboratory but a mean protection factor under normal work activities equal to 13 for a quarter-mask to 30 for a purifying powered helmet is reached in poultry production when the respirators are correctly worn (Popendorf *et al*., 2003). Poultry workers, however, often experience respirators to be uncomfortable at work. In a French cohort study 46% of the poultry workers reported never using a respirator (Gérault *et al*., 2003). The study of Popendorf *et al*. (2003) evaluated the acceptability of respirators by poultry workers: a powered helmet was preferred to a disposable or half mask because of easier breathing and communication despite increased weight. Nevertheless all respirators presented characteristics considered as inconvenient by workers, limiting their acceptability. Use of personal respiratory protection tools is mainly recommended for those tasks that expose most to dust and gas, as identified by occupational exposure studies: hen, litter and manure handling, cleaning and disinfection operations. Other personal protective equipment can be used in poultry production, including gloves, goggles or boots. Wearing respirators, gloves and goggles is required for a manipulation of hazardous chemical products such as disinfectants.

18.4.3 Prevention of zoonotic risks

Personal protective equipment and respect of biosecurity and hygiene rules are the main preventive measures that should be adopted to avoid transmission of zoonotic agents from poultry to workers. Concerning the zoonotic risk caused by avian influenza viruses, the first prevention measure is to ensure a close surveillance and a rapid detection of the virus in poultry to limit virus spread (MacMahon *et al*., 2008). In the event of outbreaks of HPAI, specific protection measures need to be applied to the workers directly exposed as defined by the health authorities. This includes protective clothing, heavy gloves and boots, goggles and masks. The experience from outbreaks of LPAI H7N7 in Italy shows that workers exposed to LPAI also need to be protected. Although vaccination against conventional human influenza strains will not prevent avian influenza infection, including HPAI infection, it could prevent dual infection and possible reassortment of the virus. Thus vaccination of workers involved in HPAI control and eradication activities is required by World Health Organization. A prophylactic antiviral drug treatment is

also recommended in case of breach in personal protection equipment. The desirability of vaccinating poultry industry staff against seasonal influenza has been the subject of a notice of the French High Council of Public Hygiene. In the absence of outbreaks of avian influenza, the probability of co-infection between human and avian influenza viruses appears negligible and professionals of poultry industry do not represent a population at specific risk concerning influenza virus infections.

As a result of the high prevalence of poultry red mite infestations in poultry buildings, much of the current research targets on developing new methods of control. Alternative methods have been developed, such as silica dust and insect growth regulators. Management of poultry flocks is also important. Vacuum cleaning the houses between flocks is essential and very efficient because it eliminates dust which is necessary for the survival of mites. Washing the houses should always be carried out because mites are very sensitive to humidity. Spraying acaricide in all crevices and corners is appropriate after washing and disinfection. Finally, in order to allow the mite population to naturally decline, the interval between depopulating and repopulating the house must be as long as possible.

18.4.4 Prevention of musculoskeletal disorders and accidents

Prevention of accidents relies on training of workers, respect of safety policies and identification and rectification of potentially dangerous situations as exposed earlier. Equipment (cages, nests) must be designed to avoid extreme body positions for workers which are the most likely to cause accidents, discomfort and strain. Therefore the participation of working environment experts would be valuable in designing husbandry material. If mechanical egg collection is now well developed in on-floor systems and aviaries, collecting mislaid eggs always causes an ergonomical problem. Therefore, enhancing nest laying by modifying husbandry practices is needed; some tools are also available to collect eggs from the floor, such as pliers with a haft.

18.5 Future trends

Pollutants present in the air of poultry houses are widely described but the number of studies on exposure assessment of workers and relation between exposure and working tasks is limited. Specific risk analyses for the most common hazards have to be developed to propose adapted preventive measures. Prevention essentially relies on risk communication towards farmers with a special emphasis on identification of risk situations, respect of security measures and hygiene rules and use of personal protective equipments. Taking into account that exposure to bioaerosols is related to a wide range of health effects, more research is needed to establish quantitative exposure assessment methods and dose–response relationships to define threshold

values specific to workers' exposure in poultry confinement buildings. The use of furnished cages and alternative housing systems is expanding greatly in the European Community as a result of the European Directive 1999/74/EC (EC, 1991). The development of these systems may lead to an increase of some occupational risks as for the exposure to aerosol in alternative systems or cause new risks as ergonomic problems for workers in on-floor systems. Implication of experts in working environment and occupational risk analysis is needed to create an environment that is acceptable for both the hens and the workers.

18.6 Sources of further information and advice

The American Thoracic Society proposed in 1998 a complete review of respiratory health hazards in agriculture focused on main epidemiological features and relations between exposures and health effects (Schenker *et al*., 1998). In a book on agriculture health and safety edited by McDuffie *et al*. (1995) two chapters are especially devoted to egg production dealing with the impact of alternative systems on accident risks and respiratory hazards. The websites of the American Occupational Safety and Health Administration (OSHA, 2010) and of the European Agency for Safety and Health at Work (OSHA-EU, 2010) propose specific sections on occupational risks in agriculture. Risk analysis, good practice guides and supports for risk communication are available for a wide range of risks in agriculture such as chemical, biological risks or musculoskeletal disorders. The World Health Organization on its website tracks the evolution of the HPAI H5N1 panzooty and regularly updates guidelines on prevention and surveillance of Avian Influenza (WHO, 2010).

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19

Environmental sustainability of egg production and processing

M. G. MacLeod, University of Edinburgh, UK

Abstract: Sustainability can be defined as 'meeting the needs of the present without compromising the ability of future generations to meet their needs'. Life cycle assessment (LCA), taking into account all inputs (including energy) and outputs (including greenhouse gases), shows that poultry, including laying hens, compare favourably with other farm livestock. This is largely because biological overheads are relatively low because of rapid maturation and high fecundity. Also, genetic selection, along with control of nutrition and environment, has allowed the industry to respond to challenges linked either with selection for productivity or with intensive housing and nutrition. Issues of pollution by poultry are often related to disposal of wastes in limited and sensitive areas rather than total quantities. Emerging challenges for the poultry industry include competition with biofuel production, itself ostensibly developed with sustainability in mind.

Key words: climate, ecology, energy, environment, laying hens, nitrogen, phosphorus, pollution, sustainability.

19.1 Introduction

The definition of sustainability quoted in the Abstract, 'meeting the needs of the present without compromising the ability of future generations to meet their needs', seems to have originated in the World Commission on Environment and Development, Brundtland Report (Brundtland, 1987). In the case of the poultry industry, meeting the needs of the present must balance economic decisions against environmental ones. Also, we must be

objective, but we should not allow less objective propagandists to present a totally negative view of the ecological sustainability of animal production, particularly the poultry industry. Life cycle assessment or analysis (LCA), taking into account all major identifiable inputs (including energy from fossil fuels) and outputs (including greenhouse gases), shows that poultry compare favourably with other farm animals. An inherent advantage of poultry systems is that the overheads of reproductive and rearing costs are relatively low because of rapid sexual maturation and numerous offspring per breeder parent. Also, the poultry industry has a history of rapid adaptation to practical difficulties; conventional genetic selection, coupled with control of nutrition and physical environment, has allowed the industry to respond to challenges (e.g. skeletal problems) purported by some to have originated either in selection for production rate or in intensive housing and nutrition. Issues of nitrogen and phosphorus pollution are often related to production and disposal in a limited geographical area rather than absolute quantities.

In common usage, the definition of sustainability has sometimes been extended to include welfare and aesthetic or sociological issues. When these issues are under discussion, it might be more precise to replace the word 'sustainability' by 'acceptability' or 'permissibility'. As an example, a less humane husbandry technique may be infinitely sustainable under an ecological definition of the word and the question is rather whether the practice is acceptable or permissible on ethical grounds. Similarly, gaseous or dust emissions from a poultry system sited near human habitation may be ecologically sustainable but the question should be whether they are acceptable or permissible under aesthetic or public health criteria. Fortunately, changes promoting global sustainability are also likely to have benefits for people living near poultry farms and are unlikely to be detrimental to the birds themselves. There are many chickens around the world which escape being included in official statistics. These are the ultimate examples of sustainability. There are large numbers of scavenging village chickens, especially in Africa, Asia and Latin America, which produce eggs and meat at little cost to their owners. These birds receive household food scraps and also forage for insects and other invertebrates.

19.2 The ecology of sustainability

All primary production (by plants) which is then used for animal or human food will finally be degraded to substances we might call waste. However, en route to this state, it passes through a varying number of processes and transient locations, the latter including poultry and ourselves. The way to minimise the demand for primary production and therefore reduce environmental impact is to intercept as much as possible of the nutrients originally 'fixed' by primary producers at every point in the food chain where we have some

control. In the case of poultry producers and their suppliers the tools at their disposal might include the following:

1. Using feed crops that need minimal inputs of nitrogen, phosphorus and also minimal combustible fuel for cultivation and transport.
2. Intercepting nutrients in byproducts/coproducts/wastes from other biologically based processes to use in poultry diets (El Boushy and van der Poel, 2000). This might include residues from processing of human food or from biofuel production. Wastes consisting of animal material may be problematical.
3. Providing the nutrients in a diet form/formulation that leads to maximum utilisation efficiency.
4. Genetic selection of birds to use feed as efficiently as possible.
5. Maximising nutrient yield from raw materials by physical or chemical techniques (e.g. enzyme addition to maximise phosphorus and nitrogen capture).
6. Keeping the birds, especially their alimentary tracts, in a healthy condition to maximise nutrient capture. All diseases are likely to have a cost in nutrient capture or utilisation. The cost may be in terms of specific nutrients or in terms of energy utilisation (e.g. to mount an immunological response).
7. Minimising mortality and 'downgrading losses' (including discarded eggs, etc.). These losses have all involved uptake of energy and raw materials which have then short-circuited the production process to become waste.
8. Keeping the birds in a thermal environment that minimises energy use by the birds without excessive replacement of feed energy with fossil fuel energy.
9. Dealing with the inevitable waste in a way that maximises recycling of nutrients. This must mean that the nutrients should finally be routed back into beneficial primary production (e.g. crops or pasture rather than aquatic micro-algal 'blooms').

It might be possible to make a positive interpretation of outputs from poultry. The carbon dioxide they produce has almost entirely originated from plant materials which the plants have synthesised by fixing atmospheric carbon dioxide. The question may be whether more would have been fixed or whether less would have been released by other biological processes if the agricultural animals did not exist. The animals could be seen as a temporary reservoir of carbon in an ongoing cycle. Whether they lead to greater atmospheric carbon dioxide burden depends on a comparison of the carbon balance of crops grown for animal consumption and the carbon balance of 'natural' vegetation. Carbon dioxide production from animals is therefore different from the burning of fossil fuels, which releases carbon which would otherwise have been bound up in non-gaseous form for a very long time. With poultry, we do not have a significant methane output

to consider. In the case of nitrogen, much depends on how much inorganic nitrogen fertiliser has been used to produce the plant proteins consumed by the bird, how much has come from fixed atmospheric nitrogen and how well the waste nitrogen products from the birds are 'recycled'. If the plant proteins consumed by birds were eaten directly by humans, the current losses due to the birds' 40% nitrogen utilisation efficiency would be avoided, but human utilisation efficiency would be much lower than currently achieved by eating meat or eggs.

19.2.1 Eggs in a lacto-ovo-vegetarian diet

Pimentel and Pimentel (2003) estimated that only one-third (at that time, about 2 billion) of the world population live on a meat-based diet. The majority of the remainder (4 billion) live on a vegetable-based diet, which is often supplemented with eggs and dairy products (lacto-ovo-vegetarian). Pimentel and Pimentel calculated that the amount of feed grains used to produce the animal component of a meat-based diet was almost twice the amount used to produce the milk and eggs of the lacto-ovo-vegetarian. This sort of analysis is supported by LCA results quoted earlier in the present chapter. Eggs can, therefore, be seen as a lower-impact way of adding animal protein to a vegetarian diet. The nutrient balance of egg protein as compared with meat protein is confirmed by Table 19.1. As would be expected when the egg must contain all the nutrients needed to support the development of the chick for the 21 days until it hatches, the amino acid balance of the egg contents is similar to that of chicken meat.

19.2.2 Primary energy use in egg production

Heating is used as little as possible in egg production. Each hen produces about 10 W of heat energy (100 hens = 1 kW). With a high enough stocking density, suitable building insulation and controlled ventilation, this is sufficient to maintain the optimal house temperature of 20–22 °C in a temperate environment. In warmer environments, the problem is rather to dissipate bird heat, which can be achieved by natural or forced ventilation, possibly assisted by air cooling at the highest ambient temperatures. With lower stocking densities, as in 'alternative' housing (including free-range and organic production), bird metabolic heat will be insufficient to maintain optimum ambient temperature. The birds will respond by increasing thermoregulatory heat production, which is supplied by an increased feed intake. The increase in feed intake is of the order of 1–2% for every 1 °C decrease in ambient temperature. The effect of this will appear as a decrease in feed utilisation efficiency (an increase in feed : egg production ratio) and will be detected as reduced efficiencies of energy and nutrient utilisation in life cycle assessments of 'alternative', free-range and organic systems. The effect may be exacerbated by increased physical activity of the birds in these systems. It should be noted that most of these

Table 19.1 Amino acid composition (g/kg protein) of protein in poultry carcass, feathers and egg

	Carcass	Feather	Egg
Alanine	69[c]	35[c]	54[a]
Arginine	69[cd]	61[c]	68[a]
Asparagine	0	68	0
Aspartic acid	93[c]	62[c]	107[a]
Cystine	11[bc]	67[c]	18[a]
Glutamic acid	136[c]	87[c]	120[a]
Glutamine	0	67	0
Glycine	82[c]	68[c]	62[a]
Histidine	29[bcd]	8[cde]	24[a]
Hydroxyproline	20	20	0
Isoleucine	43[bd]	50[de]	56[a]
Leucine	73[bc]	81[de]	83[a]
Lysine	80[bd]	20[de]	62[a]
Methionine	27[bd]	7[de]	32[a]
Phenylalanine	41[bcd]	50[de]	51[a]
Proline	61[c]	92[c]	38[a]
Serine	44[c]	91[c]	78[a]
Threonine	42[bc]	48[de]	51[a]
Tryptophan	10[b]	7[d]	18[a]
Tyrosine	30[bcd]	27[de]	40[a]
Valine	47[bcd]	76[de]	75[a]

[a]Lunven *et al*. (1973); [b]Hakansson *et al*. (1978); [c]Nitsan *et al*. (1981); [d]Hurwitz *et al*. (1983); [e]Blair *et al*. (1981).

increases in feed intake with increased thermoregulatory and locomotory costs are to supply energy. The need for specific nutrients, such as amino acids, may be almost unaffected. Environmental impacts of nitrogen, phosphorus and other losses can, therefore, be minimised by increasing the dietary energy : nutrient ratio (e.g. increasing the ME : CP (metabolisable energy : crude protein) ratio). This can be done by altering the diet formulation or by providing whole grain as well as a compound diet, allowing the birds to select a higher energy : nutrient diet.

19.3 Life-cycle analysis: a mathematical framework for integrating inputs and outputs

Pelletier (2008) noted that, until then, most published work on the environmental impacts of poultry production had concentrated on on-farm emissions. A broader assessment concluded that feed provision accounted for most of the environmental impact: 80% of energy use, 82% of greenhouse gas emissions, 98% of ozone-depleting emissions, 96% of acidifying emissions and 97% of eutrophying emissions associated with broiler production. On-farm inputs and emissions, largely related to heating and ventilation, contribute on average

only 9% of these impacts. LCA examines production systems to account for all inputs and outputs that cross a specified system boundary. Williams *et al.* (2006) used LCA to compare poultry with other agricultural species (Table 19.2) and also to compare intensive with extensive and organic poultry systems (Table 19.3). In LCA, the desired output is termed the functional unit, which Williams *et al.* (2006) defined as 1 tonne of poultry meat or 20 000 eggs (about 1 tonne). The model assumes that 80% of the poultry meat is derived from chickens and ther remainder from turkeys and that approximately 1% of the chicken and turkey market is organic. All inputs were traced back to primary resources, e.g. electricity from primary fuels (coal, oil, uranium). Ammonium-based fertilisers use methane as a raw material and as an energy source. Phosphate and potassium fertilisers require energy for extraction,

Table 19.2 The main burdens and resources used in UK animal production, scaled per tonne of meat, per 20 000 eggs (about 1 tonne) or per 10 m^3 milk (about 1 tonne dry matter) (adapted from Williams *et al.*, 2006)

Impacts and resources used	Eggs	Poultry meat	Pig meat	Beef	Milk	Sheep meat
Primary energy use, GJ	14	12	17	28	25	23
GWP_{100}, t CO_2	5.5	4.6	6.4	16	10.6	17
Eutrophication potential, kg PO_4	77	49	100	158	64	200
Acidification potential, kg SO_2	306	173	394	471	163	380
Pesticides used, dose ha	7.7	7.7	8.8	7.1	3.5	3.0
Land use	0.67	0.64	0.74	2.33	1.20	1.40

Table 19.3 The main burdens and resources used in UK egg production, scaled per 20 000 eggs (about 1 tonne) (adapted from Williams *et al.*, 2006)

Impacts and resources	Non-organic (cage and free-range)	Organic	Non-organic (cage only)	Non-organic (free-range only)
Primary energy use, MJ	14 100	16 100	13 600	15 400
GWP_{100} kg 100 yr CO_2 equivalent	5530	7000	5250	6180
Eutrophication potential kg PO_4 equivalent	77	102	75	80
Acidification potential kg SO_2 equivalent	306	344	300	312
Pesticides used, dose ha	7.8	0.1	7.2	8.7
Land use, ha	0.66	1.48	0.63	0.78
Nitrogen losses (kg)				
NO_3^-–N	36	78	35	39
NH_3–N	79	88	77	81
N_2O–N	7.0	9.0	6.6	7.9

processing, packing and delivery. Tractors and other machinery require steel and other materials for their manufacture, all of which incur energy costs, in addition to the use of diesel by the finished machine. Allowances were also made for making the plant used in industrial processes (factory or power station) as well as the energy used directly.

In agriculture, including poultry industries, nitrogen losses are more important than carbon losses in global warming potential and also contribute, along with phosphorus, to eutrophication potential. In the case of poultry, nitrous oxide (N_2O) dominates and there is relatively little contribution from methane. As Williams *et al*. (2006) point out, the poultry industry should be described as having a 'carbon–nitrogen footprint' rather than a carbon one. The same is true when the boundary of the poultry industry LCA is extended back to feed crop production, since the nitrogen cycle dominates global warming potential from crops, contributing about 80% in the case of wheat. Poultry can be envisaged as 'living on' arable land, since most of their feed consists of arable crops even if the crops are grown remotely from the poultry unit. Ruminants score over poultry in being able to utilise low quality, high cellulose feeds, often grown on lower quality or even marginal land. However, this is counter-balanced environmentally by the globally significant production of methane by ruminants. Williams *et al*. calculated that the ratio of global warming potential to primary energy consumed is about 50% higher for ruminants than for poultry. Consequently, poultry meat and egg production compare more than favourably with other agricultural species in terms of most resource uses and environmental burdens (Table 19.2).

19.3.1 Environmental burdens and impacts

Emissions to the environment and consumptions of resources are termed environmental burdens. Environmental impacts are the consequences of particular burdens; e.g. nitrate leaching is a burden, of which eutrophication is an impact. Burdens can be aggregated into environmentally functional groups of which the major ones used by Williams *et al*. (2006) are listed below.

19.3.2 Global warming potential (GWP)

GWP is calculated using timescales of 20, 100 or 500 years. The main agricultural sources are nitrous oxide (N_2O) and methane (CH_4) together with carbon dioxide (CO_2) from fossil fuel. The relative GWP of different gases varies with time because the different gases have different 'lifetimes' in the atmosphere. CO_2 was used as the reference because it has an atmospheric lifetime of > 10 000 years. CH_4 has a lifetime of only 12 years because of oxidation to CO_2 and water. N_2O has a lifetime of about 120 years. For the analysis of Williams *et al*. (2006), GWP was standardised as GWP over 100 years (GWP_{100}) as a CO_2 equivalent (Table 19.4).

Table 19.4 Global warming potential (GWP) factors for major gases using IPCC (Intergovernmental Panel on Climate Change, 2001) climate change values (adapted from Williams *et al.*, 2006)

Substance	GWP 20 years kg CO_2-equiv	GWP 100 years kg CO_2-equiv	GWP 500 years kg CO_2-equiv
CO_2	1	1	1
CH_4	62	23	7
N_2O	275	296	156

19.3.3 Eutrophication potential (EP)

Eutrophication refers to the artificial nutrient enrichment of water bodies and the resulting effects on aquatic flora and fauna (e.g. algal blooms in lakes). The greatest nutrient burdens resulting from poultry production are nitrate (NO_3) and phosphate (PO_4), eventually leaching to water and ammonia (NH_3) emissions to air. Williams *et al.* (2006) quantified nitrogen outputs in terms of phosphate equivalents: 1 kg NO_3-N and 1 kg NH_3-N are equivalent to 0.44 and 0.43 kg PO_4 respectively.

19.3.4 Acidification potential (AP)

In the case of poultry, the main source is ammonia, although there will also be sulphur dioxide (SO_2) from fuel combustion. Although ammonia is itself alkaline, it is oxidised to nitric acid. Acidification potential is expressed as SO_2 equivalents: 1 kg NH_3-N is equivalent to 2.3 kg SO_2.

19.3.5 Primary energy use

Fuel costs include the energy required for extraction and supply. The primary fuels modelled by Williams *et al.* (2006) were coal, natural gas, oil and uranium. Efficiencies varied from 1.1 MJ natural gas per MJ available process energy to 3.6 MJ primary energy per MJ of electricity. A variable but presumably increasing proportion of electricity is produced by renewable sources such as wind and hydropower.

19.4 Organic and less intensive production

The nutritional, spatial and other constraints of alternative systems have consequences for sustainability. Williams *et al.* (2006) calculated that organic poultry meat and egg production increase energy use by about 30% and 15%, respectively (Table 19.3). This was because the lower energy needs for producing organic feed crops is more than counter-balanced by lower poultry performance. The global warming potential of organic poultry meat production was 45% more than for non-organic meat. Experimental approaches (e.g.

Kratz *et al*., 2004) have also confirmed that retention efficiency of nutrients was higher in intensive indoor production than in free range or organic production; nutrient surplus was highest in organic production. Among the reasons for the differences were duration of growth period, strain of birds and feeding strategy. Providing optimally balanced dietary protein is usually practicable only with supplemental amino acids and is well known to have large effects on nitrogen losses (Kim and MacLeod, 2001). There are further possible environmentally detrimental consequences of organic production, related to restrictions on the use of 'non-organic' raw materials. For example, an analysis by IFEU (2002) shows that supplementation of 1 kg synthetic DL-methionine requires less than 16% of the energy needed to provide the equivalent amount of methionine from soybean or rapeseed meal. As with the example of synthetic amino acids, LCA on egg pigments also gives results that some might regard as counter-intuitive. Carotenoid or xanthophyll pigments are widely used as feed additives in the egg production industry. These pigments can be obtained commercially either from chemical synthesis or from processed plant materials; an LCA revealed that products in the former category had lower environmental impacts (Saling *et al*., 2006).

19.5 The role of feed and nutrition

19.5.1 Nutritional methods of reducing environmental effects

Nutrition is the most immediate and readily accessible route to reducing nitrogen, phosphorus and other losses: (1) optimising nutrient balance where possible (amino acids are the prime example); (2) not including large excesses or safety margins of nutrients (e.g. metals, chlorides, phosphorus) – this is aided by reliable data on requirements; and (3) maximising availability of nutrients so that total quantities added and then excreted are minimised; dietary enzymes, especially carbohydrases and phytases have helped with this aim. There are many published examples showing the N-loss benefits of using a balanced ('ideal') protein diet, mostly in broilers, but one illustration is by Kim and MacLeod (2001; Table 19.5). This experiment showed N retention efficiency falling from 0.66 on a near-ideal protein to 0.42 on an imbalanced diet. N retention did not change significantly, because of a constant and limiting dietary lysine concentration but there was a 2.5-fold increase in N excretion. Several authors, including Summers (1993) and Meluzzi *et al*. (2001) have demonstrated comparable effects in the laying hen.

19.5.2 Genetic selection for improved nutrient utilisation

Genetic selection may be less instantaneous than nutritional methods but it has the advantages of 'permanency' and potentially a degree of independence from diet composition. The latter may be particularly valuable when there are impediments to formulating a balanced amino acid composition, such as might

Table 19.5 Nitrogen retention and loss by broiler chickens on diets with the same lysine concentration but a wide range of crude protein content (adapted from Kim and Macleod, 2001)

Diet	1	2	3	4	5	SED	*P*
True metabolisable energy MJ/kg	13.4	13.4	13.4	13.4	13.4		
Crude protein (CP) g/kg	180	210	240	270	300		
Lysine concentration g/kg	11	11	11	11	11		
Lysine : CP ratio	0.061	0.052	0.046	0.041	0.037		
N intake (g/bird day)	4.10	4.18	5.29	5.90	6.18	0.212	<0.001
N retention (g/bird day)	2.68	2.43	2.60	2.61	2.60	0.147	NS
N loss (g/bird day)	1.41	1.75	2.68	3.29	3.59	0.168	<0.001
Efficiency of N retention	0.66	0.58	0.49	0.44	0.42	0.022	<0.001

occur with organic diets or if there is an increasing tendency to use imbalanced protein co-products from biofuel production. The clearest differences in gross efficiency of egg production occur between long-established breeds of greatly differing body weight which still have similar egg productions in terms of individual egg weight and total egg mass production. Examples include the difference between Leghorn-type and Rhode Island Red-type hens. The larger-bodied hens have a greater maintenance requirement, simply to sustain their body mass but their egg production is not proportionally greater. There are many examples of how this is reflected in energy and other nutrient requirements (e.g. MacLeod and Shannon, 1978). If body weight is adjusted for, other differences accounting for variation in maintenance requirements and, therefore, efficiency of converting feed to eggs include differences in physical activity, feather cover, area of unfeathered skin (e.g. comb, wattles and legs) and basal metabolic rate (Luiting, 1990).

There is undoubtedly still scope for exploiting the heritability of certain nutritional efficiencies. Zhang *et al*. (2003) measured the heritability of phytate phosphorus availability in a randomly bred chicken population. The heritability estimate for P availability was about 0.10. Genetic correlations between P availability and body weight, body weight gain and feed consumption were moderately negative, indicating that selection for increased P availability would have a negative effect on growth. However, the genetic correlation between P availability and feed conversion was negligible, suggesting that selection for P availability would not affect feed conversion ratio (FCR). The economics of genetically selecting poultry for improved phytate P utilisation depends on the relative cost of adding dietary phytase. We should not be complacent about further improvement in nutrient utilisation by poultry as a way of reducing environmental impacts. We are in a 'win–win' position if we can reduce outputs by increasing the efficiency of nutrient utilisation.

What has past selection for productivity done to the environmental impact of poultry? Most of the research dealing with the genetics of feed efficiency in layers has approached the problem via 'residual feed consumption'

(RFC). RFC is the difference between measured feed consumption and feed consumption predicted from a model with body weight (or metabolic body size), body weight gain and egg mass production as the independent variables. This does give an estimate of overall feed conversion efficiency but only in terms of weight. A number of laboratories have used this approach (Katle, 1991; Luiting and Urff, 1991; Bordas *et al.*, 1992; Schulman *et al.*, 1994; Gabarrou *et al.*, 1998). Possibly because of the apparently greater simplicity of the biological system, without egg production as a complication, more detailed analysis has been done on growing birds. It may be useful to include some results from these to show what is potentially also possible in layer selection.

We (MacLeod *et al.*, unpublished work) recently measured production and environment-affecting changes in modern (2006) commercial broilers (Aviagen) compared with a control line randomly bred since 1972. Table 19.6 summarises some of the changes by showing 2006 measurements as a multiple of those from the '1972' birds. Food intake to 42 days has increased 2.0-fold while weight gain has increased 2.5-fold. This gives an increase in conversion efficiency (gain : feed) of 1.2-fold. This can be explained largely by dilution of maintenance; i.e., more rapid growth, even if mainly due to increased food intake, means that each animal puts a smaller proportion of its dietary energy into maintenance and also has to maintain itself for a shorter period before slaughter. Table 19.6 shows that this has occurred with relatively small changes in the underlying efficiencies of nitrogen, phosphorus and calcium digestion and retention efficiencies. In general, therefore, selection has reduced the environmental impact of growing a bird to market weight. Also, this has been done with very little change in absolute efficiencies of nitrogen and phosphorus, confirming that there may be scope for further, more detailed, selection. In layers also, maintenance costs have been reduced as a proportion of total nutrient and energy inputs. There has been selection for increased production efficiency, which will have been greatly increased by breeding 'broodiness' out of domestic hens. Genetic improvements have been bolstered by increased control of photoperiodic effects to increase

Table 19.6 Ratios of production and environment-affecting changes in modern (2006) commercial broilers to those of a control line randomly bred since 1972

	Ratio
Food intake (to 42 days)	2.0
Weight gain (to 42 days)	2.5
Food conversion efficiency	1.2
Nitrogen retention efficiency	1.1
Nitrogen digestibility	1.0
Phosphorus retention efficiency	1.1
Calcium retention efficiency	1.0

egg production control of the thermal environment to reduce the amount of dietary energy used for thermoregulation.

The improvements which a science-supported industry has produced over the years in poultry mirror the mechanisms used to explain why poultry compare well with other farm animal species in LCA. Maintenance costs have been reduced as a proportion of productive output in both meat and egg breeds. Reproductive 'fitness' is high in that birds become sexually mature in a relatively short time and each bird then produces a large number of offspring.

19.5.3 Sustainability of poultry feed supplies

Competition between poultry and humans for the same raw materials is increasing. A particularly critical case, that of phosphorus, has arisen in recent years. There has been a huge increase in the demand for rock phosphates by the expanding arable farming necessary to supply growing and more affluent populations in developing countries. There are finite supplies, unless alternative sources, such as marine deposits, are developed. A further emerging challenge for the poultry industry is competition with biofuel production, itself developed with sustainability in mind.

Baumgartner *et al*. (2007) carried out an LCA of feed production for laying hens, highlighting the importance of the choice of raw material. The LCA showed that the substitution of Brazilian soya by European peas or beans had a favourable effect on environmental impact. This resulted from the reduction in transport costs and the reduction in the use of cereals, including maize. However, the replacement of soya had potentially negative effects on local nitrogen and phosphorus pollution, since the incorporation of peas, beans or lupins often had to be accompanied by other raw materials such as sunflower and rapeseed meal. Environmental impacts depended on the proportions of the different ingredients, which vary with stage of production and with variation in ingredient prices. The environmental burdens related to oil extraction plants and the feedmill were rather small.

19.5.4 Biofuel production versus poultry feeds

Ethanol production competes for starch, while biodiesel competes for oilseed crops. Biofuel has already affected the prices of feed grains. Ethanol production in the USA increased from 0.5 Mt in 1980 to 11.9 Mt in 2005. In the EU, it is expected that biodiesel production will grow to 12.7 Mt in 2010 from 3.2 Mt in 2005 (Windhorst, 2007). The effect of these changes on feed prices is clear; however, there is inevitably an increase in co-product/by-product production. Maize gluten meal and dried distillers grains are currently the main co-products from ethanol production. Rapeseed, sunflower, soya and palm expeller meals are the commonest co-products from biodiesel production. The increasing availability of such materials may compensate

to some extent for the increasing prices of the entire grains but there may be challenges of product quality and feed formulation.

19.6 Indirect ways of improving productivity : maintenance ratio

Poultry, including laying hens, are inherently efficient compared with other livestock because of their high fecundity and rapid rate of maturation. In the case of laying hens, fecundity must be taken to include the production of eggs even if these eggs are infertile by design. One of the limiting factors in the productive life of a laying flock is declining eggshell strength, which leads to an increasing number of breakages. If genetic selection were used further to increase eggshell quality (Dunn *et al.*, 2005), the length of the laying cycle could be increased. This would mean that fewer hens would have to be hatched and raised to maturity to produce the same number of marketable eggs.

19.6.1 Culling of male layer-strain chicks

About half the chicks produced by layer-strain breeder hens are males, which are unwanted because they do not lay eggs and also grow less efficiently than birds genetically selected for meat production. Most of these unwanted males are culled as soon after hatching and sex identification as possible. This is seen as an ethical and animal welfare problem but can also be identified as a sustainability issue. There are the obvious environmental burdens of carcass disposal and the costs of synthesising the materials of the chicks but there is also the wasteage in terms of the costs of maintaining a population of layer breeder hens, 50% of whose production is immediately destroyed. This can be put in the context of the 'ecological discussion above (point 7, page 447).

One way of avoiding the killing of hatchlings would be to identify the males within the egg and destroy them at some point before hatch. Some methods proposed for this are reviewed by Nandi and Clinton (2009). Such a strategy would avoid some of the ethical concerns but might have little effect on environmental costs of disposal, depending on how early the sex identification was carried out. The effect on raw material costs would be minimal, since all the chemicals necessary for synthesising the chick are already present in the egg when it is laid. One answer would be to find a method of converting male embryos to females. However, a recent landmark paper indicated that the sex identity of birds may be inherently more resistant to change than that of mammals (Zhao *et al.*, 2010). In mammals, the somatic cells of males and females are initially sexually undifferentiated and sex differences are imposed under hormonal control by the type of gonad that

develops. In the chicken, however, an ingenious series of observations and experiments involving gynandromorphs (found to be male–female chimaeras) and embryonic transplantation studies has shown that gonadal development and other aspects of the sexual phenotype are autonomous to the individual cell and not governed by sex hormones.

19.7 Other sustainability issues

19.7.1 Egg washing and water use

With water shortage becoming an ever-increasing problem, the use of water in egg washing or processing must be of clear benefit before it can be justified. In the EU, egg washing is currently permitted only for 'class B' eggs, which are intended for further industrial processing rather than direct consumer use. Hutchison *et al*. (2004) remarked that any benefits of washing eggs are offset by the worry that wetted eggs are prone to spoilage and water loss. However, washing contaminated eggs under optimum conditions resulted in a >10 0000-fold reduction in *Salmonella* counts from the shell surface and *Salmonella* was not isolated from the yolk or albumen of eggs washed by an appropriate method. However, contamination with *Salmonella* Enteritidis and *S*. Typhimurium was found within the egg contents when water temperature was reduced. Work by the Food Safety Agency in the UK indicated that the most important factor in reducing *Salmonella* levels on the shell surface was maintenance of a temperature >40 °C in the wash and rinse water. However, the energy cost of raising wash water to such a temperature acts against the ecological sustainability of the practice. Industry opinion tends to be against egg washing, at least partly because of the cost. The 'water footprint' of the process and the environmental burden of heating wash water to an effective temperature suggests that it is also inimical to sustainability. There certainly has to be a clear hygiene benefit to justify the practice.

19.7.2 Spent hens

When hens reach the end of their economically viable life, they are usually transported to a processing plant for conversion into meat products. As with all steps in the food production and consumption process, human consumers are only temporary repositories of energy, protein or other nutrients as they pass through the system. The material of the spent hens will end up as waste whether we eat them or not! However, it behoves us to make use of these birds – if we don't consume them, we will have to consume other foods which will need even more energy, nitrogen, carbon, etc. to produce, with all the concomitant losses and inefficiencies. There seems to be a declining demand for the meat from spent hens, so alternative methods may have to be found for disposing of carcasses. One proposal is to dispose of the hens

by an aerobic digestion process, which could potentially produce energy and agricultural plant fertilisers.

19.7.3 Eat local

It can be argued that it is not ecologically sustainable to move production overseas from (say) Europe to avoid possible carbon or nitrogen charges or even to avoid the costs of improved animal welfare. On the other hand, some types of egg production might have such low input demands (e.g. of energy) in other geographical conditions that it may be environmentally sustainable to produce abroad and incur the environmental costs of transportation. If production is local, we also have to consider the costs of importing feedstuffs which can not be produced locally. Locally produced food can sometimes consume more energy and have greater global warming potential than food imported even from considerable distances. Transporting eggs may be quite efficient per unit weight, and the environmental costs could conceivably be small compared with producing them in a less favourable environment and having to importing feedstuffs (soya, maize) which are then converted to eggs with varying efficiency. Further objective analysis (e.g. LCA) is required in this area.

19.7.4 Manure treatment

Manure treatment might sometimes rather disparagingly be called an 'end-of-pipe solution', which in some industries belongs to an earlier era of pollution control (Lee and Rhee, 2005). Ideally, we should be aiming to reduce the production of wastes at source but it is impossible to reduce nutrient losses to zero in a biological system such as egg production. Manure treatment then becomes an essential way of turning wastes into a useful resource or, at least, minimising any negative effects. The fact that poultry farms produce the nitrogen waste products in a relatively small land area can be seen as a problem in terms of public impact, but can also be seen as an opportunity for collection and recycling of nitrogen to minimise losses as ammonia and nitrogen oxides. Although it is most important to reduce nitrogen and other losses at source, research is also needed to improve or develop methods of manure management and treatment to reduce ammonia emissions (Ritz *et al.*, 2004). UK recommendations are that poultry manure applications should supply no more than 250 kg total N/ha per annum, meaning that 2–3 hectares are required per 1000 birds. The nitrogen should also be supplied at a time when crop productivity is nitrogen-limited (Chambers and Smith, 1998). Various substances have been tested for their efficacy in preventing gaseous nitrogen losses from manure during storage (Pratt *et al.*, 2006). Most manure treatments involve either substances which chemically combine with potential 'pollutants' such as nitrogen or phosphorus or substances which act as adsorbents or chelating agents. Such treatments allow the control of

environmental effects in the immediate vicinity of poultry farms or manure storage facilities but do not make the environmental burden disappear permanently. However, they do facilitate the disposal and use of the manure through such routes as agricultural fertilisers.

Burning poultry litter/manure in electricity-generating stations was once seen as environmentally friendly but would now be seen as a very direct way of re-releasing nitrogen and carbon into the atmosphere. Li *et al*. (2008) estimated that ammonia emissions from manure-belt layer houses are less than 10% of the emissions from high-rise houses, where manure may be stored in-house for a complete laying cycle. However, on-farm manure storage for manure-belt houses also emits NH_3, which is a part of the total farm emissions. Nevertheless, treating manure in storage sheds to decrease NH_3 emissions may be preferable to treatment inside the layer houses because of bird health concerns and possible detrimental effects of the treatment on the housing equipment. Li *et al*. (2008) showed that agents such as zeolite, aluminum sulphate, ferric sulphate and sodium bisulphate reduced NH_3 emission by 30–95%.

19.8 Conclusions

If we want to continue eating birds' eggs, there is no feasible alternative to poultry. Fortunately, for animal protein production (eggs or meat), poultry have lower environmental impacts than most agricultural species. If low maintenance and replacement costs are the key, the main competition for poultry would be 'cold-blooded' animals, farmed fish currently being the practical example. In fairness to other species, apart from meeting a market demand, ruminants are important in utilising feeds which are of little direct nutritional value to humans and in using marginal land. LCA suggests that organic and more extensive poultry farming are less sustainable in terms of global warming and eutrophication but these systems may be seen to have other advantages which are not currently quantifiable by LCA. The biological strategy which should benefit all husbandry systems is to continue breeding for efficient capture and utilisation of nutrients. However, to minimise environmental burdens, the benefits of genetic improvement must be supported by precise assessment of nutrient requirements and of the yields of nutrients from feed ingredients. Finally, engineering and logistic solutions must be used to minimise the environmental effects of those waste products that evade biological methods of reduction.

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20

Organic and free-range egg production

M. Hammershøj, Aarhus University, Denmark

Abstract: This chapter includes information on the development of free-range and organic egg production and their market shares in different countries. Consumer behaviour is investigated, particularly in relation to the price and availability of non-cage eggs. Regulations on the production of free-range and organic eggs and their present and future impact are examined. Nutrient supply, animal welfare, productivity, safety and environmental impact of the types of egg production are covered with a focus on the effects of using different forage materials. Egg quality parameters of eggshell, sensory properties, yolk colour and carotenoid content, egg protein and egg products are also discussed.

Key words: organic, free range, egg production, forage material, egg quality.

20.1 Introduction

Current commercial hen egg production can be divided into four different husbandry systems: cage eggs, barn eggs, free-range eggs and organic eggs. Over the years increasing attention has been paid to the differences in animal health, welfare, environmental impact, production economy, food safety and food quality within and between these production systems, both on the scientific and the applied level. Moreover, increasing consumer attention and awareness of these issues, together with cultural and political points of view, has affected the development of egg production and thereby the relative market shares of these production systems in individual regions and countries.

During the twentieth century, egg production became more intensive and efficient when hens were moved into battery cages. Battery hens were – and still are – kept four or six hens to a cage throughout their egg-producing life. This resulted in a production economy that made cage eggs cheap for the consumer and the economy and management attractive for the farmer. However, during the last 20–30 years there has been a resurgence of interest in free-range egg production, mainly in developed countries, due to animal welfare concerns (Magdelaine *et al*., 2010; Miao *et al*., 2005). The production of free-range eggs and organic eggs has therefore experienced an increase in both production and market shares. In the European Union (EU), the European Commission has put a ban on the conventional cages for laying hens (Council Directive 1999/74/EC, 1999), which will come into practice by 1 January 2012. Alternatives such as enriched cages, aviaries and free-range production systems are likely to compete on market shares together with an unknown amount of eggs which will be imported into the EU member countries from less economically developed countries (Windhorst, 2009).

20.2 Market development of organic and free-range eggs

20.2.1 Worldwide

Global egg production during the period between 1990 and 2007 experienced a remarkable increase from 35.2 million tonnes to 62.6 million tonnes, i.e. a 78% expansion (Windhorst, 2009). This growth is, however, very different worldwide. Asia, especially China and India, now dominates global egg production while Europe and North America have both lost importance as egg production regions.

Consumer interest in purchasing free-range and organic eggs has increased, which consequently has led to increased production and market shares during the last 20–30 years. However, the egg market is very different in different regions. These differences are the result of both consumer behaviour and production practices. No worldwide universal standards exist for organic food production; so many countries have established their own national standards. These include organic egg production (Blair, 2008), which will be discussed in detail in Section 20.3.1.

According to the International Egg Commission (IEC), in 2008 free-range hen flocks and those in organic housing systems made up 0.4% of the total in American countries, 23.8% of the total in Asian countries, Australia and New Zealand, and 14.6% of the total hen flocks in European countries (www.ThePoultrySite.com). In Asia especially, the higher fraction of free-range and organic egg production is dominated by small householder flocks. National differences in free range and organic egg production range from as low as 0% in countries such as Brazil, India and Mexico up to 65% in Switzerland, where conventional caged egg production has been banned since 1992, and 37.9% in the UK (www.ThePoultrySite.com).

20.2.2 The EU countries

The European egg market has slowed in growth during the last two decades and, as is often the case for mature markets, it has begun to segment into two main production areas; more processed products to meet convenience demands and more high quality 'niche' products to meet consumer demands and animal welfare and environmental protection expectations (Magdelaine, 2007; Windhorst, 2009).

Within the EU, the relative proportion of organic egg production in 2007 was highest in Denmark with 14% of total egg production and 8% of total shell egg consumption (Magdelaine *et al.*, 2010; Windhorst, 2005), followed by Austria with 8% of the total egg production and 2.2% of shell egg consumption. However, the largest producers of organic eggs by volume are Germany and France (Magdelaine *et al.*, 2010). The overall percentage of non-cage egg production systems in the EU increased from 8% in 1996 to 21% in 2005, with the Netherlands dominating with 52% and the UK followed with 32% of production being non-cage eggs (Magdelaine, 2007).

According to a calculation of the mean costs within the EU, the production costs of free-range eggs are 48% higher than those of conventional cage eggs (European Commission, 2004). These costs are partly covered by the farmer and partly by charging a higher price for free-range and organic eggs. The retail market shares for non-cage eggs are somewhat different from their production shares; in countries such as the Netherlands, Sweden, Denmark and the UK nearly 50% of eggs at the retail level are from non-cage systems and in Austria and Germany these account for ~25% of the retail market (European Commission, 2004). However, the policy of individual supermarkets to whether cage eggs should be an item available in their shops influences the relative share of free-range and organic eggs purchased. For example, the UK supermarkets Marks & Spencer and Waitrose have only non-cage shell eggs for sale in their retail stores, as does the Danish retail chain Irma.

The Danish shell egg market is dominated by two major egg packaging companies which have contracts with egg farmers to supply to their chains. The main distributors are the supermarkets and local grocery stores. However, a considerable amount of shell eggs, estimates suggest ~10–20% of all eggs, are sold at the farm directly to the consumers or from individual's backyard egg production. This segment of egg production is dominated by free-range and organic eggs which contrasts with the official production statistics.

20.2.3 Consumer behaviour and willingness to pay

The higher costs and subsequently higher price of free-range and organic eggs could lead to the assumption that the cage ban within the EU (Council Directive 1999/74/EC, 1999) will lead to consumers turning to imported eggs from battery cages outside the EU.

The expressed attitude and actual choices of consumers towards purchase

of eggs often turn out to be contradictory (Parrott, 2004). When consumers were asked what they considered the most important factor when making their decision to buy eggs, results showed that they ranked 'type of egg production system' highest, followed by 'date', 'assurance of freshness' and finally 'price' (Parrott, 2004). But only 24.3% of consumers actually checked how the eggs were produced. This highlights that the consumer knowledge of the production methods is limited and that their views are not necessarily reflected in their behaviour in a purchasing situation.

Within the EU, a survey from 2005 stated that ~57% of the consumers are willing to pay more for non-cage eggs, distributed by 25% accepting a 5% price increase, 21% accepting a 10% price increase, and 11% are willing to accept a price increase of 25% or more (European Commission, 2005a). Of the respondents, who were willing to pay more for non-cage eggs, 58% gave as their main reason for this that 'the welfare of laying hens is very bad or fairly bad' (European Commission, 2005a).

The consumer's willingness to pay for a specific husbandry category of eggs also depends very much on the local region in which they live. A study in Denmark showed that consumers in an urban area (Copenhagen) were more willing to pay for free-range and organic eggs; in fact their results were 25–30% above the average for the whole of Denmark. In rural areas, however, consumer willingness was ~50% below the Danish average, and vice versa for conventional cage eggs (Baltzer, 2004). This could be the result of difference in mentality, with urban consumers in general more oriented towards the organic ideology. The results, which are based on supermarket sales figures, can hide an actually higher consumption of free-range and organic eggs in rural areas since more people have the opportunity either to keep laying hens in their own backyard or to buy eggs from local farmers. These private egg sales and consumption would therefore not appear in the sales statistics. Recent research into the differences in consumer decision-making and behaviour with regard to organic food showed that there are differences in behavioural intentions between the North and the South of Europe (Thoegersen, 2009). The most important factors behind these differences seem to be policy implications, e.g. political support, environmental concerns and food culture. In southern Europe, the marketing channels of the organic sector are dominated by direct marketing via specialized shops, whereas in the north of Europe most of the sales are concentrated in supermarkets (>60%) and in non-specialist shops (European Commission, 2005b). This naturally increases the accessibility of organic foods for consumers in the northern countries.

The study by Baltzer (2004) also found that the effect on the Danish market of advertising and offering organic eggs at a bargain price was much lower than for cage eggs, barn eggs and free-range eggs, indicating that the market for organic eggs is less price-driven than the other egg types.

The willingness to pay may also be illustrated by the UK egg market in 2009, during the time period of the recession. The total egg sales dropped

marginally by 0.2%, but the free-range egg share of egg packing station outputs continued to increase to 37.6% compared with the same period in 2008 when it was 31.7% (www.ThePoultrySite.com, 'UK egg statistics'), including sales (Booth, 2009). During this same period, the share of conventional cage eggs declined from 58.6% in 2008 to 54.1% in 2009, and so did the market share of organic eggs, which dropped from 6% in 2008 to 4.1% in 2009. The author suggests that this illustrates the price limit that the majority of consumers are willing to pay for eggs, which is substantiated when the economic situation is bad, is near the price of free-range eggs, since their market share went up, but not at the price of organic eggs, since their market share went down.

It should, however, be noted that several UK retail chains have stopped stocking caged eggs, which may have guided consumers in the direction of buying non-cage eggs as it became increasingly difficult to find battery cage eggs (Booth, 2009). This is also true at the other end of the scale: perceived barriers to purchasing organic food, such as it being less available than conventional food, can simply make some consumers give up, even in the most mature organic markets (Thoegersen, 2009). The conclusion is that in order to increase the market share of free range and organic eggs the importance of an effective distribution system is emphasized, and that a good supply of these egg types may be able to create their own demand.

20.3 Regulations on organic and free-range egg production

20.3.1 The standards and principles of organic agriculture

As mentioned in section 20.2.1, there is no worldwide standard for organic food production, but most nationally developed standards evolve from those developed in Europe by the International Federation of Organic Agriculture Movements (IFOAM) and from the guidelines for organically produced food from 1963 developed by FAO and WHO within the Codex Alimentarius framework (Blair, 2008). The IFOAM and Codex basic standards are under review, but are intended as guidelines for states and governments to develop their own regulations. However, these standards do not certify products themselves directly. In the US in 2002, the National Organic Standard Board set guidelines that must be met by producers marketing organic eggs (United States Department of Agriculture,www.ams.usda.gov/nop). In the EU, four main principles of *health*, *ecology*, *fairness* and *care* are aimed for in organic farming (Council of the European Union, 2007). Further values of importance in organic farming, which are not covered by this regulation, are environmental protection and biodiversity conservation, animal welfare and health, and social aspects (Jespersen, 2008).

In general, the most important regulations for egg production as stated by the IFOAM and Codex Alimentarius include the following (Blair, 2008):

- The choice of hen breeds should favour stocks well adapted to the local conditions and the intended production system.
- Roughage, fresh or dried fodder or silage must be supplied in the hens' daily ration.
- Poultry should be reared in open-range conditions with free access to open-air run, when weather conditions permit this, and keeping in cages is not permitted.
- When the natural day length is prolonged by artificial light, the maximum hours of light shall be prescribed by the competent authority with respect to the species, their health and the geographical considerations.
- Buildings should be emptied between each poultry batch and outdoor runs left empty so vegetation can grow back. This may in practice mean one hen-free year for the outdoor run.
- The feedstuffs are those necessary or essential to maintain animal health, welfare and vitality and should be permitted according to national legislation on animal feeding, furthermore, feed must not contain genetically engineered or modified organisms and products hereof.
- Non-organic sources of feedstuffs can be used under specific conditions and only without the use of chemical solvents or treatments, and synthetic nitrogen compounds, i.e. amino acids, shall not be used.

Organic egg production within the EU is regulated to pursue the general objectives of (a) establishing a sustainable management system for agriculture, (b) aiming at producing products of high quality and (c) aiming at producing a wide variety of foods and other agricultural products that respond to consumer demand for goods produced using processes that do not harm the environment, human health or animal health and welfare (Council of the European Union, 2007).

In the EU, organic egg production differs from free-range egg production by specific regulations and principles. For instance the feed ingredients have to be produced organically. However, this is being achieved by steadily decreasing the non-organic proportion of ingredients in the feed as follows: ≤ 15% up to 2008, ≤ 10% to 2010, and thereafter ≤ 5% until 1 January 2012. By this final 2012 date, 100% of feed ingredients will have to be produced following organic agricultural principles (Jespersen, 2008). Other general specifications for organic egg production are that chemically produced or genetically modified (GM) products and synthesized amino acids and vitamins are not allowed to be included in the birds' diet. No preventive medical treatments are permitted with, e.g., antibiotics, and medical treatments are only approved after a veterinarian has made a diagnosis. Finally, beak trimming of the birds is prohibited.

20.3.2 Housing, outdoor area, daylight requirements, flock size, etc.

As discussed previously different regions and countries have different regulations of the husbandry systems. The Danish regulations on organic

egg production (Ministry of Food Agriculture and Fisheries, 2007) follow the EU regulations (Council of the European Union, 2007), but are for some areas even more restricted on different criteria. In the following, the Danish rules are given as an example of how free-range and organic egg production can be regulated.

In free-range egg production, the standard given by the EU for stocking density inside the henhouse is that it must not exceed nine hens per m^2 usable area (Council Directive 1999/74/EC, 1999). However, the EU has allowed a derogation to give existing producers time to switch over to the updated regulations. They were allowed to continue the 11.7 hens/m^2 stocking, but this derogation comes to an end in December 2011 within the EU. There are no requirements for daylight inside the house, but nests and perches are required, and a minimum of one-third of the floor area should be covered with straw, wood shavings, sand or peat. Beak trimming of chicks is permissible if it is carried out before they are 10 days old. There must be access to an outdoor area with a minimum of 4 m^2 per hen, and the ground should be covered with vegetation. The flock size varies from 3000 to 10 000 hens.

In many ways, organic egg production resembles free-range egg production but the major differences are the inclusion of organic feed ingredients, requirement of forage material, indoor stocking density of six hens per m^2, and that beak trimming is not permitted. Furthermore, the flock size must not exceed 3000 hens, but there may be more than one flock at the same farm. There should be more than one hen-yard, so that the outdoor area is hen-free for a year at a time. This last is for environmental reasons in order to minimize the amount of nitrogen, phosphorus and other nutrients emitted from the excreta dropped in the hen-yard.

20.3.3 Feed and forage material

The diets of free-range and organic hens are formulated to fulfil the same nutrient requirements as for all egg laying hens. The purpose of the diet is to provide an optimal supply of nutrients to the hen for body maintenance, physical functions and the components required for daily synthesis of shell, egg albumen and yolk follicle maturation and ovulation.

Usually, a standard pelleted or meal feed is offered in both systems, and in organic egg production either a supply of forage material or access to pasture is required; see Section 20.3.1 (Blair, 2008). A key aim of organic husbandry is to produce in a sustainable way and to have a high proportion of self-supply of feed ingredients, which in most cases will include the cultivation of grain crops such as wheat, corn, peas, soybeans and lupin. Often, the grains are supplied together or mixed on the farm with a feed concentrate/feed premix that is composed to give a total fulfilment of the nutrient requirements of the laying hens. Such a concentrate usually contains protein feedstuffs, minerals and vitamins (Blair, 2008). From the year 2012, the EU regulation states that all feed for organic livestock must be

composed of 100% organic ingredients (Jespersen, 2008), hence the addition of synthetically produced amino acids will not be allowed in the birds' diets. This raises a challenge for farmers in supplying the laying hens with essential amino acids, especially methionine. The supply of sufficient levels of these essential amino acids could be achieved by including an excessive level of protein in the diet, which will increase the cost of the diet and result in an inefficient use of scarce protein supplies and contribute to increased nitrogen (N) output in excreta, i.e. a potential environmental hazard (Blair, 2008). Alternatively, the supply of essential amino acids can be achieved by exploiting 'new' protein crops that can be cultivated organically. These could include crops such as lupin, quinoa or naked oat, which have been studied for usage as feed ingredients in organic egg production (Hammershøj and Steenfeldt, 2005; Steenfeldt and Hammershøj, 2009).

Organic egg farmers will need to consider whether it is possible to cultivate sufficient amounts of organic high quality protein sources to supply the organic production. The risk of health and welfare problems, e.g. feather pecking incidences, may increase, when hens lack essential nutrients (Ambrosen and Petersen, 1997). A specific feature of organic egg production is the allowance to use eggs and egg products in the feed. If it primarily, i.e. >50%, originates from the same region, in accordance with the new rule (Council of the European Union, 2007), then this could also be regarded as an amino acid source, although costs may be high.

20.3.4 Animal welfare

At present there are no European rules for the rearing of pullets used for organic egg production. At a maximum age of 18 weeks, conventionally reared pullets can be brought in as livestock for organic egg production (Magdelaine *et al.*, 2010). A few countries have defined specific rules for 100% organic pullets, i.e. Belgium, Denmark and the Netherlands, and in Austria and Germany farmers who follow private standards are obligated to use organic pullets (Magdelaine *et al.*, 2010). The rearing conditions may have welfare impacts on the pullets as the maximum stocking density and access to free range are defined differently by the standards of the above-mentioned countries: for example, the problems of hygiene and the increased mortality of young pullets who have outdoor access (Magdelaine *et al.*, 2010). Acclimatizing pullets to an outdoor range during rearing increases their use of the outdoor area as adults (Van de Weerd *et al.*, 2009).

In modern egg production, the hen breeds used in free-range and organic egg production are the same breeds that for generations have been used in conventional cage egg production. One of the major problems that has arisen from this is the feather pecking behaviour which conventional breeding appears to have exacerbated. Studies have found that the various commercial and experimental egg laying breeds differ in their feather pecking behaviour (Kjaer and Sørensen, 2002). When the hens were caged, this behaviour

could be controlled, but with the introduction of free-range systems, feather pecking became unrestricted, meaning that in severe cases all the birds had featherless areas (Kjaer and Sørensen, 2002). In severe cases of pecking behaviour, the risk of cannibalism among the hens increases, although feather pecking is not always a precursor of cannibalism (Kjaer and Sørensen, 2002; Wechsler and Huber-Eicher, 1998). For example, mortality in organic egg production was 14%, in free range 8% and in battery cages 4% in 1997 (Danish Poultry Council, 1998). This changed during the following 10 year period to 9% in organic egg production, 7% in free range and 6% in battery cages (Danish Poultry Council, 2008). A substantial portion of hen mortality in the free-range and organic systems is suggested to be due to incidences of cannibalism. Although organic and free-range egg productions are similar, there are three main differences which can be assumed to cause the higher mortality in organic egg production: (1) beak trimming of hens is prohibited, (2) the use of preventive synthetic medication for the treatment of diseases is not allowed and (3) diet formulations have fewer permissible ingredients to choose from, making it more difficult to compose a balanced diet.

Access to forage material and an outdoor range area also affects the behaviour and energy utilization of hens. In a study which compared hens in an aviary system with access to an outdoor pasture to hens with only indoor access, the mean proportion of outdoor-treatment hens actually located outdoors was 62% (Shimmura *et al.*, 2008), and the mean proportion of outdoor-treatment hens foraging was 50% (37% in the outdoor area plus 13% in the indoor), in comparison with only 21% of the indoor-treatment hens. Furthermore, during the time period of outdoor access the outdoor-hens showed significantly less feather pecking behaviour than the indoor-hens (Shimmura *et al.*, 2008). Several studies have shown that increased use of the outdoor range inversely decreases the prevalence of feather pecking (Van de Weerd *et al.*, 2009).

20.4 Productivity of organic and free-range hens

20.4.1 Laying rate, egg size and egg mass output

One challenge to free-range and organic egg production has been the genotypes available for egg production. Because conventional cage egg production was the major production system for many generations of egg layers, the selection criterion of breeding was, for decades, to obtain modern lines of poultry suited to fit the cage system, i.e. low body weights in order to use less energy for growth and maintenance and very high rates of egg production e.g. the white leghorn hens (Anderson, 2009). The usage of these modern egg layers in free-range and organic production has led to different challenges e.g. behavioral problems with feather pecking, cannibalism and health issues (Horsted and Hermansen, 2007; Van de Weerd *et al.*, 2009). In order to prevent a high incidence of behavioral problems, some heavier

genotypes like New Hampshire hens have proven their suitability in the free-range and organic egg production, due to their lower tendency to feather pecking and cannibalism. The disadvantage of these genotypes is that egg production – measured as rate of lay, egg size, egg mass output and feed conversion – is also lower than for the highly selected previous egg layer types. Selection experiments on feather pecking have proven a positive correlation between feather pecking behaviour and egg production, which indicates that continuous selection for high productivity will inevitably also mean the continuous selection of hens with a higher potential to feather peck if they are in an environment which stimulates their natural pecking behaviour (Kjaer *et al.*, 2001; Kjaer and Sørensen, 2002). For example, in Denmark the egg production per hen, initially put into production, is ~22% lower and the feed consumption ~25% higher for organic egg production than it is for cage egg production (Hermansen *et al.*, 2008). In Table 20.1, an overview of different egg production systems and the means of various performance parameters are given. However, owing to the higher retail price of organic eggs (approximately three times higher than cage eggs), the contribution margin is higher in organic production than in cage egg production, with the 2005 levels being 14.8 DKK/kg egg in organic production compared to 5.2 DKK/kg egg in cage egg production (Danish Poultry Council, 2006). The price of organic feed is often ~50% higher than for conventional feed. Within the EU, prices for organic eggs in 2001–2002 were at farm gate level on average 0.14€/egg and prices ranged from 0.30€/egg in Greece to 0.11€/egg in the Netherlands (European Commission, 2005b). At retail level a similar pattern was seen with 0.79€/egg in Greece and 0.25€/egg in the Netherlands. The prices of organic eggs were high in Greece, Spain, Portugal and Italy, being 121–231% above the price of conventional eggs (includes cage, barn and free-range), but only 17–25% higher in Denmark and Austria (European Commission, 2005b).

Free-range egg production figures for the productivity parameters are at comparable levels to the organic egg production, but owing to a lower retail price of free-range eggs the resulting contribution margin is ~50% of that for the organic egg production (Table 20.1).

The performance of hens under different housing systems shown in Table

Table 20.1 Mean egg production for different husbandry systems in Denmark 2005, values are given per hen

	Production length (days)	Eggs		Laying rate (%)	Mortality (%)	FCR (kg/kg egg)	Contribution margin (DKK)
		No.	kg				
Cage	392	341	21.4	89	4.5	2.04	18.8
Barn	364	289	18.3	83	11.0	2.47	46.1
Free range	336	262	16.4	81	8.2	2.57	56.1
Organic	336	265	16.5	82	9.4	2.61	106.9

After Hermansen *et al.* (2008) and Danish Poultry Council (2006).

20.1 is confirmed in other studies, where hens in free-range and organic systems in general have lower egg production, poorer feed conversion and higher mortality than battery cage and barn hens (Miao *et al.*, 2005; Wathes, 1981).

20.4.2 Foraging, feed consumption and energy requirement

As the free-range and organic hens have access to outdoor areas, more energy is required for body temperature maintenance and for the physical activities of running, exploring, and foraging in the outdoor range area. Energy intake therefore also needs to be higher; this can be achieved with either a higher feed intake or by providing a more energy-dense feed formulation. Either way, the FCR (kg feed/kg egg) consequently becomes higher than in the other production systems. As stated in the Organic Revision (Sundrum *et al.*, 2005), the protein sources for organic layers have to be of high quality in regard to methionine and cystine, which is important for egg performance. Methionine proportion should not be less than half of the sulphurous amino acids, and performance can be increased with a high methionine and low energy feed composition. The issue of how to produce high-value protein sources under the organic conditions requires further research (Sundrum *et al.*, 2005).

As previously mentioned, organic egg production requires outdoor pasture with either good vegetation or supplement forage materials, e.g. fresh vegetables, silages, grass and hay. The consumption of forage material by the laying hen can be considerable, up to ~120 g/hen/day (Hammershøj and Steenfeldt, 2005; Steenfeldt *et al.*, 2007), or ~70 g dry matter (DM)/hen/day when foraging at a herbage pasture containing chicory (Horsted *et al.*, 2006). Forage material intake may therefore account for more than 50% of the hen's daily diet. This figure will naturally depend on the type of forage material and especially the content of dietary fibres, which for example for alfalfa silage may be >50% (own data, unpublished), thereby limiting the amount of forage material it is possible for the hen to intake.

The requirement of forage material or green pasture in organic egg production can have an impact quantitatively on the feed intake and thereby affect the nutrient supply of the hen, which depends highly on the type of forage material. The consumption of forage material can reduce the feed consumption by up to 20% (Blair, 2008). In a study on a daily forage supplement of carrots of 70 g/hen, the feed intake decreased by 8–11 g, but the total dry matter intake was unaffected by treatment with or without forage material (Hammershøj *et al.*, 2010). In another study, the feed intake was not affected by high amounts of maize silage and carrots given *ad libitum*, resulting in a daily intake of 123 g/hen (Hammershøj and Steenfeldt, 2005), which contrasts with another study in which forage material of 108 g/hen/day resulted in a feed consumption decrease of 16 g/hen/day (Steenfeldt *et al.*, 2007). The access to forage material, regardless of whether it is supplied by the farmer or is

foraged by the hen itself in the vegetation of the hen-yard, definitely plays, at the relative high amounts reported above, a role in the nutrient supply for the hen. So far, little attention has been paid to the nutrient content of forage material with regard to the formulation of the basal feed. Further studies are needed in order to obtain information on the nutritive value of different forage materials on the basis of their composition, fibre content and especially in relation to their effects on egg production, egg quality and animal welfare. In relation to feather pecking, a recent study showed that increased feed content of non-starch-polysaccharides, e.g. fibre, during the rearing of pullets resulted in less feather damage at 49 weeks of age. It was suggested that this was due to the pullets being increasingly imprinted on feed and not feathers as pecking substrate (van Krimpen *et al.*, 2009).

20.4.3 Impact on the environment

One of the fundamental pillars on which organic production is built is sustainability by respect for the environment. This is also the case in free-range egg production, but to a lesser extent, as there are no restrictions on how to cultivate the feed ingredients. An important issue in both free-range and organic egg production systems is the environmental impact of the excretion of nitrogen (N) and phosphorus (P) to the outdoor range (which varies depending on how much is deposited on the range and how much is deposited indoors) and the emission of greenhouse gases. The emission of N to the air occurs in the form of ammonia converted microbially from N in the excreta of hens, and excess levels of nitrogen, protein and amino acids in poultry diets contribute to ammonia emission. Under organic conditions, the fractions of N and P originating from the feed that is transferred to the eggs produced are approximately 25–30% and 12–15%, respectively (Hegelund *et al.*, 2005).

Reducing the dietary crude protein (CP) level can potentially reduce N excretion by up to 40%, and reducing CP in the diet by 1.5% concurrently reduces ammonia emissions by 11% (Veens *et al.*, 2009). This has to be done carefully, however, in order to fulfil the amino acid requirement for egg production, which again puts high demands on the protein sources, especially for organic cultivation to be used in the organic egg layer diets. A CP reduction of 1%-unit in the diet significantly reduces N excretion, but also reduces the laying rate and mass of the eggs produced, although egg weight is unaffected (Roberts *et al.*, 2007). If CP becomes as low as 13.0% the egg size is reduced (Veens *et al.*, 2009).

The global warming potential (GWP) is an indicator of the impact on greenhouse gas emission expressed in CO_2-equivalents per kg of product, e.g. eggs (De Vries and de Boer, 2010). Two studies from the UK and the Netherlands showed that egg production with outdoor access required more energy 13.9–15.4 MJ/kg egg than cage egg production of 13.0–13.6 MJ/kg (De Vries and de Boer, 2010; Mollenhorst *et al.*, 2006), and that the GWP

was 4.6–4.9 kg CO_2-equivalents/kg egg and 3.9–4.2 kg CO_2-equivalents/kg egg, respectively. These differences between egg productions originate in the differing energy requirements of the two groups of hens. The hens with outdoor access need much more energy than the caged hens because of their increased physical activity and temperature maintenance. Free-range and organic egg production are thus cast in an unfavourable light, by being less environmental friendly than conventional cage production. Research is therefore required in order to bring the GWP of these production systems down.

20.5 Quality of organic and free-range eggs

20.5.1 Shell and bone quality

The macrominerals calcium (Ca) and phosphorus (P) are essential for laying hens to form and maintain the skeleton and form the eggshell. Deficiency of either one of these in poultry diets will limit the utilization of the other, because they are closely related. In laying hens, a ratio as high as 12–13:1 by weight of Ca:P is needed. The sources of Ca in both conventional and organic production are ground limestone, oyster shell and dicalcium phosphate (Blair, 2008).

Evidence shows that the shells of eggs in free-range and organic birds are more likely to be dirty than those of eggs in barn and cage systems (Ferrante *et al.*, 2009), due to a higher incidence of eggs being laid on the soil in the free-range area. The impact of this on shell contamination with microorganisms is described in Section 20.5.5.

Furthermore, there are higher frequencies of cracked eggs in barn egg production than in organic, which may be due to either a higher stocking density in the barn than in the organic henhouse or an increased shell thickness in the organic egg (Ferrante *et al.*, 2009). Hens that are kept in production systems with a free-range environment have more physical activity (Miao *et al.*, 2005), which gives them greater bone strength and may also result in better calcium resorption from the bones to the shell synthesis. Analysis of wing bones (n = 200) originating from organic egg layers showed a mean breaking strength of 270 ± 45 N at hen age up to 48 weeks (own data, unpublished), whereas the wing bone breaking strength was significantly lower in cage layers at age 50–70 weeks of 124 ± 12 N (Danish Poultry Council, 2008). Note that, there is a small difference in hen age between the two studies. Although bone strength is improved in non-cage systems and hence the risk of broken bone incidences is reduced as result of handling and transport, there are still incidences of bone breakage as other factors play an important role. The perches in free-range and organic egg production are a risk, as birds may injure themselves and even break bones when they attempt to land, but miss the perch (Botheras *et al.*, 2006).

Regardless of the husbandry system, the supply of the essential minerals for eggshell formation, the avoidance of stress factors and a low incidence of diseases that affect the oviduct are essential to obtain a high eggshell quality.

20.5.2 Factors affecting the sensory quality

When egg purchasing consumers in the UK are asked about the taste of eggs, only a small minority (5%) believe that all eggs taste the same and that the production system does not matter, whereas more than 50% of consumers believe that there is a difference in the taste of eggs, and 35% of respondents believe that 'the taste of eggs appeared to be the main distinguishing feature between free range eggs and eggs from caged hens' (Parrott, 2004).

It is well known that some feed ingredients can have a negative influence on the sensory properties of eggs, e.g. fish oil or fish meal (Hammershøj, 1995; Koehler and Bearse, 1975), rape seeds (Butler and Fenwick, 1984; Hammershøj, 1996), or have an enhancing effect on the egg flavour itself, e.g. naked oat (Cave *et al.*, 1992). Few studies have dealt with the idea of producing eggs with a higher score for positive sensory attributes which, however, appears possible by feeding, for example, thyme (*Thymus vulgaris* L.) (Tserveni-Gousi, 2001), basil (*Ocimum basilicum* L.) (Narahari, 2003), or garlic, fennel, peppermint and marjoram, resulting in a seasoned taste and aromatic flavor (Richter *et al.*, 2002).

Even fewer studies have examined the sensory properties of free-range and organic eggs in comparison with cage and barn eggs, and there appears to be no general difference between these (Mizumoto *et al.*, 2008). Nevertheless, it is possible to affect the sensory properties of eggs in free-range and organic egg production through the forage material (Hammershøj and Steenfeldt, 2009).

For free-range and organic egg production the access to vegetation or supply of forage material offers the possibility of feeding the hens plants such as spices, herbs or other aroma containing vegetables, resulting in a sensory effect on the eggs laid. Supposedly, this can increase the diversity of eggs for the consumer not only on the basis of production systems but also with a quality parameter for the consumer to select from. The sensory evaluation of eggs can be performed in many ways; as a preference test, a differentiability test, or using descriptive analysis. In the latter method, a vocabulary of attributes for the specific eggs to be judged is developed in one session by the panelists in order to describe the sensory quality. After the set of attributes is agreed upon, the sensory analysis including the scoring of the eggs follows in another session. For example, in a study on organic egg production with treatment combinations of forage materials, feeds and hen genotypes, a total of 23 attributes were developed (Table 20.2) which described the sensory quality of hard-boiled eggs (Hammershøj and Steenfeldt, 2009).

Table 20.2 Attributes with descriptors used by 10 judges to evaluate the sensory properties of hard boiled organic eggs (Hammershøj and Steenfeldt, 2009)

Egg part	Attribute	Description	Positive/ Negative*
Whole egg aroma	Fresh	How fresh is the egg aroma?	+
	Sulphur	How much does the egg smell of sulphur?	–
	Cress/green	How much does the egg smell of cress?	0
	Dirty	How dirty does the egg smell?	–
	Acidic	How acidic is the egg smell?	+
Yolk appearance	Colour	How yellow is the yolk?	0
Albumen texture	First bite hardness	How hard is the albumen at first bite?	0
	Hardness at chew	How hard is the albumen when chewing?	0
	Cohesiveness	How much sticks the albumen together?	0
Albumen taste	Fresh	How fresh does the albumen taste?	+
	Sulphur	How sulphurous does the albumen taste?	–
	Sweet	How sweet does the albumen taste?	+
	Cress/green	How cress-like does the albumen taste?	0
	Dirty	How dirty does the albumen taste?	–
	Watery	How watery does the albumen taste?	0
Yolk texture	Dryness	How dry does the yolk feel?	–
Yolk taste	Fresh	How fresh does the yolk taste?	+
	Sulphur	How sulphurous does the yolk taste?	–
	Sweet	How sweet does the yolk taste?	+
	Cress/green	How cress-like does the yolk taste?	0
	Dirty	How dirty does the yolk taste?	–
	Acidic	How acidic does the yolk taste?	+
	Bitter	How bitter does the yolk taste?	–

*Indicating whether the attribute is positive (+), negative (–) or neutral (0).

Of the forage materials studied, there was a significant positive effect from alfalfa silage on the yolk colour, the hardness of the egg albumen, and a less dirty taste to the albumen in comparison with maize silage (Hammershøj and Steenfeldt, 2009). Feeding the hens with fresh kale (*Brassica oleracea* ssp. *acephala*) leaves as forage material resulted in eggs that had significantly lower scores for sulphur aroma, and a watery taste to the albumen together with significantly higher scores for cress/green taste of the yolk and yolk colour (Hammershøj and Steenfeldt, 2009). Another interesting result of the study was related to the hen genotype, which also had an influence on the sensory quality of the eggs evaluated. For instance, the eggs of the genotype New Hampshire scored significantly higher for the negative attributes of a sulphur taste to the albumen and a dirty taste to the yolk, and significantly lower for the positive attributes, cress aroma of whole egg, fresh and sweet taste of yolk, and also lower for the neutral attributes of albumen texture in comparison with the genotype Lohmann Silver (Hammershøj and Steenfeldt,

2009). This indicates, to some extent, that the traits distinguishing the two genotypes may also have an impact on egg quality, i.e. the low body weight and high performance Lohmann Silver genotype lay eggs with a better sensory profile than the heavier and lower performing New Hampshire genotype. Whether these observed sensory properties are directly coupled with the performance difference of the two genotypes or is a result of other indirect differences cannot be concluded on the basis of this study.

20.5.3 Yolk colour and carotenoids

The egg yolk colour is affected by the feed content of carotenoids, and xanthophyll isomers, lutein and zeaxanthin, have especially high deposition efficiencies (DE), around 20–27% compared with the α- and β-carotenes with DE < 1% (Hammershøj *et al.*, 2010). Lutein and zeaxanthin have been found to be important in human health due to their antioxidant properties which help against age-related macular degeneration (Granado *et al.*, 2003).

As hens in free-range and organic production systems have access to outdoor areas with either vegetation or are supplied with forage material of plant origin, the availability of carotenoids is high compared with hens in conventional husbandry systems. However, synthetic pigments, such as canthaxanthin, are allowed to be used in feed in conventional egg production in contrast to organic egg production, which enables easy control of the yolk colour at a stable level by supplying a mixture of yellow and red pigments (Nys, 2000). Organic eggs were found to contain 2–3 times higher concentration of lutein than conventional eggs in a Danish study (Leth *et al.*, 2000). In contrast, a study from the UK revealed the lutein concentration in conventional eggs to be 2–4 times higher than in organic eggs (Surai *et al.*, 2000). These contradictory findings probably originate from the differences in lutein content of the actual feeds as well as the forage material available.

For the past five years, experiments at the Aarhus University have aimed at studying the effects of different forage materials for laying hens on the egg quality, including the colour and carotenoid composition of the yolk. The results show that forage materials such as violet carrots, alfalfa silage and kale (*Brassica oleracea* ssp. *acephala*) have a significant effect especially on the redness (a*) of the yolk colour analysed by the tristimulus Lab scale reflecting lightness (L*), redness (a*) and yellowness (b*) (Hammershøj *et al.*, 2010; Hammershøj and Steenfeldt, 2009). The *ad libitum* feeding (~120 g/hen/day) with kale as forage material proved especially effective in increasing yolk redness by 4 units on the a*-scale and in producing a 3-fold rise in the lutein content from ~2.2 to ~6.2 mg/100 g yolk (own data, unpublished). The kale was analysed and shown to contain significant concentrations of lutein, β-carotene, and violaxanthin, which are confirmed by previous analysis (de Azevedo and Rodriguez-Amaya, 2005). Violaxanthin is an orange carotenoid and was also found in egg yolks at ~150 μg/100 g of hens fed kale. It can be assumed that this also increased the redness of the yolk.

20.5.4 Egg protein

Research has shown that an insufficient supply of methionine for laying hens has a negative effect on the egg quality (Hammershøj and Steenfeldt, 2005). The egg albumen dry matter content, which resembles the protein concentration of the egg albumen, decreases, when methionine intake is below the required level of 300 mg/hen/day (National Research Council, 1994), and vice versa (Shafer *et al.*, 1996). The protein concentration of the egg albumen is an important quality parameter, when heat treating the egg to form a strong albumen gel before consumption (Hammershøj *et al.*, 2001). As described in Section 20.3.3, the risk of an under-supply of essential amino acids in the European organic egg production will probably increase by 2012 due to the regulation on 100% organic ingredients in the diets. Therefore, it is essential to secure the supply of organically grown protein crops with a suitable amino acid composition.

20.5.5 Contamination risk of parasites, microorganisms and dioxins

The egg production systems considered here offer outdoor access to the hens, and therefore may also include risks for animal health, which can result in reduced food safety due to bacterial, viral, or parasitic infections or environmental contaminants. Endoparasites are one of the major health and welfare risks of free-range and organic hens due to their access to outdoor areas. At high infection rates with roundworm or tapeworm there is an effect on the health of the hens. Infections with parasites can cause loss of appetite, thereby affecting growth and egg laying (Van de Weerd *et al.*, 2009). Some studies indicate that the supplementation of forage material to feed leads to higher counts of *Ascaridia galli* (roundworm) eggs in infected hens than unsupplemented feed (Idi *et al.*, 2005).

The hen egg is the greatest source of Salmonellae infections in humans worldwide. Today, vaccination against Salmonellae is practised in many countries. Nevertheless, the contribution and risk of infections in the cage, barn, free-range and organic husbandry systems is of great importance in order to ensure the high safety of the eggs originating from each production system. In a German study, the mean Salmonellae prevalence was 32% for 329 layer flocks analysed. The battery cage system had the highest share of Salmonellae positive flocks with 46% followed by 33% in organic flocks, and 22% in free range flocks (Methner *et al.*, 2006). *Salmonella Enteritidis* was the most dominant serovar in egg layers with a share of 78% of all types found. This pattern was confirmed in a study from Belgium of 148 laying flocks, where 30% of cage layers were diagnosed as positive for Salmonellae, and only 1% of barn and free-range layers (Namata *et al.*, 2008). These findings contradict the older data by Kinde *et al.* (1996), which showed a higher prevalence of *Salmonella Enteritidis* in free-range flocks at 50% compared with caged flocks at 2% giving rise to the thought that contamination risk should be higher in eggs produced in non-cage systems,

because of greater exposure of layers to environmentally based contaminants such as mice, cats and skunks (Kinde *et al.*, 1996). However, in practice the control of the Salmonellae risk is not necessarily better or easier in cage systems; the disinfection of cages is difficult to do efficiently and there is a higher density of hens producing larger amounts of faeces and dust per area compared with free-range and organic production systems (Davies and Breslin, 2004).

The level of bacterial contamination of eggshells in relation to the different production systems was studied on 58 farms in France, and showed that the mean eggshell contamination tended to be higher in flocks with floor access compared to those in conventional cages (Huneau-Salaun *et al.*, 2010). The reason was suggested to be the higher risk of close contact between the egg shell and the environment soiled by faeces and dust in non-cage production systems even though floor eggs were excluded from the analysis. Nevertheless, the furnished cages turned out to have a very high variation in their eggshell contamination (Huneau-Salaun *et al.*, 2010). Results are contradictory however, as other studies have not found significant differences between furnished cages and other production systems (De Reu *et al.*, 2005).

In general, the consumption of eggs includes a risk of an intake of dioxins, which are very toxic substances that cover a large group of dibenzo-p-dioxin and dibenzofuran congeners (Kijlstra *et al.*, 2007). Eggs account for ~4% of the total dioxin intake of humans, with animal fats in meat, milk products and fish as the other main sources. Dioxin levels must not exceed the EU standard level of 3 pg Toxic Equivalence Quotient (TEQ) per g fat (De Vries *et al.*, 2006). Survey studies from the Netherlands, Belgium, Germany and Switzerland have found that free-range and organic eggs have a higher risk of being contaminated with dioxins than cage eggs (De Vries *et al.*, 2006; Schoeters and Hoogenboom, 2006), whereas an Irish survey found no differences between the levels of dioxins in cage eggs and free-range eggs (Schoeters and Hoogenboom, 2006). De Vries and colleagues (2006) report egg dioxin content in different EU countries to range from 0.1–2.3 pg TEQ/g fat in eggs from conventional cages to 0.4–19 pg TEQ/g fat in free-range and organic eggs. Other EU surveys have found 95th percentile figures of 2.57 pg TEQ/g fat in cage eggs and 7.33 pg TEQ/g fat in free-range eggs (Schoeters and Hoogenboom, 2006).

The dioxins are accumulated in the yolk fat, and originate from the hen's intake of worm and insects (20 g/hen/day), soil (2–10 g/hen/day) and herbs, grass and feed (De Vries *et al.*, 2006). The transfer of dioxin from the hen's intake to the egg is estimated to be ~25%. Interestingly, a significant effect of flock size on the egg dioxin content was found in a study including 34 organic farms with flock sizes from <50 hens to >10 000 hens, which found that the dioxin level was significantly higher in flocks with fewer than 1500 hens (Kijlstra *et al.*, 2007). This may be explained by the fact that the larger the flock, the higher is the tendency (for unclear reasons) of the hen to stay inside (Hegelund *et al.*, 2005).

In summary, the free-range and organic egg production systems may affect food safety by their contamination with parasites, Salmonellae and dioxins. However, many countries have high standards and survey systems to handle these risks, thereby ensuring the safety of consuming eggs.

20.5.6 Organic egg products

The relative share of eggs being industrially processed by separation, pasteurization, spray drying, fermentation, etc. into liquids or powders of albumen, yolk or whole egg is continuously increasing; the reasons being the high convenience and microbial safety in using egg products. Although, recent statistics on the amount of eggs being processed are difficult to obtain, the share reached ~20% in the EU, 31% in the US and 40% in Japan in 1997 (Hammershøj *et al.*, 2004). In 2005, in the EU an average of 24% of eggs were used for egg products, whereas Italy and Germany had the highest share with 38% of eggs being further processed (Magdelaine, 2007). It is therefore to be expected that the processing and marketing of organic and free-range eggs into products have increased and may continue to do so.

Recent statistics of the market share for organic egg products are scarce, although it is believed to represent a relatively small fraction of the egg products that are processed from organic eggs. The incorporation of eggs from non-cage production systems is still low in the industrial production of egg products (Magdelaine, 2007). For the organic food segment, this is mainly due to the following reasons; first, the ideology of organic production does not fit well with the increased processing degree and, secondly, it is required that the processing industry keep certified organic products strictly separated from the production of non-organic egg processing, which increases the costs for cleaning and labour.

Nevertheless, some organic egg products are available in catering size or small containers as refrigerated versions of pasteurized yolks, albumen or whole egg with a shelf life of up to 56 days or as frozen (www.Eggsite-Pro.dk) in the US and some European countries.

Some of the described quality differences caused by the husbandry system could be expected to influence the properties of egg products. The egg yolk content of total tocopherol, α-tocopherol and lutein, known to have antioxidant activity, is found to be significantly higher in eggs from a free-range system compared with battery cages (Pignoli *et al.*, 2009). However, this did not result in significant differences in lipid oxidation of freeze-dried egg yolk.

The demand of egg products based on the husbandry system is not believed to increase. However, some specific qualities may originate or are possible to obtain from the use of forage material in the egg production, for example, and could turn out to be niche products on the egg product market in time.

20.6 Future trends

One future scenario for free-range and organic egg production may occur as consequence of the cage ban in the EU by 2012. This could mean that more egg producers choose these husbandry systems and, at the same time, the European egg market may change into a higher proportion of imported eggs from outside the EU. The consumer willingness to pay for the higher production costs of free-range and organic eggs will be challenged at this point. The strength of consumer concerns about animal welfare and environment protection will also be a determinant of how the future egg market will be composed.

There is a huge challenge and opportunity in offering free-range and organic eggs and egg products, which have a higher diversity range in quality aspects. This could be, for example, in the flavour of the egg to meet certain high quality expectations, and also by offering the consumer an extraordinary flavour experience in eggs tailor-made for certain purposes in relation to how they are going to be served and eaten. Spices and herbs in the forage material can be useful tools for such a task.

The EU regulation which will come in effect in 2012, saying that the feed used in organic husbandry has to be composed of 100% organic ingredients, creates a huge challenge for scientists, feed companies and egg producers in order to meet the hen's requirement of some specific nutrients. This is especially a significant issue for the essential amino acids methionine and lysine, which no longer can be added as synthetic concentrates. The search for new protein-rich crops that can be grown organically has to be carried out promptly and further studies of their effects on egg production and egg quality parameters are needed.

Both free-range and organic egg production methods face challenging futures in their aims to increase consumer interest and willingness to pay for the values of animal welfare, environmental respect and food quality.

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21

Production, composition, and quality of duck eggs

J. F. Huang and C. C. Lin, Council of Agriculture, Taiwan

Abstract: The duck egg has become increasingly more important in the world because of its nutrition and less capital input is required to produce it. This chapter discusses duck egg production, duck breeds, and productive styles in the world. A comparison of duck eggshell, egg white, and egg yolk composition between different avian species is given. Duck products include the salted egg, pidan (peedan, century egg, thousand-year egg, alkalized egg), balut (embryonic egg), ω-3 fatty acids-enriched egg, and yolk pigment-enhanced egg. Last, we discuss the factors affecting the quality of duck eggs and products.

Key words: production, composition, quality, duck egg.

21.1 Introduction

The duck egg has been consumed in many Asian countries for a very long time. It is an important source of nutrition in many developing countries due to the low capital input required to raise laying ducks. Duck egg consumption accounts for 10–30% of total egg world consumption (Pingel, 2009). The top ten countries for egg production (excluding hen eggs) in the world are listed in Table 21.1. Although the values obtained from FAOSTAT are eggs excluding hen eggs, these numbers in most of the Asian countries in Table 21.1 are believed to be mostly derived from duck eggs. China is the largest duck egg production country. Duck eggs are usually not consumed in the countries in America and Europe due to the potential *Salmonella* risk (Chang, 1992). Brazil, Romania, and the United Kindom may produce other

Table 21.1 Top ten egg production (excluding hen eggs) (1000 tons) countries in the world

Country*	Year							
	2001	2002	2003	2004	2005	2006	2007	2008
1. China	3300.8	3342.9	3380.4	3527.7	3580.2	3685.3	3663.1	3821.1
2. Thailand	292.7	297.5	304.0	304.0	305.0	310.0	310.0	310.0
3. Indonesia	141.0	157.6	169.7	185.0	173.2	195.0	193.6	207.5
4. Philippines	73.0	73.8	74.4	74.0	72.0	72.0	72.0	73.0
5. Brazil	60.0	65.0	59.0	61.1	66.2	74.8	74.2	78.6
6. Bangladesh	53.1	57.3	61.9	67.0	66.9	78.7	75.9	76.0
7. Taiwan**	31.1	30.7	31.0	27.1	31.7	30.3	33.0	31.5
8. South Korea	21.3	23.5	25.0	28.0	26.0	28.0	28.0	28.5
9. Romania	22.7	23.8	33.0	30.6	34.0	10.2	14.8	14.8
10. United Kingdom	15.3	17.2	17.9	18.1	16.3	16.0	16.6	13.9
World	4058.6	4139.3	4209.5	4383.2	4430.0	4565.3	4550.5	4727.9
% change		1.99%	1.70%	4.13%	1.07%	3.06%	–0.33%	3.90%

Source: FAOSTAT (2010); Council of Agriculture (2008).
*Country is ranked by average production of 2001–2008.
**The data for Taiwan are derived only from duck eggs.

avian eggs, e.g. quail eggs (Table 21.1). Year on year, annual growth rate of duck egg production in the world ranges from 1.07 to 4.13%, except for –0.33% between 2006 and 2007 (Table 21.1).

21.2 Breeds of laying ducks

There are several varieties of laying duck breeds in the world, with deviations in plumage color and body size (Huang *et al*., 2008a). In Taiwan, the major laying duck is the Brown Tsaiya. The laying ducks raised in China include the local breeds Shaoxing, Jinding, Youxian partridge, Sansui, Liancheng White, and Putian Black, and some imported breeds such as Khaki Campbell and Cherry Valley (Qiu, 1988; Wang *et al*., 2005). In Thailand, the laying duck breeds commonly include Khaki Campbell, native ducks Paknum and Nakonphatom, and Khaki Campbell–native duck crossbreds (Thongwittaya, 2007). Malaysia has laying duck breeds, including the local Itik Java, Khaki Campbell, and the crosses (Yimp, 2007). In Vietnam, the most popular local common ducks are the Co duck (also named Tau duck in the south) and Bau duck; Khaki Campbell, CV 2000, and Triet Giang are also raised in this country (Hanh and Tieu, 1999; Tuyen, 2007). Bangladesh raises the major laying duck breed Deshi duck and other breeds including Khaki Campbell, Indian Runner, Jinding, etc. (Shafiuddin, 1985; Khatun, 2005). In India, the laying duck breeds include Sythetmete, Nageswari, Indian

Runner, and Khaki Campbell (Shukla and Nayak, 2009). Philippines raise the 'itik' for egg production (Villar and Ramil, 2007). Two common egg-laying varieties, the Kahki Campbell and the Indian Runner, are used in modern duck breeding farms in Iran (Nimruzi, 1998). In Cambodia, Teah Angkam, Teah Sampov, and the combination of the two genotypes are raised (Chhum Phith, 1999).

21.3 Productive styles of laying ducks

21.3.1 Backyard raising

Since little care and supplementary feeds are used in backyard duck raising, this system is still popular in some countries. Because of low input and imbalanced nutrition, the production efficiency is low, e.g. only 60–90 eggs are laid annually per duck (indigenous) in Bangladesh (Huque, 2006). In addition, poor-quality feed and husbandry render ducks more susceptible to non-infectious (e.g. leg weakness and aflatoxicosis) and infectious diseases (duck virus hepatitis, duck virus enteritis, cholera, etc.) (Aini, 2006).

21.3.2 Herding rearing

In one herding system, ducks are kept in the rice paddy between the crop routines (Fig. 21.1). The fallen rice accounts for 5% of the total rice yield, which provides energy source for ducks (Tuyen, 2007). The most extensive system is to herd ducks moving from one rice field to another. Usually they are pastured until the age of sexual maturity (Prasetyo and Iskandar, 2007; Villar and Ramil, 2007). The group may travel up to 100 km away from their original place (Prasetyo and Iskandar, 2007). Another herding system

Fig. 21.1 Herding rearing of laying ducks.

raises ducks on the shore of a lagoon, with paddy whole grain or dried rice remnants at about 110 g/duck/day, provided twice a day as the energy source. The herding ducks get their protein source from snails, clams, small shrimp, small fish, insects, crabs, etc. Algae, seaweed, leaf materials, and organic matter provides fiber and other nutrients (Harimurti *et al*., 2009).

21.3.3 Duck–fish integrated production

The duck–fish integrated system can be employed by duck farmers for both meat ducks and laying ducks. The fish species raised depend on the market demand in different countries (Liao and Luo, 2005; Huang *et al*., 2008a). To meet hygiene requirements, it is suggested the pond sediment be removed and cleaned properly each year end. Introduction of ground water or surface water is conducted in some areas to maintain the pond in good condition for both ducks and fish (Lee *et al*., 1997). In addition to duck–fish system, the duck–rice and duck–fish–rice systems have also been studied (Minh *et al*., 2007).

21.3.4 Confined rearing

Countries raising laying ducks have different ratios of confined rearing. In Taiwan, all laying ducks are confined in a duck house with some areas for bathing and resting in addition to a litter area for laying during midnight to early morning. The size of laying ducks farms is 3000–70 000 birds per farm (Fig. 21.2; Huang *et al*., 2007a). Most laying ducks in China are also raised in a confined system (Lee S R, personal communication). Approximately 24% of duck reared in the Philippines are confined. The floor in this system is characterized by a slat–litter combination. The ducks are fed with commercial feeds in the form of mash, crumbles, and pelleted forms (Villar and Ramil, 2007). In Vietnam, about 20–25% of ducks are raised in intensive and semi-intensive systems (Tuyen, 2007). Only a very small proportion of the ducks raised in Indonesia involve the fully intensive system (Prasetyo and Iskandar, 2007). In some countries, a wading/drinking channel is constructed for duck production, using either underground water, rain water, irrigation water, or springs (Bird, 1985). Although the duck is a waterfowl, water for swimming is not absolutely necessary (Lee *et al*., 1991). Better egg production and feed conversion efficiency is obtained when laying ducks are raised in cages equipped with nipples or cut-in-half pipes as compared with being raised on a floor with a water area for swimming (Lee *et al*., 1991).

A recent study was conducted to compare the environmental parameters and laying performance of caged Tsaiya ducks between the water-pad and non water-pad duck house during the hot season. The water-pad duck house had lower room temperature and higher relative humidity (>93% vs <88%). Higher egg production and lower mortality was observed in the Tsaiya ducks raised in the water-pad duck house (CY Lin *et al*., 2006).

Fig 21.2 Confined rearing of laying ducks.

Table 21.2 Avian egg weight and percentage of egg white, yolk, and eggshell

Species	Egg weight (g)	Egg white (% of egg weight)	Egg yolk (% of egg weight)	Eggshell (% of egg weight)
Hen	50.1–68.5	57.0–63.4	28.0–30.6	8.1–12.4
Quail	8.4 ± 1.7	62.0	30.2	7.9
Pheasant	26.6 ± 1.3	58.5	31.6	9.9
Goose	143.2 ± 18.6	58.7	30.4	10.8
Duck	60.0 – 90.0	45.0–58.0	28.0–35.0	11.0–13.0
Turkey (Japan)	104.7 ± 8.6	62.0	28.7	9.4
Turkey (USA)	85.0	55.9	32.3	11.8
Pigeon	17.0	74.0	17.9	8.1

Source: Chang (1992); Chen (2001).

21.4 Duck egg composition and characteristics

Owing to the varieties in laying duck species worldwide, the duck egg weight range is 60–90 g. The weight percentages of the eggshell, egg white and egg yolk to that of the whole egg, accounts for 11–13%, 45–58, and 28–35%, respectively (Table 21.2; Burley and Vadehra, 1989; Chang, 1992; Chen, 2001). Duck eggs have a relative higher percentage of egg yolk compared

with other avian eggs. This favors duck eggs when the products utilize the egg yolk instead of whole egg, e.g. rice dumpling in the Dragon Festival and 'moon' cake in the mid-autumn festival in Chinese community (Chang, 1992; Lin, 2000a). The duck egg shape index ranges from 70.3 to 72.8 (Tai *et al*., 1985a,b; Chen *et al*., 2003; Huang *et al*., 2009a). Duck eggs are more stable during storage at room temperature than chicken eggs. Duck egg albumen and yolk indices and Haugh unit score are not severely affected by long storage times like those of chicken eggs (Jalaludeen *et al*., 2009). During the storage period of 97–98 days at 44 °C, egg white index and egg yolk index decreased by 56.5% and 66.7%, respectively in hen eggs compared with the storage temperature at 2 °C. Duck egg white and egg yolk indices decreased by only 22.8% and 15.6%, respectively (Table 21.3; Lin, 2000a). The duck egg also has a special flavor that most of the respondents (72.1%) in a survey can distinguish from hen eggs (J H Lin *et al*., 2006).

21.4.1 Eggshell

Eggshell thickness of duck eggs is 0.36–0.42 mm, higher that that of hen eggs. Eggshell strengths of Tsaiya duck, Muscovy duck, and Kaiya duck are 3.97–4.30, 4.16, and 4.06 kg, respectively (Tai *et al*., 1985a,b; Chen, 2001). Eggshell color is the focus because of its relationship with eggshell quality and consumer preference in Taiwan (J H Lin *et al*., 2006; Huang *et al*., 2009a). It has been proven that the blue eggshell contains biliverdin and protoporphyrin, while only protoporphyrin was observed in the white eggshell (Liu *et al*., 1998). A study on pigment deposition in Tsaiya duck eggs indicated that the genes controlling blue eggshell had a dominant effect over those for white eggshells (Liu *et al*., 1998). The ultra-structure of Brown Tsaiya duck and White Leghorn hen eggshells showed the duck eggshell palisade was compact in the palisade layer compared with many hollow vesicles in the hen eggshell (Chen and Shen, 2000). There is higher organic matrix (2.31 vs 1.72%) and lower Mg contents (0.12 vs 0.45%) in the duck eggshell than those in the hen eggshell. This may play some role on the better eggshell quality in ducks compared with hens. Similar Ca level was observed between the two species and both eggshells have only calcite crystal (Chen

Table 21.3 Comparison of egg quality during storage between hen and duck eggs

Storage		Hen			Duck		
Temperature (°C)	Period (day)	pH	Egg white index	Egg yolk index	pH	Egg white index	Egg yolk index
2	97	9.0	0.062	0.42	8.9	0.079	0.45
44	46	9.5	0.051	0.23	9.5	0.072	0.40
44	98	9.7	0.027	0.14	9.6	0.061	0.38

Source: modified from Lin (2000a).

and Shen, 2000). Wang *et al*. (1997a) reported that better eggshell quality was observed in the blue-shell duck eggs compared with white-shell ones. The cuticle on the blue duck eggshell was fine and firm compared with a dispersed interstitial structure on white duck eggshell. The palisade of blue eggshell was more compact than that of white eggshell. Blue eggshells had more regular and smooth mammillary knob than the white ones. A cohesive state of shell membrane fibers of the blue eggshell was observed, but not in the white eggshell (Wang *et al*., 1997a).

21.4.2 Egg white

The duck egg white is clear compared with light yellowish green in hen eggs (Lin, 2000a). The average height of the egg white is approximately 8.50 mm (Tai *et al*., 1985a). The approximate egg white composition of avian species is shown in Table 21.4. Moisture, protein, fat, and ash in duck egg are 88.3, 8.8, 0.13, and 0.53%, respectively. The amount of protein in hen and duck egg white is listed in Table 21.5. The major proteins in duck egg white are

Table 21.4 Approximate composition (%) in egg white of avian eggs

	Species	
	Hen	Duck
Moisture	88.2–89.3	88.3
Protein	9.4–11.5	8.8
Fat	0.01–0.06	0.13
Ash	0.56–0.78	0.53

Source: modified from Chang (1992).

Table 21.5 Amount of proteins in hen and duck egg white (%)

Protein	Hen	Duck
Ovalbumin	54	40
Panalbumin	0	0.1
Conalbumin	12	2
Ovomucoid	11	10
Ovomucin	3.5	3
Lysozyme	3.4	1.2
Ovoinhibitor	1.5	–
Ovomacroglobulin	0.5	1.0
Ovoflavoprotein	0.8	0.3
Avidin	0.05	0.03
Others	15	42

Source: modified from Lin (2000a).

ovalbumin, ovomucoid, ovomucin, conalbumin, and lysozyme (Lin, 2000a). The major amino acids in the duck egg white are glutamate, serine, aspartate, leucine, alanine, and lysine (Wang *et al.*, 1997a). The whipping quality of duck egg white is not as good as that in hen eggs, which may hinder duck egg utilization in making cakes (Chang, 1992; Lin, 2000a). Lemon juice or citric acid could be added to adjust the pH value and whipping quality of duck egg whites in making angel cakes (Lin, 2000a). In addition, there is a significant difference in coagulation temperature between duck and hen egg whites. When duck egg white is heated at 50 °C, its viscosity is greatly increased, while the temperature needs to be 55 °C to obtain the same viscosity in hen egg whites. In order to get visible coagulation the duck egg white needs to be heated to 55 °C for 10 minutes compared with 30 minutes for the hen egg white (Lin, 2000a).

21.4.3 Egg yolk

The approximate egg yolk composition of avian species is shown in Table 21.6. Duck egg yolk has relatively higher fat content than hen egg yolk, with a trade-off in lower moisture and protein contents. The cholesterol content in duck egg yolk is 17 mg/g yolk, higher than 14 mg/g yolk in hen eggs (Chang, 1992; Chang and Huang, 2001). Yang and Chen (2001) reported that the total cholesterol contents in fresh duck yolk and chicken egg yolk were 12.05 mg/g and 11.64 mg/g, respectively, with no significant differences observed. In the aspect of fatty acid composition of neutral lipids in the egg yolk of avian species, duck egg yolk has higher 18:1 content but less 16:0 and 18:2 contents than hen egg yolk. In terms of fatty acids in the complex lipid, duck egg yolk has higher levels at 20:4 but lower at 16:0 and 18:2 levels (Lin, 2000a).

21.5 Duck egg products

Duck eggs are consumed as table eggs in many countries. There are many varieties of duck egg recipes, for example boiled eggs, stuffed eggs or deviled eggs, coddle eggs, roasted eggs, egg omelet, scrambled eggs, poached eggs,

Table 21.6 Approximate egg yolk composition of avian eggs

	Approximate composition					
	Hen	Quail	Pheasant	Turkey	Goose	Duck
Moisture	47.7–48.1	48.8	47.6	47.7	44.7	44.7
Protein	16.3–17.8	16.4	15.4	17.2	17.3	15.8
Fat	30.2–33.3	30.0	34.3	32.2	35.8	36.8
Ash	1.63–1.82	1.58	1.61	1.77	1.39	1.70

and so on (Jalaludeen *et al*., 2009). Except for being consumed as table eggs, duck eggs in some countries are processed into salted eggs and pidan (peedan, century egg, thousand-year egg, alkalized egg; Huang *et al*., 2007a; Tuyen, 2007). There are different names for pidan in China, e.g. songhua in Beijing, tsaidan in the southern, and nidan in the north western (Lin, 2000b). The manufacturing of pidan from hatched unfertilized duck eggs has also been studied to enhance their utilization (Wang and Ho, 1984). Balut (embryonic eggs) is a nutritious delicacy in some South East Asian countries, such as the Philippines, Vietnam, and Laos (Jalaludeen *et al*., 2009; Villar and Ramil, 2007; Tuyen, 2007).

To meet the demand of functional food, eicosapentaenoic acid (EPA)- and docosahexaenoic acid (DHA)-enriched duck eggs have been studied (Chen *et al*., 2000a, 2000b; Hwang, 2002). Yolk color-enhanced duck egg is also produced in some places to suit consumer preference. Recently, functional components in duck eggs have been attracting attention (Chen, 2009; Wang *et al*., 2009). The anti-oxidative activities of hydrolysates from duck egg white using enzymatic hydrolysis were investigated (Chen *et al*., 2009). When duck egg white was hydrolyzed with papain, trypsin, chymotrypsin, alcalase, or flavourzyme, the hydrolysate from papain had the highest peptide contents and the best anti-oxidative activities (Chen *et al*., 2009).

21.5.1 Salted eggs

Although salted eggs are made mainly from duck eggs (Fig. 21.3), hen eggs can also be made into salted eggs (Chang, 1992). There are two methods to produce salted eggs. The immersion method pickles fresh eggs in a salt solution containing wine, tea, etc.; or using only saturated salt water. The coating method coats the eggs with a macerated paste containing salt, wood ash, red clay, wine, etc. (Lin, 2000b). The pickling period is dependent on salt content in the pickling solution and environmental temperature. Hwang

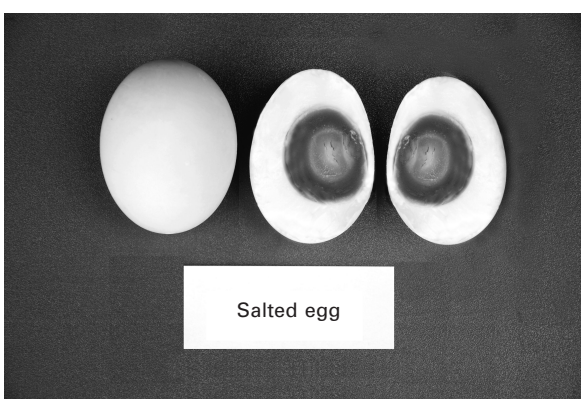

Fig 21.3 Salted eggs.

(2002) used the coating method to produce salted eggs. After 35 days of pickling, the egg white of the salted eggs had lower moisture content, pH value, emulsifying capacity, and emulsifying stability, but higher ash and salt content than that of fresh eggs (Hwang, 2002). A similar electrophoresis pattern was observed in salted egg white and fresh egg white. After heating, the microstructure analysis showed that there were larger but more irregularly arranged ball-shaped proteins in the salted duck egg white than in fresh duck egg white (Hwang, 2002). Separated duck egg yolk can also be pickled so that egg white separated before pickling can be utilized better (Lin, 2000b). Hwang (2002) pickled separated duck egg yolk in a 20% salt solution with 0.3% citric acid and 0.2% ferric citrate at 25±2 °C. After 32 hours, a good separated duck egg yolk similar to a traditional duck egg yolk was obtained. The salt and solid contents of the egg yolk, hardness of the egg yolk, thickness of the hardening layer and volume of the extracted oil increased. The weight and moisture content of the egg yolk decreased with pickling time (Hwang, 2002).

21.5.2 Pidan

There are three methods for processing pidan (Fig. 21.4): immersion method, coating method and a combination the two methods (Lin, 2000b). Although there are a lot of recipes for making pidan, the main ingredients include salt, tea or tea leaves, and alkaline materials with sodium hydroxide most frequently used (Chang, 1992). The immersion method involves placing fresh eggs into a pickling solution. The coating method coats the eggs with an alkaline paste. The third method is a combination of the immersion and coating methods. Fresh eggs are pickled in the solution for 10–14 days, then taken out and coated with alkaline paste for another period of time (Lin, 2000b). In either method, the pidan processing period is very much dependent on the recipe and environmental temperature. Although most pidan is made from duck eggs, hen and quail eggs can also be processed into pidan (Chang, 1992). A less alkaline environment is needed to coagulate duck eggs than to coagulate

Fig 21.4 Pidan.

hen eggs, which is believed to be associated with the egg white protein composition (Lin, 2000a). After being made into pidan, lysine, arginine, cystine, and serine contents in the duck egg white and yolk decrease, with cystine decreasing most strikingly (Lin, 2000a).

21.5.3 Balut

Consumption of balut (embryonic duck eggs) is familiar in the food customs of people in the Philippines, Vietnam, Laos, Cambodia and Thailand. This is a duck egg that has been incubated approximately 17 days and removed for boiling and consumption. It is considered a delicacy and is highly nutritious (Jalaludeen *et al*., 2009; Tuyen, 2007; Villar and Ramil, 2007). Penoy is an infertile incubated duck egg or egg with dead embryos, which is also boiled for 20–30 minutes like balut but sold at a lower price (Villar and Ramil, 2007).

21.5.4 EPA- and DHA-enriched duck eggs

EPA and DHA contents in the duck egg yolk are increased by fish oil dietary inclusion (Chen *et al*., 2000a; Huang *et al*., 2000), and the EPA and DHA contents are increased with increased fish oil in the diet (Chen *et al*., 2000a). When 6% fish oil is included in the laying Tsaiya duck, the yolk EPA is increased by 20-fold (0.038% vs 0.77%), and DHA increases by 5.6-fold (0.86% vs 4.97%) (Chen *et al*., 2000a). In the traditional herding system, when ducks are raised on the shore of a lagoon, EPA and DHA in eggs is 1.63% and 1.16%, respectively, which is higher than those in the eggs produced by the confined ducks fed a corn–soybean-based diet (Harimurti *et al*., 2009). Dietary inclusion of 4% fish oil does not affect smell, taste, and overall acceptability sensory evaluation scores. The EPA and DHA levels do not change during 3-week storage at 25 °C (Chen *et al*., 2000b). It was also shown in the same study that the addition of 100–400 ppm vitamin E does not decrease TBA values in EPA and DHA-enriched duck eggs (Huang *et al*., 2000). Dietary inclusion of 2–6% fish meal also increases EPA and DHA contents in the duck yolk. The sensory evaluation of duck eggs was not affected by the fish meal inclusion (Lin *et al*., 2003).

When the EPA- and DHA-enriched duck eggs are immersed in the 30% saltwater for 28 days to be made into salted eggs, the EPA and DHA levels are not changed by the salting treatment (Huang *et al*., 2001). Furthermore, when the ω-3 fatty acid-enriched duck eggs are cooked, and then stored at 3 or 25 °C, yolk EPA and DHA levels are not changed significantly (Huang *et al*., 2001). However, Hadiwiyoto (2009) reported that a decrease in EPA and DHA levels during salting and storage was observed. The different observations between Huang *et al*. (2001) and Hadiwiyoto (2009) are probably due to different salting mediums, cooked vs uncooked after salting, or storage conditions.

21.5.5 Yolk pigment-enhanced duck eggs

People in some countries prefer an orange-red duck yolk and duck eggs with deeper yolk color can be sold at a higher price (Chang, 1992). In practice, xanthophylls, shrimp meal, or some food-graded pigment are added in the duck diet for deeper yolk color (Chang, 1992). Different supplementary levels of citranaxanthin (CIT) and canthaxanthin (CAN) have been studied in the corn–soybean-based diet to investigate their yolk-color enhancing efficiency for laying ducks (Tai *et al*., 1985b). In contrast to an average score of 8.9 by the DSM color fan, a score of 15 was reached by supplementation of 4–6 ppm CAN. The average score for the group of dietary supplementation of 9, 11, 13, and 15 ppm CIT was 12.2, 13.0, 12.8, and 13.3, respectively. When the milo–soybean meal basal diet was formulated into four isocaloric and isonitrogenous treatments containing 0, 3, 6, and 9% alfalfa meal for 21 days, the yolk color scores were 2.62, 3.92, 4.66, and 5.43, respectively (Chen *et al*., 2003). In addition, the color scores of egg yolk of salted duck eggs were higher than those of fresh eggs before they were made into salted eggs (Lu *et al*., 1991; Chen *et al*., 2003).

21.6 Factors affecting quality of duck eggs

21.6.1 Genetics

The heritabilities of characteristics in Tsaiya duck egg has been reviewed (Table 21.7; Huang *et al*., 2008a). Eggshell strength can be differentiated between Tsaiya populations after one generation of divergent selection on eggshell strength (Huang, 2004). A Tsaiya duck line for producing blue-eggshell eggs has been established (Liu *et al*., 2001). The egg weight, eggshell quality, and yield rate of pidan among Brown Tsaiya ducks with blue eggshell (BS), with high eggshell strength (HES), and from commercial hatchery (CM) have been compared in both our research institute and in the field (Huang *et al*., 2007b, 2009a). The results showed that the eggshell color and eggshell strength of

Table 21.7 The heritabilities of characteristics in the Tsaiya duck egg

Breed*	Characteristic	Age (week)	h^2_s	h^2_d	h^2_{s+d}	Reference
WT	Albumen height	50	0.344	0.141	0.242	Tai *et al*. (1985a)
WT	Shell thickness	50	0.316	0.204	0.260	Tai *et al*. (1985a)
WT	Shape index	50	0.011	0.623	0.317	Tai *et al*. (1985a)
WT	Yolk weight	50	0.467	0.128	0.298	Tai *et al*. (1985a)
WT	Yolk index	50	0.150	0.358	0.254	Tai *et al*. (1985a)
BT	Green egg color	52	0.38	0.98	0.68	Hu *et al*. (1993)
BT	Green egg color	33	0.15	0.27	0.21	Liu *et al*. (2001)
WT	Yolk color	40	0.018	0.55	0.28	Hu and Tai (1993)

Source: Huang *et al*. (2008a).
*WT: White Tsaiya; BT: Brown Tsaiya.

both BS and HES ducks were better than those of CM ducks. However, the CM ducks had numerically higher egg production than that of BS and HSE ducks. The CM ducks had the highest egg weight, followed by HES and BS ducks; and ducks raised in the field had heavier egg weight than those raised at our research institute (Huang *et al*., 2007b).

In terms of eggshell quality, Huang *et al*. (2009a) reported the HES ducks had the thickest eggshell, followed by BS and then CM ducks. Ducks raised in the field had a less consistent trend in the order of eggshell thickness at the same age and there was a fluctuation in eggshell thickness when the ages of the ducks increased. In addition, the BS ducks had deepest eggshell color, followed by HES and then CM ducks. The BS and CM ducks had the lowest and highest culling rate of fresh eggs, respectively when eggs were detected to pick up the good-quality ones for production of pidan. At 50 weeks of age the BS ducks had a culling rate of less than 50% compared with those of over 60% in the other two populations. The BS ducks also had the highest yield rate of pidan followed by the HES ducks and then CM ducks. There were similar shape index among the three populations (Huang *et al*., 2009a). Wang *et al*. (1997a) reported that pH value and ammonia nitrogen content were similar between pidan made from blue-shelled and white-shelled Tsaiya duck eggs.

21.6.2 Nutrition

Egg weight increases with increased dietary crude protein (CP) levels. When Brown Tsaiya ducks were given a diet containing 14.5–19.0% CP, the 19.0% CP group had an egg weight of 64.7 g, significantly higher than that in the 14.5% CP group 62.2 g (Huang *et al*., 2008b). In addition, He *et al*. (2003) reported that average egg size of Shaoxing ducks was increased with the dietary methionine content between 2.6 g/kg diet and 4.0 g/kg diet when the basal diet contained 17.5% protein and 11.5 MJ/kg metabolizable energy; however, average egg size was not further increased when dietary methionine level was above 4.0 g/kg. In Peking ducks, an increase in egg and yolk weight of 11.1 g was observed at ages between 26 and 31 weeks. However, minimal age difference in egg weight was observed thereafter due to feed restriction (Applegate *et al*., 1998). When Tsaiya ducks were raised on floor and fed a severely restricted grower diet, they reached their peak egg weight at 40 weeks of age. The ducks raised in the cage and fed a less-restricted grower diet reached peak egg weight at 35 weeks of age (Huang *et al*., 2007b).

Eggshell plays an important role in the utilization of duck eggs. For example, only eggs with good eggshell quality can be used to produce pidan. A level of 3% calcium is recommended for Tsaiya ducks (Shen, 1988). Ducks and hens respond to low calcium diet differently. When the two species are given diet with 1.98% calcium, a significant decrease in eggshell strength is observed in hens compared with the diet with 3.89%, whereas this decrease

is not significant in ducks (Ding *et al.*, 1992). Eggshell strength tends to be decreased when ducks and hens are fed the diet with 250 mg/kg magnesium compared with the diet with 2200 mg/kg magnesium (Ding *et al.*, 1992). When Tsaiya ducks are fed a diet containing 3% calcium, requirement of available phosphorus is 0.4–0.6%. The diet with available phosphorus level at 0.3% gives rise to less eggshell weight; and at 0.2% causes lower eggshell strength, eggshell thickness, eggshell weight, and percentage of eggshell (Li *et al.*, 1997).

Eggshell quality is also influenced by dietary electrolyte balance (dEB). When Brown Tsaiya ducks are fed diets of dEB values of 100–400 meq, the best eggshell quality (eggshell weight, eggshell thickness, and eggshell strength) is obtained in the diet of 228 meq dEB in the hot season (Huang *et al.*, 2002). Higher cholesterol contents in duck eggs to some degree hinders duck egg acceptance in some countries. Therefore, developing duck eggs with lower cholesterol is an important issue. It has been reported that yolk cholesterol was decreased by dietary addition of 4% fish oil (Huang *et al.*, 2000).

21.6.3 Age

Aging of ducks causes eggshell deterioration (Lee, 1997). Eggshell strength reaches its peak when ducks are at 30 weeks of age, and it decreases gradually thereafter (Huang *et al.*, 2007b). When eggs are detected for pidan production, ducks at 33 weeks of age have the lowest culling rate of fresh eggs irrespective of the duck population and then the culling rate is increased with age (Huang *et al.*, 2009a). Because only good-quality duck eggs can be used to produce pidan, a comparison of the yield rate of pidan between different ages of Brown Tsaiya ducks was made. The yield rate of pidan was over 85% when the ages of Brown Tsaiya ducks were 20–34 weeks old. However, the yield rate decreased after 36 weeks of age (Chen and Wang, 2001).

21.6.4 Raising environment

Ducks raised in individual cages had higher egg production and smaller eggs, and there was less breakage than those raised on the floor (Lee *et al.*, 1991). In addition, ducks raised on the shore of a lagoon had the benefit of lower cholesterol in the egg than those produced by confined ducks (Harimurti *et al.*, 2009). Foraging of ducks in the rice paddy might give rise to some residues of pesticides in the eggs, which attracts increasing attention due to stronger demand for food safety (Liao and Luo, 2005; Anitha *et al.*, 2009; Harimurti *et al.*, 2009). Total organochlorine residues in the crop and body fat of ducks were 0.0018–0.0152 and 0.0077–0.0419 ppm, respectively (Anitha *et al.*, 2009). Although these levels are well below the Maximum Residue Limits, this issue cannot be ignored (Anitha *et al.*, 2009).

Minh *et al*. (2007) reported that insecticides on the system without ducks was 4.20–11.61 mg/kg dry paddy and 0.05 mg/liter water from pond or field, which was considered very dangerous. It is also necessary to pay attention to the potential contamination of duck eggs by some industrial by-products such as dioxins due to improper treatment and disposal of industrial waste since laying ducks are usually raised on outdoor floor. When the dioxins-contaminated ducks are moved to cages from floor, the toxic equivalents of dioxins in eggs decrease dramatically (Huang *et al*., 2007c).

Microbial contamination on the eggshell and in the egg contents are highly related to the raising environment and the condition of post-harvest. Under the conditions of 23 °C and 90% relative humidity, the microbial load of air inside duck shed is >300/square feet/minute and total viable count (TVC), coliform count (CC), and yeast and mould count (YMC) of the litter is 9.12×10^{10} CFU/g, 9.17×10^{7} CFU/g, and 5.97×10^{4} CFU/g, respectively. The TVC bacterial count on the eggshell and in the contents is 8.29–9.02 log 10 CFU/eggshell and 6.74–6.87 log 10 CFU/g egg contents, respectively (Thomas *et al*., 2009). The CC on the eggshell and in the contents was 4.30–4.50 log 10 CFU/eggshell and 3.37–5.07 log 10 CFU/g egg contents, respectively; and the YMC on the eggshell and in the contents was 2.95–3.13 log 10 CFU/eggshell and 0.32–0.38 log 10 CFU/g egg contents, respectively (Thomas *et al*., 2009). Study by Baker *et al*. (1985) showed clean unwashed and dirty unwashed duck eggs had bacterial loads of 9×10^{1}/shell and 9×10^{5}/shell, respectively. *Salmonella enteritidis* and *Salmonella badar* were recovered in some samples (Baker *et al*., 1985). Therefore, it is important to improve the hygiene of the raising environment and have proper post-harvest measures to decrease the risk to public health.

21.6.5 Ambient temperature

There are only a few reports discussing the effects of ambient temperature on egg quality. The main reason is that the major duck production countries are located in Asia where plenty of surface water is available; ducks can dissipate remarkable amounts of heat in water through their feet and bills (Hagen and Heath, 1980; Scott and Dean, 1991). Laying ducks raised in the duck house with a bathing area still experience lower feed intake and egg production, poorer eggshell quality, and lower pidan yield rate in subtropical summer. Huang *et al*. (2009b) compared laying performance and eggshell microstructure of the Brown Tsaiya ducks raised in an individual cage under the ambient temperature of 12, 22, and 32 °C, respectively (with relative humidity of 80–85%) for nine weeks. The results showed that the 32 °C treatment group had significantly lower egg production, egg weight, eggshell thickness, and feed intake than the other two groups. The high ambient temperature also changed the eggshell microstructure. However, there were no significant differences in eggshell strength, number of gas pores, and Haugh unit.

21.6.6 Processing and marketing of egg

Duck egg quality is affected by the processing, storage, and marketing conditions. The cholesterol contents in the processed duck egg yolk are 12.27–12.50 mg/g yolk and 7.69–7.87 mg/g yolk in salted duck eggs and pidan, respectively (Yang and Chen, 2001). The main cholesterol oxidation products (COP) are 20-hydroxycholesterol and 7-β-hydroxycholesterol; other COP are not detected. In addition, the yolk in cooked salted eggs had significantly higher TBA value than that in the raw salted eggs and pidan made with immersing method or coating method (Yang and Chen, 2001). A regression equation for the correlation between additives in the pickling solution for making pidan and their residues in the pidan has also been established (Wang and Hsieh, 1995).

To improve the stability of heavy metal-free pidan, efforts have been put to investigate the effects of coating treatments (hot air drying, coating with mineral oil or albumen) on the storage quality of pidan. Groups of both hot air drying and albumen coating had the best performance in colour stability during the 12-week storage period (Wang *et al*., 1997b). Temperature control was later employed to stabilize the processing procedure for pidan without the addition of heavy metal. Optimal pickling period of pidan is 17, 13, and 11 days when the environmental temperature was 20, 25, and 30 °C (Wang *et al*., 1998). The salt percentage in salted eggs can be increased during processing and storage. Hadiwiyoto (2009) reported the salt content (% wet base) in the uncooked salted egg increased from 0.29% to 1.61% after 16 days of coating with a salting material, and it increased to 1.94% and 2.65% when stored at room temperature and low temperature (ca. 10 °C) for 16 days, respectively. It was also reported that the change in fat appeared to be a combination of salting time and storage time effects as analyzed by response surface methodology (Hadiwiyoto, 2009).

Potential microbial contamination in duck eggs has attracted attention. Fu *et al*. (1993) surveyed fresh eggs and environmental specimens including soil, duck feces, straw, and water from duck farms and processed egg from retail stores. *Salmonella* was not detected in all the egg yolk of duck eggs. Examination of the swabs of seven duck eggshell and 23 environmental samples showed *Salmonella typhimurium* was detected in only one soil sample from duck shed. Chen *et al*. (1994) later investigated the microbiological quality of the fresh duck eggs and processed duck eggs in the supermarket and wet market. Total plate counts in fresh duck egg, salted egg, and pidan were 4.5×10^3–6.8×10^5, 1.0×10^4–1.1×10^6, and 1.5×10^4–9.3×10^4 CFU/g, respectively. There were 3.6–9.1, 3.6–4.3, and 3.0–3.6 *Staphylococcus*/g in the fresh duck eggs, salted eggs, and pidan, respectively.

Fu and Su (1997) inoculated 50–100 CFU/egg yolk of *Salmonella enteritidis* CCRC 10744 and strain SEP in fresh eggs, respectively, before they were processed into salted eggs and pidan. For salted eggs, salt content increased from 0.56% to 0.98% after 30 days of pickling in the 35% (w/v) saltwater. In the first two days, the viable cells reached 10^7–10^8 CFU/g yolk and

maintained at 10^7/g yolk throughout processing. Similar growth curves were observed in the two strains. After processing, both strains were observed in most salted eggs after 2 months of storage at 25–30 °C. In terms of pidan, the pH value increased from 6.5 to 10.3 during processing for 30 days in the pickling solution containing major components of 7% salt and 4.3% sodium hydroxide. The changes in viable cell count were similar to those in salted eggs, with 10^6 CFU/g yolk at the end of processing. It decreased to 10^5 CFU/g yolk after one month of storage at 25–30 °C. After two and three months of storage, *Salmonella enteritidis* CCRC 10744 could be still detected in two and one egg yolks of the five sampled eggs, respectively. The strain SEP was observed in all five sampled eggs after two or three months of storage (Fu and Su, 1997).

Another study examined survival of *Salmonella* in the pickling solution. After 2×10^{10} *Salmonella* was inoculated into the 3 liter pickling solution containing 4% salt (w/v) and 5% sodium hydroxide (w/v) to process pidan for 19 days, *Salmonella* was not detected either in the pickling fluid or in the pidan after 19 days of pickling (Chen Y P, unpublished data).

21.6.7 Egg washing

There is a relative scarcity of studies on the washing effect on duck egg quality compared with hen eggs due to low entrepreneurship in the duck egg industry, selling behavior and consumption habit of duck eggs. Washing breeder duck eggs with chlorine sanitizer reduced the bacterial count on the duck eggshell (Baker *et al*., 1985). Lin *et al*. (2004) evaluated the effects of washing procedures on microbial and physical properties of duck eggs. The weight of washed duck eggs decreased by 2.6–3.4% and the pH increased after four weeks of storage at 25 °C. During this storage period, the Haugh unit dropped by 25–30 units due to thick egg white being turned into thin egg white. *Salmonella* was not detected either on the eggshell surface or in the egg contents. It is recommended that the washed duck eggs be stored less than three weeks at 25 °C (Lin *et al*., 2004).

21.7 Conclusion

The duck egg is an important source of nutrition in many Asian countries. The major duck egg producing countries have experienced increased growth in duck egg sales in past years. It is believed this trend will continue for some time. Although laying ducks are confined in some countries, in many areas in Asia ducks are still raised in backyards and duck herds. This situation hindered the prevention of avian influenza dissemination because duck herds become a virus reservoir if infected. The possible contamination of duck eggs by pesticides has also become an increasing concern. Attention needs to be paid to the risk of *Salmonella* contamination in duck eggs. It is also important

to note that duck eggs may be contaminated by some industrial by-products when developing countries become more industrialized and the areas for industrial and agricultural purposes are not clearly defined. Therefore, confined duck production is a necessary step for duck egg production sustainability. Duck production hazard analysis and the establishment of a comprehensive market survey system will become more important in the future.

21.8 References

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22

Production and quality of quail, pheasant, goose and turkey eggs for uses other than human consumption

A. Tserveni-Goussi and P. Fortomaris, Aristotle University of Thessaloniki, Greece

Abstract: The avian egg is an important source of nutrients required for the developing embryo and also a valuable material for the investigation of fundamental physiological processes. Non-table eggs are those produced from avian species that are not directed to food market. Their use is limited to the species reproduction, for research purposes and, to a lesser extent, for other industrial applications. The aim of this chapter is to provide the available information on the production, quality and composition of non-table eggs produced by different avian species (quail, pheasant, goose and turkey).

Key words: non-table egg production and quality, quails, pheasants, geese, turkeys.

22.1 Introduction

The avian egg, especially the hen's egg, has been used as a major food source by humans since antiquity because of its adaptability to many different types of recipes and its nutritive value. Being an important source of nutrients, containing all of the proteins, lipids, vitamins, minerals and growth factors required by the developing embryo, the avian egg is a valuable material for the investigation of fundamental physiological processes, including developmental processes, the response of the developing embryo to foreign substances, the production of immune antibodies and hydrolysis by enzymes (Romanoff and Romanoff, 1949).

The egg industry is dominated by hen's egg production (*Gallus gallus domesticus*) and there are no accurate data relating to the contribution of all

the other commercially important species. The food industries absorb the largest share of the hen's eggs with pharmaceutical and research laboratories utilizing a smaller proportion. Inedible eggs and hatchery discards are also used to some extent as raw materials for the manufacture of a small number of products. According to estimations from the US Department of Agriculture (USDA), for the year 2009, more than 80% of the hen eggs produced globally were used for consumption, while approx. 15–18% were hatching eggs. The remainder was used for other products.

Although there is a lack of official data, a very small proportion of the produced eggs from other avian species are marketed as table eggs. Duck and goose eggs, as well as smaller eggs such as quail eggs, are only occasionally used as a gourmet ingredient. The market of these eggs, especially those from geese and pheasants, is very limited because of their small and seasonal egg production. For instance, the breeding season of geese extends from October to May in Taiwan (Wang *et al.*, 2009), from January to June in France (Sauveur, 1982), and from October to Match in Israel (Pyrzak *et al.*, 1984). In quail a large variation of production type (meat or egg) is observed among different countries of the world; Japanese quails are produced mainly for eggs in Asian countries and in Brazil (Minvielle, 2004). Pheasant and turkey eggs are also edible but are even less widely available in comparison to the above species.

Non-table eggs can be defined as the eggs produced from avian species and are not directed to food market; their use is limited to the species reproduction, for research purposes and, to a lesser extent, for other industrial applications. As mentioned above, almost 15% of the hen eggs produced can be regarded as non-table ones, while for the other avian species such as quails, pheasants, geese and turkeys, the vast majority of the produced eggs are considered to be non-table.

The objective of the present review is to provide the available information on the quality and composition of non-table eggs produced by different avian species (quail, pheasant, goose and turkey). Furthermore, general information on the commonly used breeds and the egg production characteristics of the above species are also provided as well as their potential use in the field of research. Because of the commercial importance of ducks in their own right, the information relating to this species has already been presented in Chapter 21.

22.2 Uses of non-table eggs

The avian egg is a valuable raw material for many industrial products due largely to its colloidal nature; its ability to form and stabilize emulsions and the coagulability of its proteins gives it special importance for the makers of food products (Romanoff and Romanoff, 1949). Other properties enable the egg to be used in such diverse ways as in manufacturing and in various applied

arts and sciences. Moreover, eggs also contain substances with biological functions beyond basic nutrition, and extensive research has recently been undertaken to identify and characterize these biologically active components (Kovacs-Nolan *et al.*, 2005).

The use of eggs in the animal feed industry was reported as early as the beginning of the 20th century (Romanoff and Romanoff, 1949). Although experiments have shown that eggs are as nutritionally valuable for animals as for human beings, eggs and egg products have generally been considered too expensive to be fed to animals. However, eggshell waste has been used in the animal feed industry as a source of calcium. Since eggshells contain approximately 93% calcium carbonate, they are as good a source of this mineral for chicks and laying hens as the oyster shells and limestone generally used for this purpose. In a report prepared by ADAS Consulting Ltd (2002) it was estimated that 10 000–11 000 tonnes of eggshell have to be disposed of each year by egg processors and producers of hard cooked eggs. In the same report, the long-term solutions proposed included the separation of the eggshell from the membrane and their usage for several purposes such as the use of membrane in material science and the use of purified eggshell in the paper industry and in agriculture as a lime substitute or calcium supplement. The use of eggshell in soil science has also been recently proposed as a stabilizing material (Amu *et al.*, 2005), while ground eggshell was found to be an effective dye absorbent in aqueous solutions (Tsai *et al.*, 2008).

The use of eggs in the field of medicine and medical research is well documented. Bacteriological laboratories use large quantities of eggs each year in the preparation of culture media. Because of its high nutritive value, the egg is a good enriching agent. Egg yolk is an excellent source of lecithin, protein and phosphoric acid, and egg white is able to supply all the nitrogen required by some bacteria. As an ingredient for culture media the egg has the advantage of being easily obtainable. Media containing eggs are quickly prepared and therefore are valuable for comparative studies.

The embryonated chicken egg has long been one of the most widely used host systems for the isolation, propagation and characterization of avian viruses and the production of viral vaccines. Fertile eggs have also become very important in the production of immunizing sera and vaccines against human and animal diseases. In general, any type of egg (e.g. quail, duck or turkey) could be used for this purpose (Cottral, 1978). In practice, the poor laying rate and the high cost of these eggs make the commercial production for pharmaceutical use quite difficult (M. Kock, 2010, personal communication). The total number of eggs used for this purpose is currently estimated at about 600 million eggs per annum (Kock and Seemann, 2008). The market for fertile eggs, commonly called ‘vaccine eggs’, consists of different segments with specific requirements concerning production and quality; in general, there are two major categories of eggs for vaccine production: ‘Clean Eggs’ and ‘Specific Pathogen Free’ (SPF) Eggs (Kock and Seemann, 2008).

Antibodies deposited in the avian egg have been found to be a platform for the production of a diverse array of safe commercial products for improving animal health and the efficiency of their products; egg antibody technology has resulted in a broad range of products that can be used in animal feeds, human foods and animal/human pharmaceuticals (Cook and Trott, 2010). New biotechnology is being used to develop genetically modified chickens that produce compounds that can be harvested from the eggs, such as insulin for the treatment of diabetes. As new knowledge is gained in this area, designer eggs in the future may be produced that result in a range of antibodies for treatment against various health problems (Jacob and Miles, 2008). Furthermore, eggs are largely used for manufacturing purposes involving the leather industry, art materials, fertilizers and various synthetic products.

22.3 Non-table egg production, composition and quality

The production, composition and quality of non-table eggs from different avian species, although they share common characteristics, are really quite diverse. In the following sections egg production characteristics of the most commonly used breeds are given, then the chemical composition and quality characteristics according to the species are presented.

22.3.1 Egg production characteristics

Quail

Quails are mid-sized birds belonging to the order Galliformes of the Phasianidae family. Among the various genera of the quails worldwide the most used representative in egg production is considered to be the Japanese quail (*Coturnix japonica)*. Japanese quails are migratory game birds (Sanford, 1957; Weatherbee and Jacobs, 1961). Since the domestication of Japanese quail during the last few decades, they have been used extensively for production purposes. The Japanese quails have been also used widely as a model species in research on poultry breeding and genetics of growth traits (Wilson *et al*., 1961; Marks, 1990; Baumgartner, 1994). They have been used for studies in nutrition, embryology, genetics, toxicology, physiology, endocrinology, oncology and gerontology, and in biomedical research including virology (Ratnamohan, 1985; Shanawany, 1994). However, Japanese quail research measured by the number of published papers has gradually diminished over the past years and the trend appears to be due to the decrease of works using Japanese quail as an animal model or for biological studies (Minvielle, 2004).

Accurate data on Japanese quail table egg production worldwide are difficult to obtain despite significant quail farming in Europe, America and

Asia (Minvielle, 2004). The leading countries in quail table egg production are China, Japan and Brazil (7.0, 1.8 and 1.7 million eggs annually, respectively; Minvielle, 2004). However, it is difficult to estimate the number of non-table eggs produced by quails.

Japanese quails are usually kept in cages under commercial conditions but they can also be kept in deep-litter floor systems. A minimum space allowance suitable for quails is 145 cm^2 when they are raised in floors and 125 cm^2 when raised in cages. Quails raised under intensive conditions require proper care and disease prevention and control programmes.

In nutritional studies in breeder quails Yannakopoulos and Tserveni-Gousi (1989) reported the following traits: initial body weight 167.9 g (at day 42), final body weight 212.6 g (at day 105), gain 44.7 (42–105 days), food consumption 31.4 g/bird/day, egg production 87.1%, egg number 54.9 eggs/bird, average egg weight 10.8 g and feed/egg 36.0 g. Similarly production traits recently reported by Abdel-Mageed *et al*. (2009) in Japanese quail breeders were: initial body weight 204.03 g/bird, final body weight 227.00 g/bird, feed intake 19.95 g/hen/day, feed conversion ratio 2.10, egg production 81.91%, 0.82 eggs/hen/day, egg weight 11.62 g, egg mass 9.52 g/hen/day.

Good layer strains of Japanese quail reach a peak production of 92–95% and produce between 300 and 320 eggs during the laying cycle. Bobwhite (*Colinus virginianus*) strains reach a lower peak between 80 and 85% and produce around 250 eggs in a year (Shanawany, 1994).

Quail eggs are nearly one-fifth of the size of chicken eggs. Generally, egg weight ranges from 6 to 16 g, with an average weight of 10 g. This represents about 8% of the body weight of the quail layer, in contrast to chicken and turkey eggs, which represent about 3.5% and 1.0% of body weights, respectively (Panda *et al*., 1979; Bitman and Wood, 1980). Unlike the eggs of chickens and turkeys, the first quail egg of a sequence is smaller than the succeeding eggs (Woodard and Wilson, 1963).

The main reproduction characteristics of the Japanese quails can be summarized as follows: age in maturity 6–7 weeks, age in maximum egg production 7–8 weeks, life expectancy 2.5 years, body weight of adult male 100–140 g, and adult female 120–160 g, incubation and hatching period 17–18 days, egg weight 6–16 g, egg production up to 280–300 eggs/bird in the first year (Reddish *et al*., 2003; Sezer, 2007).

Pheasants

Pheasants are also members of the Phasianidae family. Being large birds, pheasants are characterized by strong sexual dimorphism with male birds being highly ornate with bright colours and adornments such as wattles and long tails. There are 35 species of pheasant belonging to 11 genera; the best-known is the common pheasant or ring-necked pheasant (*Phasianus colchicus*), which has different subspecies and is widespread throughout the world in introduced feral populations and in farm operations. Pheasants

are native to Asia, but have been introduced elsewhere as a game bird. Today, the common pheasant lives either in the wild or in breeding facilities (pheasantries), from where it is released and subsequently hunted.

Pheasants are kept under commercial conditions in range systems while in intensive ones can be kept both in cages and in deep-litter floor. A minimum space allowance suitable for pheasants is 4–8 m^2 per bird in range conditions and only 0.5 m^2 per breeder when kept intensively. Pheasant breeder facilities are usually external aviaries with a sex ratio of one cock to every five to seven hens (Larbier and Leclercq, 1992). According to Deeming and Wadland (2002) the most favourable effects in reproduction characteristics of pheasants were observed when the mating ration is 8:1 (females: males) rather than 12:1.

Pheasants are seasonal breeders. Hens will begin laying eggs in about the middle of April and continue into June. Pheasant eggs are used almost exclusively for reproduction and the hatch results depend on their biological value; the hatch results are most considerably affected by egg weight, shape index, shell thickness and its porosity, and the share of the yolk, albumen and shell in the egg (Kuźniacka *et al.*, 2005). The mean egg production in pheasants has been found to vary between 19.38 and 54.09% in different studies (Çetin *et al.*, 1997; Tepeli *et al.*, 2002; Kirikçi *et al.*, 2003; Demirel and Kirikçi, 2009). According to Tserveni-Gousi and Yannakopoulos (1990), egg production through 10 weeks of lay is affected by the higher summer (June–July) temperatures; the peak production was reached during the third week of lay, and average egg production per hen was 42.3 eggs (51.4%).

Variation in pheasant egg weight has been also reported with values ranging from 28.1 g to 33.4 g (Woodard and Snyder, 1978; Woodard *et al.*, 1983; Blake *et al.*, 1987; Slaugh *et al.*, 1988; Tserveni-Gousi and Yannakopoulos, 1990; Çetin *et al.*, 1997; Kirikçi *et al.*, 2005; Garip *et al.*, 2010). Esen *et al.* (2010) reported differences in egg production characteristics of ring-necked pheasants according to age, with values being decreased over time. The pheasant egg weight is lower than the hen's egg weight as this parameter is positively correlated with the adult bird and chick body weight. Over the time, the pheasant's natural selection was directed only to increase the embryo survival, while the laying hen's egg is the result of high selective pressure directed to increase the production and weight of eggs (Mangiagalli *et al.*, 2003).

The main reproduction characteristics of the common pheasants are summarized as follows: age at first laying 40 weeks, age at maximum egg production 44–47 weeks (4th–7th laying week), life expectancy 3–5 years (reproductive periods: 2–3), body weight of adult male 900–1200 g, and adult female 700–900 g, incubation and hatching period 23–28 days, egg weight 28–34 g, average annual egg production 30–50 eggs/bird (Tserveni-Goussi and Yannakopoulos, 1990; Kuźniacka *et al.*, 2005; Krystianiak *et al.*, 2007).

Geese

Geese belong to the family Anatidae and were one of the first domesticated animals, probably in Egypt about 3000 years ago (Buckland and Guy, 2002). Despite this, geese have never been exploited commercially as much as chickens or even ducks have been.

Geese are found worldwide. They can adapt equally well to hot climates as to cold climates – as seen in their ability to withstand northern winters outdoors with the minimum of shelter. In spite of this broad adaptability, commercial goose production is only important in relatively few countries in Asia and Europe. The FAO's Animal Genetic Resources database (AnGR) identifies 204 different breeds or varieties of geese. Crosses between the domestic breeds which have originated from two species of wild geese (*Anser anser*: greyland goose and *Anser cycnoides*: swan goose; Silversides *et al.*, 1988) are fertile and in fact have resulted in a number of recognized breeds. Many of these are thought to have little economic importance because of their relatively low production or performance levels, or a limited geographical distribution. In addition to these breeds, both old and new, there are a number of commercial cross-breeds made available by companies specializing in goose breeding.

According to published data, although the great majority of the world's geese are concentrated in Asia, there is considerable breed diversity to be found in Europe (Romanov, 1999). To judge from recent estimates (FAO, 2008), the leading goose producer in the world is China, with 86% of the world's stock and 92% of goose meat production. Considerable native goose genetic resources have been accumulated in the countries of Eastern Europe (Romanov *et al.*, 1996), especially in Russia, Ukraine and Poland. Geese are raised in practically all parts of the United States, although they total only 0.2% of the poultry population.

Management systems applied to breeding geese are generally of the following two types: intensive (in premises–deep litter, cages or slats) and extensive (on pasture; Bogenfürst *et al.*, 1997). Preference for either type depends on the breeding and production traditions and the objectives of raising birds (Romanov, 1999).

The management of geese has a considerable impact on egg production (Bogenfürst *et al.*, 1997). Geese are not prolific egg producers, laying only 30–50 eggs each year according to breed, mostly in spring. Because the number of eggs laid by each goose during one laying cycle is relatively low, a high mortality of embryos is particularly disadvantageous (Rosinski and Bednarczyk, 1997). Domestic geese are seasonal breeders and among all poultry species, have the lowest reproductive capacity, a reflection of poor semen quality and low fertility and hatchability rates. Geese can live for 20 years or more, but their reproductive potential remains at an acceptable level only up to the fifth reproductive season. They are usually kept for three to four seasons in breeding practices; however, in the first reproductive season the egg fertilization and hatchability rates are lower than those in the next

seasons. In the study of Rosinski *et al*. (1995) reproductive data for two breeder flocks over annual lay periods that extended for 12 successive laying seasons showed that egg weight increased from the first until the sixth season, while egg number and fertility were suppressed from the eighth laying season. Furthermore, it has been shown that goose breeds that originate from *Anser cycnoides* (e.g. the Kuban goose) demonstrate, in general, better reproductive performance (egg number, fertilization and hatchability) than breeds that originate from *Anser anser* (e.g., the White Italian goose) (Bednarczyk *et al*., 1985; Smalec, 1991).

The main reproduction characteristics of geese can be summarized as follows: age in maturity 32–35 weeks (2–3 weeks later in males), age in maximum egg production 40–45 weeks, life expectancy 15–20 years (limited to 4–5 reproductive seasons), body weight of male breeders 3.4–12 kg (depending on the genus), body weight of female breeders 2.9–9 kg (depending on the genus), incubation and hatching period 28–31 days, egg weight 120–180 g (more than 200 g in specific breeds), average annual egg production 30–50 eggs/bird (120–130 eggs for specific breeds).

Turkeys

Turkeys are large birds belonging to the order Galliformes of the Phasianidae family and Meleagridinae subfamily. There are two species of turkey, *Meleagris gallopavo* and *Meleagris ocellata,* both being domesticated many years ago. The modern domesticated turkey descends from the wild turkey, *Meleagris gallopavo*. Although domesticated more than 500 years ago turkeys were initially selected for their plumage; only in the 20th century were they exploited for their meat. Today, eight breeds of domestic turkey are recognized by the American Poultry Association, while others exist as officially unrecognized variants or as recognized breeds in other countries. The most used breed worldwide in poultry industry is the Broad-Breasted White, which developed from the cross of White Holland and Broad-Breasted Bronze breeds.

Commercial turkey breeding showed a dynamic growth since the 1970s with the main production goal being turkey meat. Genetic selection applied in turkey breeding resulted in hybrid strains that grow faster with more efficient feed conversion ratios. However, the turkey's reproduction cycle is still the shortest of any other poultry species while their egg production is similarly the lowest. One component of the poor reproductive efficiency of commercially bred female turkeys is their relatively poor egg production, which is related to the early induction of incubation behaviour and the cessation of egg laying (El Halawani *et al*., 1988). Commercial turkey breeders rely on management procedures to extend turkey hens' egg laying period and discourage nesting and incubation. Breeding stock is usually kept in environmentally controlled houses that provide control of heating, ventilation and lighting. The latter is of major importance due to novel findings (El Halawani, 2006), suggesting that turkeys' egg laying activity is regulated

by two light pathways, an inhibitory pathway and stimulatory pathway. Egg production is dependent upon the relative activation of the two pathways. The inhibitory pathway is activated by stimulating retinal photoreceptors by the green band of the spectrum, and the stimulatory pathway is activated by the direct action of the red band on photoreceptors in the brain. It has been shown that photostimulation of breeders with separate light sources, one stimulatory (red) and another inhibitory (green), enable one to optimize the red/green band ratio as well as the stimulatory wavelength and its energy level to maximize egg production.

In terms of egg production turkeys are grown to a wide range of weights and sizes according to their genotype. In wild turkeys, egg production is seasonal; egg production starts in March to April and does not persist past July to August (Bailey and Rinnel, 1967). In commercial turkey breeders (e.g. British United Turkeys, Aviagen, 2010) the number of eggs per hen in the 25–28 week breeding period does not exceed 120, with egg weight varying from 80 to 95 g in the start and the end the production cycle, respectively. The average male body weight may exceed 35 kg while female birds weigh no more than 12.5 kg in the end of their laying period.

Egg production characteristics are largely dependent on feed restriction programmes applied to turkey breeders; the general notion is that food restriction of turkey breeders, during the growing period results in superior reproductive performance as well as a reduction in feed cost, with age of breeder, season of implementation and length of physical feed restriction having significant effects on the reproductive performance of turkey breeder hens (Crouch *et al.*, 2002).

The main reproduction characteristics of the avian species discussed so far in this chapter are given in Table 22.1.

Table 22.1 Main reproduction characteristics in quail, goose, pheasant and turkey breeders

Reproduction characteristics	Quail	Pheasant	Goose	Turkey[1]
Age in maturity (weeks)	6–7	40	32–35	33
Age in maximum egg production (weeks)	7–8	44–47	40–45	35–38
Life expectancy (years)	2.5	3–5	15–20	10
Male body weight (kg)	0.1–0.14	0.9–1.2	3.4–12	37
Female body weight (kg)	0.12–0.16	0.7–0.9	2.9–9	12.5
Incubation and hatching period (days)	17–18	23–28	28–31	28
Egg weight (g)	6–16	28–34	120–180 (>200)	80–95
Egg production (eggs)	280–300	30–50	30–50 (120–130)	120

[1]Values obtained from BUT, *Big 6 Performance Goals*, 5th Edition.

22.3.2 Egg quality and composition

Quail

Quail is one of the most studied avian species in terms of egg composition and quality. Physical composition of quail eggs is broadly similar to the domestic fowl eggs with the exception of the egg size (Panda and Singh, 1990). Results from earlier studies reviewed by the same authors showed that the average moisture content of the whole quail egg is about 74.0%, total protein content is 13.0%, total fat is 11.0% and the total ash content is 1.1%.

Quality of the breeding eggs has an overall significance on economic breeding. Traits related with external quality of the eggs have effects on the hatchability and development of the chicks (Shanawany, 1987; Tserveni-Gousi, 1987). Quality characteristics in quail eggs have been reported in several studies concerning quail breeders. Yannakopoulos and Tserveni-Gousi (1986) and Tserveni-Gousi (1987) reported that the quail egg weight tended to increase with increasing age of birds, with the overall value for egg weight in both study being about 12.0 g.

Kumari *et al*. (2008) reported data on egg quality traits of 607 black and brown strains of Japanese quails, at 16 weeks of age, obtained from 109 sires in their study on the effect of strains, generations and hatches. According to their results the following overall means were found for egg quality characteristics: egg weight 13.71 g, egg length 34.12 mm, egg width 26.98 mm, shell weight 1.17 g, shell thickness 0.21 mm, albumen length 43.14 mm, albumen width 33.81 mm, albumen height 4.88 mm, albumen weight 7.80 g, yolk diameter 25.19 mm, yolk height 11.29 mm, yolk weight 4.74 g, yolk colour 5.37, shape index 79.23, albumen index 0.13, yolk index 0.45 and Haugh unit score 58.27. The albumen, yolk and shell constituted 56.83, 34.61 and 8.56% of the egg weight, respectively. Brown Japanese quails exhibited significantly higher means for egg width (27.07 mm), shell weight (1.18 g), albumen length (44.03 mm), yolk diameter (25.30 mm), yolk colour (5.50), shape index (79.57) and percentage of shell (8.51), while black quails were found superior for albumen width (33.98 mm), albumen height (4.98 mm), albumen index (0.13) and Haugh unit score (59.50), which showed the existence of significant genetic differences. Significant effect of strains on egg quality traits was also reported by Praharaj *et al*. (1989) and Oroian *et al*. (2002).

Roshdy *et al*. (2010) studied the effect of two different housing systems (floor pens vs battery cages) on productive and reproductive traits of Japanese quail. The results of this study showed that quails kept on floor pens had significantly higher values in laying rate, egg number, egg weight and egg mass than those kept in battery cages. Also, Haugh unit values were higher in caged birds than those on floor pens, whereas yolk index values were significantly higher on floor than in the cage system. Further studies in egg quality characteristics from quail breeders reported varying results. These results are summarized in Table 22.2.

Table 22.2 Quality characteristics of Japanese quail eggs

Characteristics	Yannakopoulos and Tserveni-Gousi (1986)	Florou-Paneri *et al.* (1997)	Nazligul *et al.* (2001)	Kumari *et al.* (2008)	Bonos (2010)
Egg weight (g)	12.23	12.25	10.41	13.71	11.58
Egg albumen (%)	59.93	–	61.22	56.83	59.72
Egg yolk (%)	32.50	–	31.55	34.61	31.64
Egg shell + membrane (%)	7.75	8.57	7.89	8.56	8.64
Egg specific weight	–	–	–	–	1.071
Egg shape index	78.28	78.30	79.90	79.23	79.94
Eggshell thickness (mm)	0.193	0.190	0.206	0.210	0.223
Eggshell deformation ($mm \times 10^{-3}$)	–	–	–	–	36
Egg yolk diameter (cm)	–	–	–	2.52	2.46
Egg yolk colour–L*	–	–	–	–	77.55
Egg yolk colour–a*	–	–	–	–	7.57
Egg yolk colour–b*	–	–	–	–	59.23

Quail eggs are characterized by a variety of shell colour patterns, ranging from dark brown to blue, white or speckled as a result of the deposition of ooporphyrin and biliverdin, the major constituents of egg shell pigments (Poole, 1965). Woodard and Mather (1964) found that pigmentation of the shell occurs approximately 3.5 h before oviposition in *Coturnix* quail laying on a 25 h rhythm. Quails are ground nesting birds and their multicoloured eggshells give this necessary advantage to protect their eggs from predators (Sezer and Tekelioglu, 2009). Unlike other plain pigmented avian eggs, colourful quail eggs, as a model system, provide more opportunities to study a wide variety of questions such as the metabolism of pigment deposition, its relationship to overall bird physiology, egg quality and sexual behaviours (Sezer and Tekelioglu, 2009).

Among the quality characteristics of quail eggs, the fatty acid profile of the egg yolk lipids is of major importance due to the yolk's role in embryo development and its survival (Donaldson and Fites, 1970). Lipids are the major nutritive components of the yolk; the oxidation of yolk-derived fatty acids provides the embryo with almost all its energy needs and the transport and transformation of yolk lipids represent the predominant metabolic features of the embryo (Speake *et al.*, 1998). In bird species, the only source of polyunsaturated fatty acids (PUFA) for the embryo is the yolk; for this reason, the yolk fatty acid composition is essential for the optimal development of neural embryo tissues (Fronte *et al.*, 2008).

There is a reasonable amount of published information on the fatty acid profile of egg yolk lipids in quail breeder eggs. The results of the most recent relevant studies are summarized in Table 22.3.

Table 22.3 Fatty acid profile in egg yolk lipids of Japanese quails

Fatty acids	Choi *et al.* (2001)	Aydin and Cook (2004)	Da Silva *et al.* (2009)	Bonos (2010)
Myristic acid (C14:0)	0.6	0.52	0.56	0.23
Palmitic acid (C16:0)	27.4	29.54	29.4	22.81
Palmitoleic acid (C16:1)	6.6	3.61	3.90	2.62
Stearic acid (C18:0)	8.3	11.66	9.78	12.40
Oleic acid (C18:1, n-9)	44.5	42.2	38.3	40.77
Linoleic acid (C18:2)	9.1	9.99	12.9	16.44
Linolenic acid (C18:3, n-3)	0.2	0.76	0.25	0.74
Saturated fatty acids (SFA)	36.4	41.71	39.7	35.44
Monounsaturated fatty acids (MUFA)	51.6	45.81	44.7	47.38
Polyunsaturated fatty acids (PUFA)	12.0	12.17	15.5	17.18

Pheasants

The physical composition of pheasant eggs is also similar to the other avian species with an average moisture content being 72.0%, total protein content being 12.5% and total lipids being 13.8% of the whole egg (Mangiagalli *et al.*, 2003). Out of all the morphological traits of the egg, the albumen is of greatest importance, constituting protection for the yolk and the embryo from pathogenic microorganisms and providing water, protein and other nutrients indispensable for appropriate growth and development (Benton and Brake, 1996; Narushin and Romanov, 2002). The main quality characteristics in pheasant eggs reported in the literature concerning pheasant breeders are summarized in Table 22.4.

In general, pheasants lay eggs of different shell colour: dark-brown, light brown, olive and blue (Krystianiak *et al.*, 2000). The physical traits and quality characteristics of eggs with different shell colour show significant variation (Krystianiak and Kontecka, 2002). For instance, blue-shelled pheasant eggs weigh less than those of other shell colour (Kirikçi *et al.*, 2005; Table 22.4) and are also characterized by thinner shells (45.9 mm on average) than olive and dark-brown eggs (Hulet *et al.*, 1985; Richards and Deeming, 2001; Krystianiak *et al.*, 2005; Koźuszek *et al.*, 2009).

As seen in Table 22.4, the shape index of white eggs of blue, brown and olive green eggs is similar to the value of 80.24 reported by Tserveni-Gousi and Yannakopoulos (1990), while yolk and albumen weights determined in brown eggs and olive green eggs are heavier than the respective values of 9.78 and 16.0 g, reported by Tserveni-Gousi and Yannakopoulos (1990).

The information on the fatty acid profile of egg yolk lipids in pheasant breeders is rather limited. Figure 22.1 shows the fatty acid profile in egg yolk lipids of common pheasants as reported by Choi *et al.* (2001) and Mangiagalli *et al.* (2003). Furthermore, Speake *et al.* (1999) reported that the fatty acid profiles of the yolk lipid classes of the wild pheasants differed in many respects from those of their farmed counterparts. These differences

Table 22.4 Quality characteristics of pheasant eggs with different eggshell colour (Kirikçi *et al*., 2005)

Characteristics	Eggshell colour			
	White	Blue	Brown	Olive green
Egg weight (g)	28.10	26.71	31.89	31.16
Shape index	77.87	81.24	80.55	80.98
Yolk index	43.05	40.65	42.27	43.75
Albumen index	1.40	1.32	1.39	1.37
Eggshell thickness (mm)	0.202	0.210	0.230	0.220
Membrane thickness (mm)	0.003	0.004	0.003	0.003
Eggshell weight (g)	2.789	2.768	3.210	3.166
Eggshell weight (%)	9.88	10.40	10.07	10.18
Membrane weight (g)	0.541	0.740	0.530	0.592
Membrane weight (%)	1.93	2.77	1.67	1.91
Haugh Unit	83.96	79.91	82.12	81.41
Yolk weight (g)	9.03	9.57	10.72	10.13
Yolk weight (%)	31.99	35.91	33.65	32.57
Albumen weight (g)	16.28	14.37	17.96	17.79
Albumen weight (%)	58.13	53.65	56.28	56.98

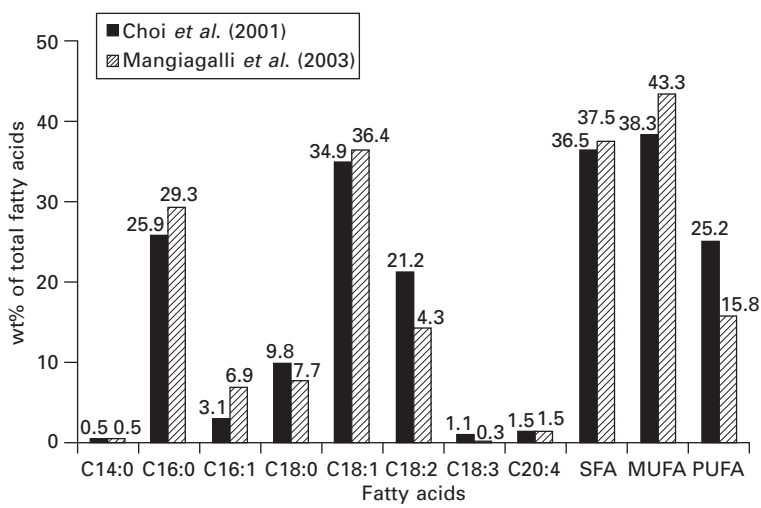

Fig. 22.1 Fatty acid profile in egg yolk lipids of common pheasants.

in egg lipid composition between wild and captive pheasants are shown in Table 22.5.

Geese

Data on the physical composition of geese eggs are limited. The few studies on the geese egg composition show that in general geese eggs are similar in terms of chemical composition to the eggs of the other avian species.

Table 22.5 Fatty acid profile in egg yolk lipids of farmed and wild pheasants (Speake *et al*., 1999)

	Triacylglycerol		Phospholipid		Cholesteryl ester	
	Farmed	Wild	Farmed	Wild	Farmed	Wild
Lipid class (wt% of total lipid)[1]	67.1	67.3	25.4	25.4	1.2	1.3
Fatty acid (wt% of total fatty acids)						
Myristic (C14:0)	0.6	0.7	0.1	0.1	0.0	0.0
Palmitic (C16:0)	26.9	24.2	27.1	25.5	13.5	12.0
Palmitoleic (C16:1n-7)	4.6	3.7	1.6	1.2	3.3	2.1
Stearic (C18:0)	5.9	7.2	16.5	19.8	4.9	7.4
Oleic (C18:1n-9)	43.1	25.4	25.0	26.4	39.7	32.3
Linoleic (C18:2n-6)	14.3	8.7	18.5	10.3	31.5	17.2
α-Linolenic (C18:3n-3)	1.6	27.9	0.4	4.6	1.7	25.5
Arachidonic (C20:4n-6)	0.1	0.1	3.3	3.2	0.0	0.0
Docosahexaenoic, DHA (C22:6n-3)	0.1	0.1	4.2	3.7	1.7	1.4

[1] The amounts of total lipid (wt% of fresh yolk) were 33.6 (farmed) and 32.8 (wild).

With respect to the quality characteristics of geese eggs, in a study regarding the effects of origin and storage time on some interior and exterior egg properties in 1-year-old Armutlu, Tatlicak, Başkuyu and INRA geese, Tilki and Inal (2004) concluded that the mean values were 145.1, 148.5, 147.2 and 144.2 g for egg weight, 19.4, 21.6, 20.0 and 18.7 g for shell weight, 13.7, 14.8, 13.8 and 13.3% for shell ratio, 0.54, 0.58, 0.55 and 0.55 mm for egg shell thickness, 67.1, 70.3, 66.7 and 68.0% for shape index, 72.9, 73.7, 74.1 and 77.0 g for albumen weight and 51.2, 50.6, 51.2 and 54.6% for albumen ratio. Mean values for Haugh unit, albumen index and yolk index of freshly broken eggs (without storage) were 76.9, 9.19 and 37.1%, respectively.

The published information on the fatty acid profile of egg yolk lipids in geese eggs is very limited. Speake *et al.* (1999) in their study in the lipid composition between wild and captive geese found that the proportion of 18:3n-3 in triacyglycerol was extremely low in eggs from housed geese but was 10 times greater in eggs from free-range geese and was a further 3 times greater in eggs from feral Canada geese. Although 18:3n-3 was only a very minor constituent of goose yolk phospholipids, its representation was 11 times greater in the free-range compared with the housed samples and a further 2.5 times greater in the feral eggs. With regard to the cholesteryl ester fraction of the goose eggs, a 10-fold increase in the proportion of 18:3n-3 in the free-range compared with the housed samples was accompanied by a commensurate decrease in the level of 18:1n-9. Table 22.6 shows the fatty

Table 22.6 Fatty acid profile in egg yolk lipids of housed (H) free-range (FR) and feral (F) geese (Speake *et al.*, 1999)

	Triacylglycerol			Phospholipid			Cholesteryl ester		
	H	FR	F	H	FR	F	H	FR	F
Lipid class (wt% of total lipid)	6.9	69.4	69.9	23.7	24.8	24.6	1.1	1.3	1.5
Fatty acid (wt% of total fatty acids)									
Myristic (C14:0)	0.3	0.4	0.4	0.1	0.1	0.1	0.7	0.9	0.6
Palmitic (C16:0)	24.8	26.4	22.7	30.3	29.2	29.9	14.5	13.6	12.1
Palmitoleic (C16:1n-7)	2.4	3.9	3.9	0.8	1.3	1.3	2.5	2.8	2.9
Stearic (C18:0)	4.5	3.8	3.8	12.1	13.1	12.4	4.3	5.1	3.0
Oleic (C18:1n-9)	62.1	49.6	41.2	33.2	30.8	33.5	56.2	46.6	45.7
Linoleic (C18:2n-6)	3.9	5.1	4.7	6.5	6.1	4.9	13.5	12.6	9.6
α-Linolenic (C18:3n-3)	0.6	6.4	19.3	0.1	1.1	2.7	0.4	4.2	11.8
Arachidonic (C20:4n-6)	0.1	0.1	0.2	9.3	7.5	7.1	2.3	2.5	1.5
Eicosapentaenoic, EPA, (C20:5n-3)	0.0	0.1	0.3	0.4	1.2	1.8	0.0	1.0	1.2
Docosapentaenoic, DPA, (C22:5n-3)	0.1	0.2	0.3	0.5	1.0	0.9	2.1	2.9	1.3
Docosapentaenoic, DHA, (C22:6n-3)	0.0	0.2	0.3	4.8	5.7	3.8	–	–	–

acid profile in egg yolk lipids of housed (H) free-range (FR) and feral (F) geese (adapted from Speake *et al*., 1999).

Turkeys

Physical composition of turkey egg does not differ from those of the avian species already discussed. In a very recent comparative study, Sinanoglou *et al*. (2011) showed that the variation in the moisture content in turkey, quail and goose edible egg yolks was less than 10% (values 43.5%, 46.2% and 44.9%, respectively). In the same study, the average ash content ranged from 2.6 to 2.9%, while the fat content in quail egg yolks was found the lowest among these species (values being 32.3%, 27.5% and 32.6% for turkey, quail and goose egg yolk fat, respectively).

The number of studies on the quality characteristics of turkey eggs is limited to breeder eggs mainly because the observed changes in these characteristics are closely related to the egg fertility and the embryo survival. Table 22.7 shows the quality characteristics of turkey eggs as reported by various workers in this field. Interestingly, differences in the overall values shown are small and indicative of the stability of egg quality characteristics over time.

Considering the various egg sizes, data suggest that the larger the egg, the greater the weight of poults, yolk and solid yolk components; this is similar to the observation of Tindall and Morris (1964) that larger eggs develop larger poults, and the observation of Bray (1965), who reported a high correlation between egg weight and hatching weight (Reidy *et al*., 1998). As Carey *et al*. (1980) pointed out, the amount of nutrients needed for embryonic development is fairly constant between species and extra yolk could be used for posthatch needs.

Applegate and Lilburn (1996) and Christensen *et al*. (2001) showed that in turkeys the weight of eggs laid at the age of 33–36 weeks was significantly smaller compared with those laid by birds at the age of 54–55 weeks. According to Bains (1994), the optimal egg albumen and eggshell quality was observed by the time of peak egg production age of 35 weeks.

Table 22.7 Main quality characteristics of breeder turkey eggs

Characteristics	Ricklefs (1977)[1]	Godwin *et al*. (2005)	Hristakieva *et al*. (2009)
Egg weight (g)	85.0	80–93	84.7–88.9
Eggshell weight (g)	10.0	7.7–8.2	9.4
Egg albumen weight (g)	44.2	48.2–55.6	49.2–50.9
Egg yolk weight (g)	27.4	24.2–32.0	26.0–28.9
Egg shape index (%)	–	–	72.3–73.3
Eggshell thickness (mm)	–	0.47–0.53	0.43–0.44
Egg yolk colour (Roche scale)	–	–	4.5

[1]Adapted from the work published by Romanoff and Romanoff (1949).

Applegate *et al.* (2005) reported that the egg, yolk and albumen weight of fertile commercial turkey eggs (34- and 44-week-old hens) was 86.63 g, 24.18 g (27.97%), and 52.64g (60.70%), respectively.

Turkey hen age at sexual maturity is positively correlated with egg size and egg size is positively correlated with poult weight (Reinhart and Moran, 1979). Increased egg weight with advancing hen age, however, is not positively correlated with proportional increases in egg components. Reidy *et al.* (1994) reported that the weight of eggs from commercial turkey breeders increased approximately 11% between the onset of lay and 24 weeks of production. During this time period, yolk weight increased 21% but albumen weight only increased 7%. The magnitude of these changes differed slightly depending upon commercial genotype.

Studying hen production age-associated changes in egg weights from Nicholas turkey hens, Applegate and Lilburn (1996) reported an increase in the yolk to albumen ratio from 0.53 to 0.62 from two hen production ages (36 to 41 weeks of age) with only a 0.1 g increase in egg weight over this production period. As egg weight increases during the initial 10 weeks of production, there is a concomitant increase in the yolk to albumen ratio from 0.50 to 0.58 (Applegate and Lilburn, 1998). The differences reported in hen production age-associated changes in egg weights and egg component weights between these studies may be largely a reflection of measuring different strains over differing hen production periods. With increasing hen production age, there is proportionately more yolk deposited in the egg at the expense of albumen, although this does not appear to influence the poult weight at hatch relative to egg weight at set (Applegate and Lilburn, 1998).

Similar changes in the relative proportion of egg components with increasing hen production age have also been reported in previous studies with turkeys (Moran and Reihart, 1980; Reidy *et al.*, 1994). Furthermore, the proportion of yolk in an egg can vary between altricial (19.8%) and precocial (35.1%) birds (Romanoff and Romanoff, 1949) and between strains within a species. Romanoff (1944) indicated that for the White Holland and Bourbon Red strains of turkey, average yolk mass varies from 11.25 to 12.18 g, respectively. In addition to strain effects, the stage of embryonic development and genetics (dam effects) can also affect the yolk sac size (Jull and Heywang, 1930; Jaffe, 1964).

Of major importance in terms of production is the content of egg yolk in lipids. It has been estimated that yolk lipid fatty acids account for more than 90% of the total energy requirements for developing avian embryos (Freeman and Vince, 1974). Research data on turkey breeder hen eggs suggest that the pattern of maternal dietary long chain fatty acids (LCFA) will influence their proportional incorporation into yolk lipids (Feifenbaum and Fisher, 1959; Wheeler *et al.*, 1959; Couch *et al.*, 1973). The fatty acid profile of the maternal diet will also influence the fatty acid profile of the embryonic tissues (Donaldson, 1967; Couch *et al.*, 1973; Vilchez *et al.*, 1992; Cherian

and Sim, 1993) and this has the potential of influencing embryonic fatty acid metabolism. Medium chain fatty acids (MCFA, $C_{6:0}$ to $C_{12:0}$) are more easily metabolized than other LCFA due to their carnitine-independent transport into hepatic mitochondria (Babayan, 1987). These shorter chain fatty acids can be added to the diet via synthetic mixtures of triglycerides containing high percentages of MCFA (45% $C_{8:0}$, 20%$C_{10:0}$) or as a proportion of intact fats (Ding and Lilburn, 1997). MCFA have been shown to have beneficial effects on the overall growth of young chicks (Mabayo *et al.*, 1993).

Approximately 80% of yolk lipid is mobilized during incubation by turkey embryos with the largest proportion being utilized during the last week of incubation (Ding *et al.*, 1995; Ding and Lilburn, 1996). Moran and Reinhart (1980), working with Small White turkeys, indicated that poults with larger yolk reserves hatched from large eggs, implying the potential of increased energy stores for the new hatchling.

The yolk fatty acid profile of two different breeder strains' eggs (British United Turkeys of America (BUTA) and Nicholas) as reported by Reidy *et al.* (1998) is given in Table 22.8.

There was a significant strain by sample time interaction for palmitic acid content of yolk indicating a larger decrease in this yolk sac fatty acid (with the Nicholas vs BUTA strain in the first 24 h posthatch). A number of strain differences are seen in fatty acid development in the embryo, although these are quite small and may not be of sufficient magnitude to account for any differences in early poult viability and/or growth (Reidy *et al.*, 1998).

Figure 22.2 shows the fatty acid profile of edible egg yolks from different

Table 22.8 Fatty acid profile of yolk lipids from commercial turkey breeders (Reidy *et al.*, 1998)

Main effects	Palmitic C16:0	Palmitoleic C16:1	Stearic C18:0	Oleic C18:1	Linoleic C18:2
	(% of total fatty acids)				
Sample time (D)					
At set	29.14	3.90	7.65	43.72	15.50
25 days incubation	28.88	2.58	8.93	43.71	15.74
At hatch	26.85	0.72	10.43	45.96	16.04
24 h posthatch	25.77	0.00	11.29	46.90	16.04
Egg weight (EW)					
70–76 g	27.87	1.57	9.53	44.11	16.77
80–86 g	27.78	1.72	9.47	45.40	15.62
90–96 g	27.24	1.96	9.82	45.72	15.19
Strain					
BUTA[1]	28.01	1.60	9.41	44.59	16.30
Nicholas	27.26	1.91	9.80	45.59	15.40

[1]BUTA = British United Turkeys of America.

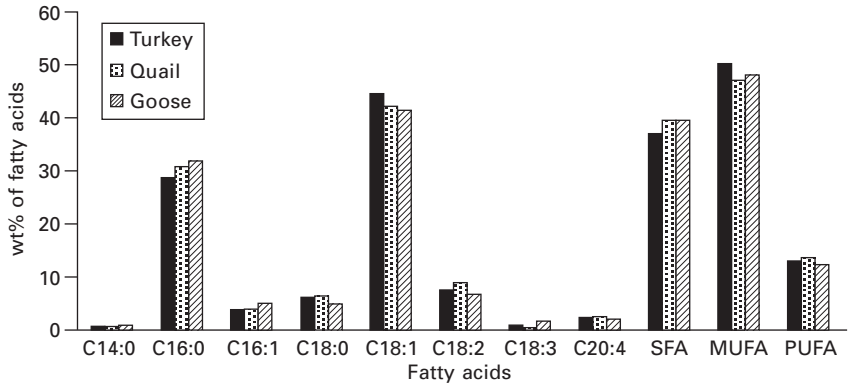

Fig. 22.2 Fatty acid profile in yolk lipids of turkey, quail and goose edible eggs (Sinanoglou *et al*., 2011).

avian species as reported recently by Sinanoglou *et al*. (2011). Fatty acid analysis in egg yolks of these species exhibited noticeable similarities with high proportions of monounsaturated (MUFA) and saturated fatty acids (SFA), while an interestingly low value of ω-6/ω-3 fatty acid ratio was recorded for the turkey and goose egg.

22.4 Other quality characteristics

22.4.1 Eggshell ultrastructural organization

One of the important characteristics of egg quality is the eggshell structure. Researchers began to study eggshells with microscopes in the 1940s and found that the structure of the eggshell was unique for each species, and useful for bird classification and evolution studies (Heyn, 1963; Becking, 1975; Sai *et al*., 1996; Chang *et al*., 2000; Rodriguez-Navarro *et al*., 2002). Panheleux *et al*. (1999), found that the general structural organization of the different avian eggshells was similar but specific differences are observed in the ultrastructure of the mammillary layer; species of the same taxonomic family could be grouped according to their structural analogies: breeder hen, turkey and pheasant resembled that of the domestic fowl, guinea fowl was unique and goose and duck were quite similar with large and confluent mammillary bodies.

In pheasants the analysis of blue eggshell ultrastructure showed a shallower mammillary layer than brown and olive coloured eggshell, and thus an abnormal structure of the shell; all these factors resulted in poorer hatchability results from blue eggs (Krystianiak *et al*., 2005). About 1–2% of blue-shelled pheasant eggs collected are often discarded at settings (Deeming and Wadland, 2002), while their hatching rate is around half the one of brown and olive eggs (Anonymous, 1993). Koźuszek *et al*. (2009) concluded that

the thinner and more porous eggshell as well as smaller number of Haugh units and height of the thick egg indicate worse quality of the shell and content in blue-shelled eggs. However, the high lysozyme content found in these eggs suggest a higher protective barrier in blue-shelled eggs (Koźuszek *et al*., 2009).

Disturbances in the shell formation process in turkeys are quite common, as confirmed by a high number of eggs which differ from regular eggs in terms of pigment colour, pigmentation pattern or shell surface (Mróz 1996, 1998). The number of eggs with shell surface faults depends on the origin of turkeys, reaching up to 10% in heavy-type birds (Mróz *et al*., 2002, 2007) and up to 39% in medium- and light-type birds (Mróz *et al*., 1997; Mróz, 1998). Mróz *et al*. (2007), studying the hatchability of turkey eggs differing in shell structure and functional properties found distinguishing features of normal shell microstructure in turkey eggs. Structural faults of eggshell can significantly decrease the reproductive performance of turkeys (Mróz *et al*., 2008). The surface structure of rough shells and shell with pigment spots suggest that the glands responsible for the production of shell mass and pigment continued to function although the next shell layer (cuticle) had already been formed. The reasons for this phenomenon have not been clarified, but it seems that the egg moving backwards in the duct before being laid could stimulate further secretion from those glands. The hatchability of eggs with pigmented spots on shells as well as of rough-shelled eggs are lower by 7.47 and 5.23% points, respectively, than of eggs with regular (normal) quality shells (Mróz *et al*., 2008). Many turkey flocks are characterized by a high proportion (>30%) of eggs with shell surface faults (Mróz, 1996, 1998; Mróz *et al*., 1997, 2002), while varied percentages of eggs with defective shells (7.5–27.6%) have been observed in inbred turkey flocks (Baran *et al*., 1994). A higher inbreeding rate is associated with an increase in the number of eggs considered unsuitable for incubation.

22.4.2 Trace elements content in eggs

Data on the 'normal' or baseline levels of trace elements in eggs of various domestic avian species are very limited. The contents of 21 elements in the eggshell of wild and captive Reeve's pheasants (*Syrmaticus reevesii*) were compared by Chang *et al*. (2007). Among the elements that made up the eggshell of the wild pheasant, the content of Ca, Mg, P and S was much higher, $\omega > 1$ mg/g, with ω (Ca) being higher than 40% of the eggshell. The contents of Na, Si, Sr, K and Al were $\omega = 0.1–1$ μg/g, while Fe, Zn, Pb, Mn, Cu, V and Ti had lower concentrations ($\omega = 1–100$ νg/g). The ω of Ni, Cr, Co, Se and Cd were lower than 1 μg/g.

Richards (1997) studied the mechanisms involved in the transfer of trace minerals from stores within the tissues of the hen to specific sites within the egg and ultimately to stores within the tissues of the developing embryo. He concluded that the transfer of trace minerals from the turkey hen to the

egg involves two possible routes: (1) via the ovary to the yolk and (2) via the oviduct to the albumen, the shell membrane, and the shell. Vitellogenin purified from the plasma of laying turkey hens has been analysed and found to contain significant amounts of zinc, copper and iron. Furthermore, Richards (1997) found that in the day 21 turkey embryo the concentrations of zinc, copper, iron and manganese are greater in the yolk sac tissue compared to the yolk contained within it. This finding indicates the ability of the yolk sac to concentrate trace minerals derived from the yolk.

The third main interest in the trace element levels of eggs relates to research by environmental scientists on the embryotoxic effects of various trace elements on birds (Nisianakis *et al*., 2009). Moreover, trace mineral levels in eggs of wild birds, as well as in those of reptiles or amphibians, are used as bioindicators to monitor pollution trends (Burger, 1994; Burger and Gochfeld, 1995, 2004; Lam *et al*., 2006).

There is an extensive variation in trace element content of eggs among species. Moreover, according to the findings of Nisianakis *et al*. (2009), the levels of trace elements in yolks are far higher than those in the albumen (Figure 22.3).

22.4.3 Egg cholesterol content

The cholesterol content of egg yolk has received intense criticism over the past years, due to the connection of blood cholesterol levels and the risk of atherosclerosis. As a result, most health authorities advised consumers to limit their egg consumption. However, recent health studies indicate that there is no link between egg consumption and atherosclerotic heart disease, which is supported by the theory that cholesterol levels in the bloodstream are not primarily dictated by ingestion of cholesterol from food.

The content in cholesterol in egg yolks of various avian species has been studied over the past decades. In a classic study, Bair and Marion (1978) showed that the cholesterol content in different avian species varied from 12.77 to 21.99 mg per gram of yolk.

Sinanoglou *et al*. (2011) in their comparative study, found the cholesterol contents in turkey, quail and goose edible eggs were 16.52, 13.61 and 13.94 mg/g of wet weight basis of egg yolk, respectively. According to the USDA Nutrient Data Laboratory the cholesterol contents in the whole egg (mg/100 g of food portion) were 933.0, 852.0, 844.0 and 423.0 for turkey, goose, quail and hen egg, respectively.

22.5 Conclusion

Non-table eggs from avian species such as quails, pheasants, geese and turkeys are mainly used for the species reproduction and for research purposes. The avian egg is not only an important source of nutrients required for the

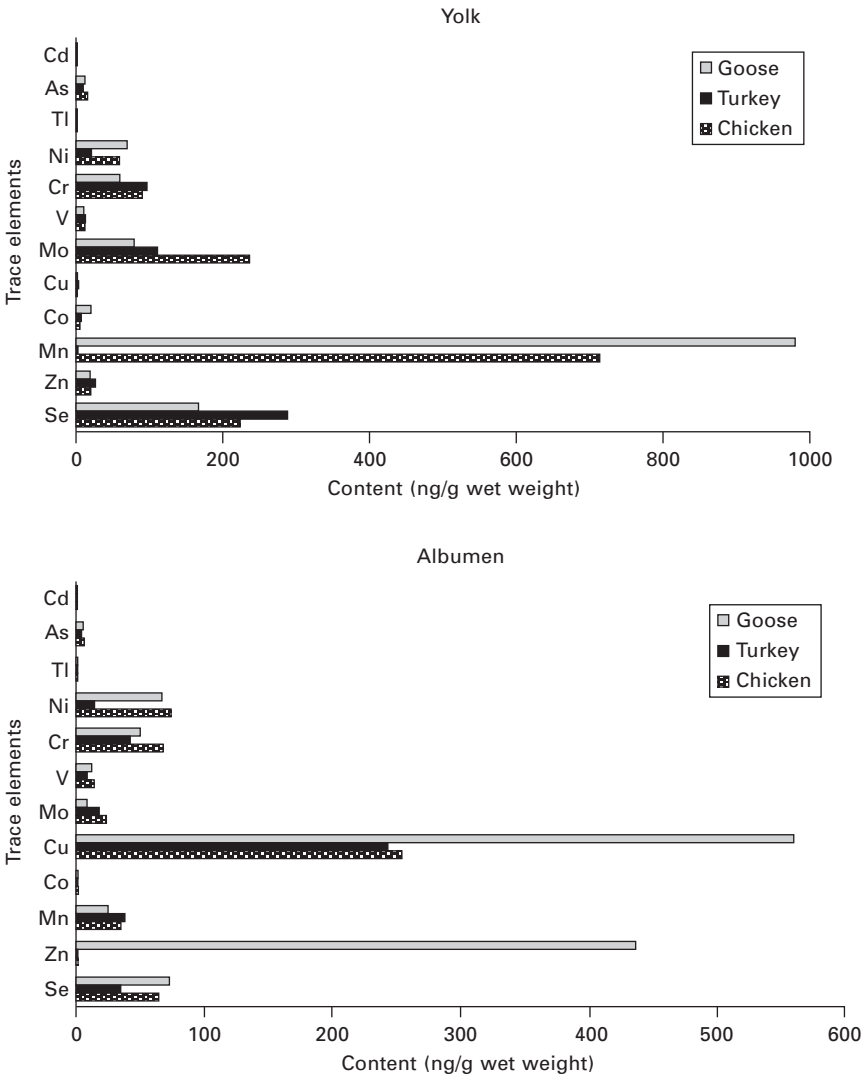

Fig. 22.3 Trace element content (ng/g wet weight) in egg of domestic avian species (Nisianakis *et al*., 2009).

developing embryo and its survival, but also a valuable material for the investigation of fundamental physiological processes.

In this chapter the characteristics and properties of non-table avian eggs have been discussed in an overall attempt to highlight the directions of the scientific research in the respective areas. This research is characterized either extended or limited and, in many cases, declined. However, the continuous streaming of information from the novel developing research fields will be useful in the direction of better understanding the mechanisms ruling

the avian egg. The implementation of the acquired knowledge that will be addressed to animal production and public health will be the major outcome of this effort.

22.6 References

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23

Processed egg products

V. Lechevalier, T. Croguennec, M. Anton and F. Nau, Institut National de la Recherche Agronomique, France

Abstract: Egg product industry developed concomitantly with the food industry to provide food manufacturers with safe and ready-to-use eggs. Pasteurised whole egg, egg yolk and egg white are thus proposed either in liquid, frozen or powder forms. Processes applied in the egg product industry aim at improving egg products' hygienic and functional quality. One of the main difficulties is the high heat sensitivity of egg products, thus limiting the possibilities for bacterial decontamination. That is the reason why much research is carried out on alternative stabilisation technologies, which are less denaturing than heat treatment. Nowadays all the 'first processing' egg products offer a high hygienic quality, and thousands of references have been developed to satisfy various demands of food industry for product functionalities. Egg product manufacturers have also developed specialty egg products which are formulated and/or cooked eggs intended to mass catering or directly to consumers.

Key words: egg product, processing, egg white, egg yolk, whole egg, pasteurisation, drying, dry heating, alternative stabilisation technology, functional property.

23.1 Introduction: industrial egg products

Egg products are defined by Commission Regulation EC No. 853/2004 as 'processed products resulting from the processing of eggs, or of various components or mixtures of eggs, or from the further processing of such processed products'. According to Commission Regulation EC No. 852/2004, processing consists of 'any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction,

extrusion or a combination of those processes'. Egg products from 'first processing' (liquid, frozen or powdered, white, yolk or whole egg) can be distinguished from speciality egg products (formulated and/or cooked eggs). The first type comprises ingredients almost exclusively intended for the food industry, even if in some countries they may be available for consumers; the second type comprises products designed for consumers, either directly or through mass catering.

23.2 Industrial production of liquid egg products

23.2.1 Shell eggs quality and storage

Eggs used for egg products are often by-products of eggs sold as shell eggs. Eggs used for this purpose are out of grade eggs (too small or too big) or downgraded eggs (with micro-cracks, dirty marks or abnormal calcification). However, laying hen farms are also especially set up for the egg products industry.

Small laying farms designed for producing, grading and packaging of shell eggs sometimes invest in small breaking units in order to valorise downgraded eggs; such units produce mainly pasteurised liquid whole egg for local markets. But the biggest egg product companies are industrial units specifically dedicated to egg breaking, cracking and formulation. Some of them are established within dedicated laying farms, in which eggs are directly transported from henhouses to the breaking machines by conveyors, without being previously packed. For those units which are not integrated into farms, shell eggs can be obtained either from packaging centres, or from farms under contract.

In order to balance the supply in shell eggs and the demand in egg products, shell eggs are usually stocked before processing. Depending on environmental conditions, water loss can be significant during storage; it mainly affects egg white, either due to evaporation through the shell, or due to water transfer from the white to the yolk (Silversides and Budgell, 2004). CO_2 loss through the shell also occurs; the resulting pH increase (from 7.4 to 9.3 in 10 days) may have consequences on the efficiency of pasteurisation page 544).

Egg white foaming properties (affecting, e.g., the volume of angel cakes) decrease after shell egg storage for 8 weeks at 4 °C (Jones, 2007), whereas they increase (foam volume) when shell eggs are stored for 10 days at 21 °C (Silversides and Budgell, 2004). Thick egg white foam stability is maximum after shell eggs storage for 20 to 40 days at 4 °C (Hammershøj and Qvist, 2001).

During storage, ovalbumin, the major egg white protein, partly changes its conformation into *S*-ovalbumin, a more thermostable form of the protein. *S*-Ovalbumin induces a decrease of gel firmness after heating. Egg white

gelling properties are optimum after storage of shell eggs around 14 days at 4 °C, resulting in conditions leading to a pH increase to around 9, and weak *S*-ovalbumin concentration.

Vitelline membrane resistance, which determines the quality of egg white/yolk separation (page 541), is decreased by vibration during transport and storage, especially at high temperature (Chen *et al.*, 2005; Berardinelli *et al.*, 2003). Temperature is thus of prime importance during shell egg storage. As well as genetics, feeding and hen age, the changes in temperature of shell eggs explains some of the variability of shell egg quality observed upstream of the breaking machine, accounting for up to 70% of the variability of pasteurised liquid egg white foaming properties (Lechevalier *et al.*, 2005a).

23.2.2 Shell eggs breaking

Mechanical breaking of shell eggs is a relatively recent technology. Equipment manufacturers first developed mechanical grasp, breaking and shell opening devices, and then technology to mechanise shell eggs supply and white/yolk separation.

Handling

The mechanisation of breaking machines has been considerably improved in the last ten years. Nowadays, completely automated machines are able to break from 80 000 to 160 000 eggs per hour. The next step will be automatic depalletising of shell eggs flats. However, this implies both identical palletising plans and materials for the whole plant and eggs with good shell integrity to enable the handling of flats at a high rate. Eggs are grasped by suction grips using a partial vacuum and deposited on the conveyor belt that supplies the breaking machines. The system must prevent shell eggs from being damaged as they land on the conveyor.

Washing devices have been designed to wash shell eggs effectively in 15 to 60 s. These technologies are thus theoretically suitable for upstream breaking operations in order to improve the hygienic quality of egg products. They are, however, not widely used in Europe because of the rapid fouling of the washing machines which limits their effectiveness. Technological improvements are, however, expected in this area, since the development of alternative hen housing systems may decrease the microbiological quality of shell eggs.

Breaking

Whatever the mechanical device used, the operating principle in shell breaking is always the same: the use of a blunt tool to break the lower side of the egg to allow draining of the yolk. The breaking of the shell is easier when they are placed horizontally, using either knives that cut into the shell or a hammer which rests on the upper part of the egg. Knives have to move away quickly in order not to break the egg yolk. The breaking part of the

machines is either fixed or mobile on horizontal or vertical carousels. Some specific devices have been developed to minimise shocks when eggs arrive at the machine (Kristensen *et al.*, 2007). The draining time for the shell may vary from 0.2 to 2 s, after which shells are quickly moved towards crushing devices.

Egg white/yolk separation

The quality of egg white/yolk separation is essential to guarantee egg white quality. Indeed, even small yolk traces (0.022%) impair egg white foaming properties (Wang and Wang, 2009) and pasteurisation efficiency. Optical devices have been developed to detect yolk traces in egg white.

The size of the yolk bowl increases with hen age, making separation more difficult. Vitelline membrane resistance decreases during shell eggs storage, decreasing the quality of separation. Poor maintenance of knives, or the use of cracked eggs, may also cause the breakdown of the vitelline membrane, before or during separation. To achieve separation after shell breaking, each egg yolk bowl is dropped into a small dish (Fig. 23.1) or pipe (Fig. 23.2). The egg white drains off the punched bottom of the dish or the slit in the middle of the pipe. The final yolk dry matter depends on the total dry matter in the yolk bowl and on the quantity of egg white sticking to the vitelline membrane. It is thus influenced by the storage conditions of shell eggs, breaking temperature and technology.

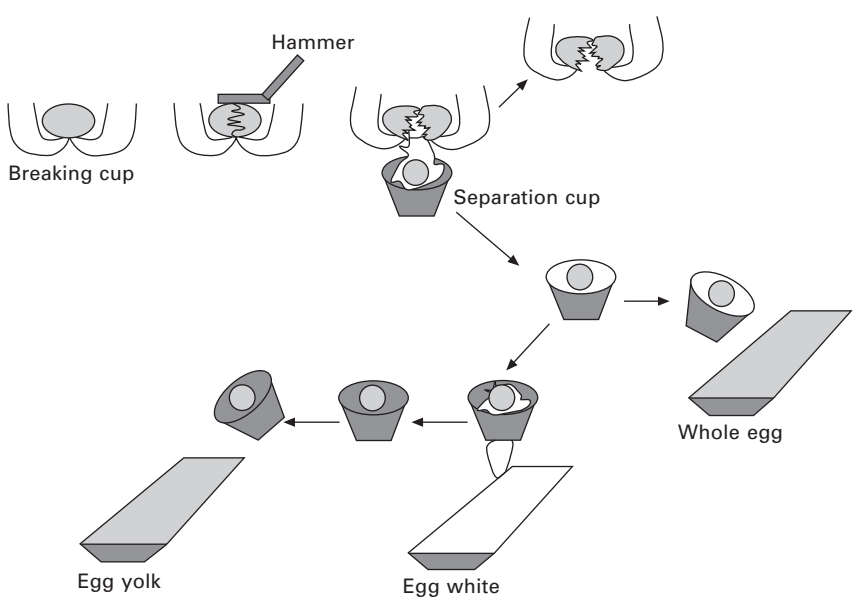

Fig. 23.1 Schematic diagram of a breaking part machine with egg white/yolk separating system by dishes.

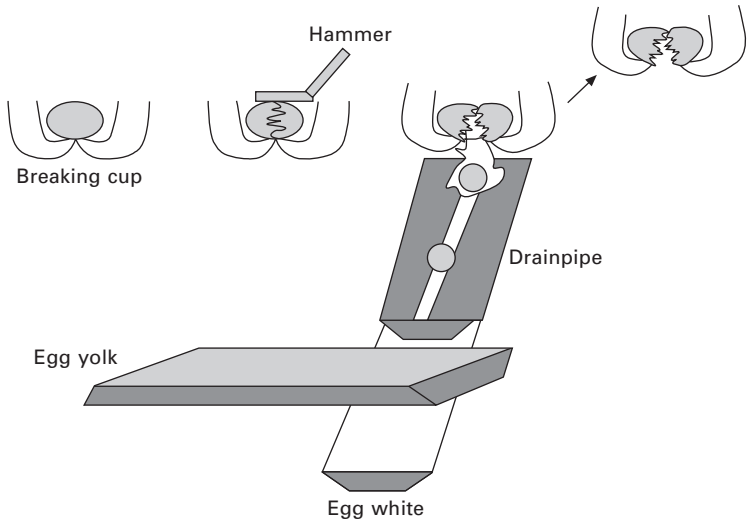

Fig. 23.2 Schematic diagram of a breaking part machine with egg white/yolk separation through drainpipes.

Filtration and cooling

Because shell fragments inevitably fall into the product, filtration is undertaken immediately after breaking. Auto-wash filters reduce the contact time between shells and product, greatly improving hygiene. Egg white and yolk are cooled after filtration and then stored in refrigerated tanks before pasteurisation.

23.2.3 Pasteurisation of liquid egg products

Pasteurisation aims to destroy the majority (5 to 6 decimal reductions) of vegetative microorganisms, including some pathogens (see Chapter 14). Pasteurised products can then be stored at 4 °C from between a few days to a few weeks. However, because egg proteins are particularly heat sensitive, heat treatments applied to egg products, and especially egg white, have to be mild.

American recommendations aim for 5 decimal reductions of *Salmonella* in egg yolk and whole egg. This is achieved with time/temperature combinations between 3.5 min, 60 °C and 3.5 min, 61.1 °C. These recommendations are lower than those applied in Europe, where a 5–6 min holding time at 65 °C or higher is typical. However, these milder heat treatments applied to egg white are not always sufficient to reach 5–6 decimal reduction of *Salmonella* Enteritidis and *Listeria monocytogenes*.

Batch pasteurisation

Batch pasteurisation is sometimes used in small units where on-line pasteurisation is not economic because of the small volumes treated.

The equipment used is an airtight, pressure-resistant tank equipped with a heating and cooling device. The tank is pressurised with sterile air to prevent contamination from outer air during cooling. On the biggest tanks (3 000–8 000 L), a recirculation system equipped with a heat exchanger allows for more efficient heating and quicker pasteurisation cycles. For whole egg and egg yolk, temperatures between 60 and 62 °C for less than 15 min are applied. It is, however, difficult to find operating conditions that simultaneously enable bacterial destruction and preserve satisfactory foaming properties.

Batch pasteurisation does not allow heat recovery. However, there is less product loss through dilution. As a result this technology is still used as an alternative to in-line pasteurisers. It is used, for example, for viscous products, small volume production, pasteurisation of products with high dry matter, pasteurisation with long holding times, or to allow degassing during heating. The development of some enzymatic treatments has also increased interest in this technology.

Hot-filling

Hot-filling consists of maintaining a high temperature during filling and cooling after packaging closure. A wide range of time/temperature combinations are described in patents: from hot-filling at low temperatures (54–56 °C) with cooling over long periods (12–72h), to hot-filling at 60 °C with a few minutes' holding time. The risk of foaming during hot-filling and rapid cooling to 4 °C in the centre of the packaging are technical difficulties that remain to be solved.

This process is widely used for small packs (less than 5 kg) of whole egg and egg yolk. But it is still difficult to hot fill egg white at conventional temperatures (from 55 to 57 °C) without damaging functional properties (Wang and Wang, 2009). There, is however one treatment (42–45 °C during 1 to 5 days) which can produce egg white which is stable at room temperature (Liot and Anza, 1996).

Online pasteurisation: standard equipment

Pasteurisers typically are plate heat exchangers or tubular exchangers with an initial heat recovery section in which the cold product is preheated by the warm pasteurised product. The following section heats the egg product to the pasteurisation temperature; in this section the temperature difference between the hot water in the exchanger and egg product has to be less than 1 °C. After holding (3–10 min) and precooling in recovery section, the last section cools the product below 4 °C.

Because egg yolk viscosity, especially in salted yolk, is much higher at low temperatures than egg white, adaptations are required in the cooling section: either slightly higher temperature coolant, the use of an accelerating pump before the cooling section, or specifically designed plates (Gut *et al.*, 2005). The plate heat exchanger is the more common technology for egg

yolk, since it is difficult to reach a turbulent flow in a tubular configuration because of egg yolk viscosity.

On-line pasteurisation: high temperature/short time (HTST) equipment
The high temperature/short time (HTST) concept was applied to egg products in the USA in the mid-1980s in order to lower protein denaturation. Hamid-Samimi *et al*. (1984) and Ball *et al*. (1987) showed that heat treatment should not decrease protein solubility by more than 5% to retain good functional properties.

HTST processing typically uses tubular exchangers since they can withstand higher pressure loss, require a shorter holding time than plate heat exchangers and are easier to clean. Though not exactly HTST systems, 'tube in tube in tube' designs are able to reach a higher temperature than standard plate heat exchangers (from 67 to 68 °C for whole egg) with holding times from 1 to 3 min. As well as these systems, the current passage tube achieves higher temperatures for short periods. This heating process (Actijoule®) involves applying a low voltage current to a stainless steel tube, which provides electrical resistance, thus producing direct heating inside the wall of the tube (known as the Joule effect). A constant temperature difference between the product and the inner surface can be obtained all along the tube. It is thus easier to control the temperature precisely in order to limit fouling and to increase cycle times. Moreover, the Actijoule® system design increases the temperature limits for whole egg from 66 °C with classical tubular devices to 70 °C thanks to the high turbulence generated ($5000 < Re < 7000$) with product held from 10 to 120 s. Actiflash® is a technology based on the Actijoule® system, characterised by a smaller tube diameter and thus a higher turbulent flow; it enables thermal treatments between 71 °C for 60 s to 90 s and 74 °C for 10 s to 20 s for whole egg. Such treatments are much more bactericidal than a classical treatment of 65 °C for 6 min, while limiting fouling and denaturation.

On-line pasteurisation: specific aspects of egg white pasteurisation
Liquid egg white pasteurisation is a tricky process because egg white proteins are very heat sensitive. Moreover, despite well-established rules for thermal destruction of bacteria (cf. Chapter 14), the number of parameters make the calculation of heat treatment efficiency difficult. Firstly, thermal sensitivity is very variable from one strain to another one, and bacterial populations are always very complex in actual industrial environments. Secondly, D_θ and z values available in the literature have been generally determined in laboratory conditions which are significantly different from industrial environments. Thirdly, it is sometimes difficult to estimate bacterial populations and characteristics in industrial machinery. The physicochemical characteristics of egg products, especially pH, considerably modify the thermal sensitivity of bacteria. Froning *et al*. (2002) reported that, for a mix of five strains of *Salmonella* and an egg white treatment at 56.7 °C, D_θ decreases from 2.47 min at pH 7.8, to 0.85 min at pH 9.3.

23.3 Egg product dehydration

Egg powders are now mainly technical products with particular functional properties which cannot be found in liquid egg products. Egg yolk powder is less widespread since yolk is naturally rich in dry matter, meaning that dehydration does not result in a high volume reduction. In contrast, egg white powder is a major egg product on the international market since dehydration does result in a large volume reduction (× 3); moreover, the functional properties of egg white powder may be enhanced by dry heating (page 560). Egg yolk and whole egg are always pasteurised before drying, while raw egg white is dried before the powder is pasteurised.

23.3.1 Treatments of liquid egg products before dehydration

Desugarisation

In egg white, glucose is involved in Maillard reactions (non-enzymatic browning) which induce changes of powder colour during storage. For whole egg powders, off-flavours, colour changes and solubility loss are also observed during storage. Storage temperature has a major effect on these changes, especially if it gets close to the glass transition temperature (55–65 °C for whole egg powder). Desugarisation is thus a crucial step in egg powder manufacture, especially for egg white powder. Several processes have been developed to eliminate glucose, upstream or downstream from the concentration process (page 546). Bacterial fermentation enables conversion of glucose into organic acids. The use of *Enterobacter aerogenes* and *Klebsellia pneumoniae* (Imai, 1976; Shehab *et al*., 1978), as well as *Streptococcus* and *Lactobacillus* (Mulder and Bolder, 1988) for glucose conversion has been described. *Lactobacillus brevi* and *Lactobacillus casei* in particular result in powders with good foaming properties.

Bakery yeast (*Saccharomyces cervisiae*) causes efficient desugarisation of egg white in few hours. Optimal conditions are a pH of 6.0 to 7.5 and a temperature of 32 °C (Shehab *et al*., 1978). Cell growth is very weak during deglucosylation and can be easily stopped by cooling, so limiting taste defects. Yeast fermentation is a good alternative to bacterial fermentation for whole egg since it prevents off-flavours.

Enzymatic fermentation is easy to achieve and produces products with consistently high organoleptic quality. Glucose oxydase (EC1.13.4) catalyses the oxidation of glucose into gluconic acid. Hydrogen peroxide is produced during this reaction, and a catalase (EC1.11.1.6) must be added to prevent its accumulation. Commercial preparations combining both enzymes are specially formulated for egg white desugarisation, requiring an optimal pH of around 6.0. An improvement of this process consists of binding enzymes on ion exchange resins and positioning them in a column which creates oxygen bubbles to accelerate the reaction (Sisak *et al*., 2006).

pH modifications

pH adjustment is often performed before drying to optimise desugarisation processes and/or to control the pH of final powders. Egg white pH increases from 6.0, 6.5 or 7.3 before drying to 6.88, 8.55 and 9.40 respectively after drying (Mine, 1996). Without pH adjustment, the pH of whole egg powder is 9.0 to 9.5, which is not good for taste or colour stability. Decreasing pH using organic acids is a common method to obtain a slightly alkaline pH (7.5 to 8.5) in reconstituted whole egg powder.

Gelling properties of egg white or whole egg increase when pH increases. 'Super-high gel' egg white powders, with a pH around 10.0, can be found on the market. However, a pH over 9.5 decreases protein solubility, damages organoleptic qualities and generates anti-nutritional factors such as lysino-alanine residues. As a result, the pH of egg white gelling powders is most often 6.5 to 8.5. Egg white foaming powders have rather a neutral or slightly acidic pH, since it improves their organoleptic qualities and can also reduce raw material variability (Lechevalier *et al.*, 2007).

Co-drying

Protein-polysaccharide complexes can be obtained by co-drying with polymers such as guar gum hydrolysate; the resulting emulsifying and gelling properties are excellent (Kato *et al.*, 1993; Nakamura *et al.*, 1998; Matsudomi *et al.*, 2002). Protein phosphorylation by phosphate addition before egg white drying and dry heating also improves foaming properties (Hayashi *et al.*, 2009).

Co-drying with saccharose or maltodextrins can restore the foaming properties of whole egg. Around 40% of these carbohydrates in the overall powder is required for this purpose. This effect is almost proportional to carbohydrate content, though short sugar chains are more effective (Kline *et al.*, 1964). However, over 20% of some carbohydrates in the powder can produce off-flavours. Long maltodextrin chains can be added in higher proportions without producing off-flavours, since they also increase the powder glass transition temperature. Commercial whole egg powders with 10–40% saccharose or maltodextrin, depending on customer requirements, are available. Salted whole egg powder is less common with salt content rarely over 5% of the powder. Further addition of maltodextrins or glucose syrup produces products with very good organoleptic and flow properties (Lai *et al.*, 1985).

Adding 20% saccharose restores the foaming properties of yolk powder (Schultz *et al.*, 1968). Sweet yolk powder (10–20% sugar) is often available for pastry mixes or desserts. Salted (5–10% NaCl) yolk powders are more common for ready meals or sauces.

Concentration

The first objective of concentration before drying (using a non-thermal technology such as membrane cross-flow filtration) is to reduce process costs

during the drying phase. The second objective is to obtain denser powder which is cheaper to store and to transport.

Egg white is typically concentrated using organic membranes before drying. Reverse osmosis and low molecular ultrafiltration can be used to concentrate egg white, resulting in limited protein loss in the permeate. Permeation flow density, investment, operating and energy costs favour ultrafiltration. Moreover, mineral transfer into the permeate induces partial demineralisation of egg white. As a result powders produced using ultrafiltration have a higher protein/minerals ratio, appropriate for diets needing a high protein but low sodium content.

Whole egg concentration before drying is very limited. Ultrafiltration with organic membranes is difficult, with low permeation flow densities even at 40–45 °C. Moreover, these conditions favour bacterial growth which decreases product quality and shelf-life.

23.3.2 Drying technologies and powder properties

Egg products are relatively easy to dry since they do not contain hygroscopic compounds. Spray-drying by liquid atomisation in a hot air flow is the most common technique. The physical properties of powders can be adjusted in different ways to fit consumers' needs.

Spray-drying

Depending on composition, egg white, whole egg and egg yolk powders have a residual humidity of 6–8%, 3–5% and 3–4%, respectively. Because they are sensitive to heat, whole egg and yolk powders can undergo colour losses or significant functional changes during drying, depending on temperature, residence time and residual water content. For egg white, the effect of thermal treatment on proteins is mainly responsible for the significant loss of foaming properties, rather than the high shear rates undergone by the product during spraying (Lechevalier *et al*., 2007). Fortunately, foaming properties can be restored to a large extent after further heating of powder (page 548). The temperature of the air used for further heating is typically 180, 165 and 145 °C for egg white, whole egg and yolk, respectively. Liquid temperature, spraying pressure and nozzles number are the only other parameters that can be modified to control product quality.

Box-dryers are predominantly used in the egg product industry, even though they have been discontinued in many other sectors that generally prefer vertical dryers. Box-dryers offer real advantages for egg products: air flow limits powder dispersion in the chamber, and variation in residence times is low. The main disadvantage is that cleaning is much more difficult.

Standard whole egg and yolk powders are a necessary compromise between productivity and final product quality. However, different drying conditions can be used to produce specific products. Low drying temperatures and/or low spraying pressures produce whole egg and yolk with high viscosity

(>1000 mPa s) after rehydration, or 'high foam' and 'high gel' egg white powders. High temperatures and/or high pressure improve the emulsifying properties of whole egg powders (Franke and Kießling, 2002).

Other drying technologies

An old drying technology, called 'pan-drying', is still used to produce 'crystal' egg white. Concentrated, desugared egg white is placed on plates in a hot room (54 °C maximum) in which slow concentration and drying occur without coagulation. A vitreous product is obtained which can be ground to different sizes according to requirements (mainly confectionery) (Bergquist, 1995). Freeze-drying is a gentler technology particularly suitable for aromatic and/or heat-sensitive products. A freeze-drying process for egg yolk has been recently developed, producing a powder with excellent functional and organoleptic properties (Jaekel *et al.*, 2008).

Powder properties

Powder density is important in reducing transport and storage costs and in specific applications where a given volume has to correspond to a preset weight. Density depends on chemical composition, and operating parameters such as concentrate dry matter, temperature, spraying pressure, type of nozzle, etc. Egg powder density is typically around 400–450 kg m^{-3}, and is higher if drying is slow.

Flow parameters affect handling, dosing or coating characteristics of powders. Lipids in egg yolk and whole egg powders are the main cause of poor flow properties. Co-drying with a hygroscopic compound improves this parameter (Lai *et al.*, 1985). Another solution is to blend additives with good flow properties using dry mixing (Graindorge and Ilari, 1991).

Rehydration properties affect users' process productivity, the formulation of ready-to-use products and functional properties. The physicochemical composition of egg (with no hygroscopic components) does not promote rapid rehydration. Co-drying with a hygroscopic ingredient is a simple solution for egg yolk and whole egg powder. Grain agglomeration to increase grain size slightly improves powder rehydration (Schuck *et al.*, 1994; Gaiani *et al.*, 2007).

23.3.3 Egg white dry-heating

Egg white powder heating (or dry-heating) is an original process in the egg product industry, allowing higher pasteurisation limits than those for liquid egg white (Baron *et al.*, 2003). The most common technology involves heating and maintaining the temperature of egg white powder in its final packaging in a hot room controlled for temperature and, if possible, humidity. The position of pallets/boxes/bags in the room, air flow and air moisture regulation are used to maximise the efficiency and uniformity of heating without drying out the powder. Dry-heating treatment varies from 65 to 85 °C over 5 to 14

days (with the length of heating required increasing at lower temperatures), depending on the powder functionalities required (minimal treatment is enough for bacterial destruction). Egg powder properties are determined by variations in time/temperature, water content and pH (Kato *et al.*, 1989; Mine, 1996, 1997; Baron *et al.*, 2003). Mild treatments (70 °C over 3 days, 75 °C over 3 or 5 days and 80 °C for 1 day) retain egg white foaming properties while saving energy (Talansier *et al.*, 2009). Low protein denaturation explains the improvement in powder functionality (Desfougeres, 2009).

Dry heating can also be carried out in a conical mixer with a rotating mixing screw to increase heat transfer (Fig. 23.3). This process allows shorter thermal treatments at higher temperatures (85–100 °C); for example, heating at 90 °C over 21 h is enough to obtain superior powder foaming and gelling properties than those in corresponding liquid product (Hammershøj *et al.*, 2004). Continuous dry-heating in a fluidised bed has been compared to heating in a conical mixer (Hammershøj *et al.*, 2006a,b). This process

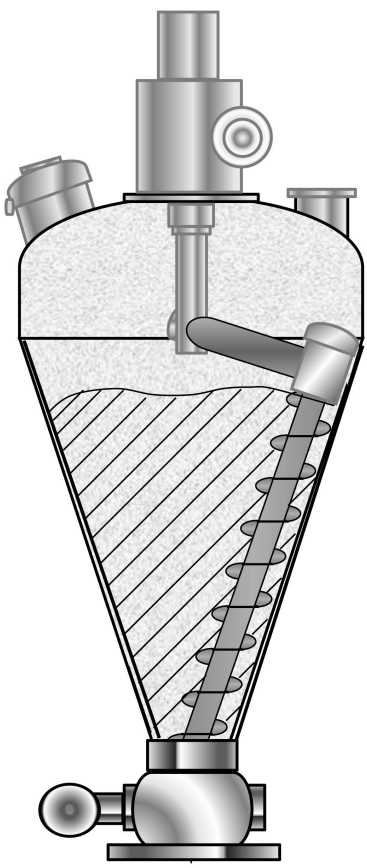

Fig. 23.3 Diagram of conical mixer with rotative mixing screw (hatched area shows product).

requires a minimum treatment of 1 h at 115 °C but is limited by the difficulty in reconciling pasteurisation efficiency and good functional properties in the heated powder.

Because many different unit operations can be used to make egg white powder (reverse osmosis or ultrafiltration; enzymatic, bacterial or yeast desugarisation; altering pH; drying and dry-heating technologies), combined processes are typically used in industry. Such combinations offer a wide range of functionalities with the potential to adapt the product to meet a specific set of requirements.

23.4 Alternative technologies for egg product stabilisation

23.4.1 Water activity (A_w) decrease

Water activity (A_w) is a measurement of free water content in a product and is an essential factor affecting the growth of microorganisms. The lower the A_w, the harder it is for microorganisms to survive. Liquid egg products have an A_w close to 1. Processing such as drying, freezing, sugar or salt addition lowers A_w below 0.85, producing more concentrated egg products which have a long shelf-life, even at room temperature. Salt addition can even reduce bacteria: a reduction by 3 log cfu of *Salmonella* Typhimurium in 10% salted egg yolk, but an increase by 1 log cfu was observed in whole egg and 10% sugared egg yolk (Musgrove *et al.*, 2009). Concentration can be performed before or after sugar and/or salt addition, using ultrafiltration (Liot, 1982) or plate vacuum evaporation. Because salt addition increases bacteria thermal resistance (Denis *et al.*, 1995), heat treatments of salted egg products have to be adapted accordingly.

23.4.2 Preservatives

The addition of ethylenediaminetetracetic acid (EDTA), polyphosphates, lactic acid, hydrogen peroxide or nisin increases microorganism heat sensitivity in egg products (Doyle and Mazotta, 2000; Doyle *et al.*, 2001). However, their use can be limited by constraints of cost, legal restrictions and consumer attitudes to some additives.

Nisin exhibits antimicrobial activity towards a wide range of Gram-positive bacteria, and is particularly effective against spores. It shows little or no activity against Gram-negative bacteria, yeasts and moulds (Delves-Broughton, 2005). Nisin addition reduces the time required for pasteurisation of liquid egg white (Boziaris *et al.*, 1998). Addition of 5 mg L^{-1} nisin in liquid whole egg increases its shelf life from 6–11 days to 17–20 days; it also has a protective effect from *Bacillus* cereus growth (Delves-Broughton *et al.*, 1992). Nisin has also synergistic effect when combined with high-pressure processing (Ponce *et al.*, 1998; Lee *et al.*, 2003) and pulsed electric field (PEF) treatment (Lado and Yousef, 2002).

Hydrogen peroxide addition in liquid egg white inactivates *Salmonella* Senftenberg almost completely (Muriana, 1997). Preheating of liquid whole egg increases the bactericidal efficiency of hydrogen peroxide against *Salmonella* Typhimurium (Unluturk and Turantas, 1987).

23.4.3 High pressure

High hydrostatic pressure treatment (from 100 to 1000 MPa) has many advantages: it has a minimal heat impact, gives products a similar shelf-life as pasteurisation but with better quality, needs a smaller amount of energy than thermal treatment and can be used to process packaged food (Pereira and Vicente, 2010). However, it is not typically a continuous process and requires large investment costs (Ahmed *et al.*, 2003). Moreover, it can affect the structure of large molecules such as proteins (Pereira and Vicente, 2010).

Salmonellae seem particularly sensitive to high pressure treatments in liquid whole egg: total inactivation is observed at 450 MPa, at either 50 or 20 °C (Ponce *et al.*, 1999; Isiker *et al.*, 2003). At 250 MPa, Velazquez-Estrada *et al.* (2008) obtained 3.2 log cfu ml^{-1} reduction of *Salmonella* Senftenberg. *Escherichia coli* is also partially inactivated in liquid whole egg after 5 min at 400 MPa (–5.5 log), and 886 s at 250 MPa or 200 s at 300 MPa (> 2 log reduction) (Yuste *et al.*, 2003; Lee *et al.*, 2003); *E. coli* inactivation is higher at 5 °C than at 25 °C (Lee *et al.*, 2001). Batch processing is generally more efficient than continuous treatment (Ponce *et al.*, 1998, 1999; Huang *et al.*, 2006). Coagulation occurs at over 300 MPa for egg white or 400 MPa at 25 °C for whole egg (Ahmed *et al.*, 2003; Lee *et al.*, 1999). At higher temperatures, coagulation occurs at lower pressure (1 h at 150 MPa and 45 °C for whole egg) (Lee *et al.*, 1999). Van der Plancken *et al.* (2005) showed an increase of egg white turbidity, surface hydrophobicity and susceptibility to enzymatic hydrolysis at a pressure range from 400 to 700 MPa; in the same time, protein solubility, total solfhydnil group (SH) content and denaturation enthalpy decreased. Protein denaturation by high pressure is related to hydrogen bond cleavage (Hayakawa *et al.*, 1996), with a potential effect on foaming properties. Richwin *et al.* (1992) measured a higher foam expansion for egg white treatment up to 400 MPa, whereas Strohalm *et al.* (2000) did not notice any significant change in foam volume and little or no effect on foam density. In contrast, Iametti *et al.* (1999) obtained denser foams after 5 min at 600 MPa and 25 °C. Seregély *et al.* (2006) showed little change in the organoleptic quality of high-pressured liquid egg white (15 min at 400 MPa and 4 °C) compared with non-treated egg white.

23.4.4 Gamma-irradiation

Ionising radiations can be applied to shell eggs or liquid egg product. In 2000, the US Food and Drug Administration (FDA) approved the use of 3 kGy dose to reduce the level of *Salmonella* in shell eggs (FDA Fed Reg 65). Efficiency

against salmonellae and *Clostridium* strains depends on whether irradiation is applied to shell eggs or liquid whole egg (Verde *et al*., 2004). Serrano *et al*. (1997) suggested that 1.5 kGy is sufficient to eliminate *Salmonella* Enteritidis from both whole shell and liquid eggs, without significant adverse effect on egg quality. In some cases, gamma irradiation is more efficient than heat treatment (Vanlith *et al*., 1995). However, irradiated shell eggs (1–3 kGy) can show some quality defects: decrease in Haugh units, yolk colour, whole egg and egg white viscosity (Ma *et al*., 1990; Tellez *et al*., 1995), with sensory analysis showing a significant difference between control and irradiated eggs. However, at the same time, egg white emulsifying and foaming properties increased, as well as foam stability and gel rigidity. But these results were contradicted by Min *et al*. (2005) who found that irradiation of shell eggs did not affect yolk colour, but decreased egg white viscosity and foaming properties, with generation of volatile sulphurs.

In conclusion, despite the efficiency of irradiation in eliminating *Salmonella* in egg products (Brooks *et al*., 1959; Mossel, 1960), its negative impact on organoleptic and functional properties limits its use, especially for yolk. Radiation (1–4 kGy) decreases yolk solubility and viscosity, carotenoid and free SH content and increases free fatty acid content and peroxide value, suggesting an oxidation effect (Ma *et al*., 1993; Badr, 2006). Its effects are less for egg white (Badr, 2006; Song *et al*., 2009). However, the effects of irradiation on functional properties remain open to debate since Ma *et al*. (1993) observed no or only a slight effect on frozen egg white and yolk foaming, gelling and emulsifying properties, whereas Clark *et al*. (1992) and Song *et al*. (2009) obtained an increase in egg white foaming ability when egg white or yolk was irradiated in liquid or powder form. As for the irradiation of shell egg, Seregély *et al*. (2006) reported changes in the volatile components of liquid egg white after irradiation at 3 kGy, whereas at the same dose Badr (2006) did not detect any effect on sensory characteristics of liquid egg or scrambled eggs. An interesting application of ionising radiation could be the reduction in the allergenicity or antigenicity of proteins through the destruction of epitopes (Kume and Matsuda, 1995; Byun *et al*., 2000).

23.4.5 PEFs

PEF technology consists in the application to the product of high voltage pulses (20–80 kV cm^{-1}) for an extremely short duration (1–100 µs). Several events such as resistance heating, electrolysis and disruption of cell membrane can occur (Pereira and Vicente, 2010). It is thus an interesting technology to replace or supplement conventional processing for the decontamination of heat-sensitive products. However, this technique is not able to inactivate spores (Lado and Yousef, 2002).

The factors that affect the efficiency of PEF treatment are not yet fully understood (Pereira and Vicente, 2010). However, it is clear that inactivation depends on the product treated (whole egg, egg white or yolk), the target

bacteria, electric field intensity, treatment duration, temperature and whether the product is recirculated during processing or not (Table 23.1). *Salmonella* Typhimurium inactivation is also pH dependent (Jin *et al.*, 2009). However, PEF treatment would not be sufficient by itself to decontaminate egg products satisfactorily, and it has to be combined with other decontamination techniques (Jeantet *et al.*, 2004; Monfort *et al.*, 2010). Combination with thermal treatment or the addition of nisin, citric acid or antimicrobial cocoa extract, increases the effectiveness of PEF treatment (Bazhal *et al.*, 2006; Calderon-Miranda *et al.*, 1999; Gongora-Nieto *et al.*, 2001; Pina-Perez *et al.*, 2009). On the other hand, Huang *et al.* (2006) did not notice any synergistic effect with high hydrostatic pressure and ultrasound treatment.

According to research, PEFs have produced varying changes in protein solubility, surface hydrophobicity and structure, colour, viscosity, gelation and lysozyme activity (Qin *et al.*, 1995; Ho *et al.*, 1997; Martin-Belloso *et al.*, 1997; Jeantet *et al.*, 1999; Fernandez-Diaz *et al.*, 2000; Ma *et al.*, 2000; Perez and Pilosof, 2004; Zhao and Yang, 2008). It seems that electrical conductivity of the solution is an important factor in explaining these differences. Changes in protein structure are irreversible.

23.4.6 Other processing methods for egg products

UV radiation

UV radiation can be a cost-effective alternative non-thermal process to achieve microbiologically safe liquid egg products with longer shelf life (Bintsis *et al.*, 2000). The benefits of UV treatment in comparison with other methods of disinfection are that it is a non-thermal process using no chemicals and causes fewer changes in colour, flavour, taste or pH with no residues. UV treatment efficiency is higher when product absorbance is lower. A higher reduction of *E.* coli is thus obtained in egg white, compared with egg yolk and whole egg; –2.2, –0.675 and –0.316 log cfu ml^{-1}, respectively, for 20 min at 1.3 mW cm^{-2} with a fluid depth of 0.153 cm (Unluturk *et al.*, 2008). Increasing temperature also increases UV treatment efficiency (Geveke, 2008). This technique could thus be used as a pre-treatment process.

Ultrasonic waves

Ultrasonic waves induce cavitation which is lethal for many bacteria. The efficiency of high intensity ultrasounds (10–1000 W/cm^{-2}) in decontaminating egg products depends on the volume treated (Huang *et al.*, 2006), but is always very low: 1 and 2 log cfu ml^{-1} reduction for *Salmonella* Enteritidis and *E. coli*, respectively, after 5 min at 40 W cm^{-2} in liquid whole egg (Huang *et al.*, 2006; Lee *et al.*, 2003). Even combination with high hydrostatic pressure is not effective (Lee *et al.*, 2003).

Ohmic heating

Ohmic heating occurs when an alternating electrical current is passed through a food, resulting in the internal generation of heat due to the electrical

Table 23.1 Bacterial inactivation of different species in liquid egg product by pulsed electric field (PEF) technology (Baron *et al.*, 2010)

Bacteria	Product			PEF intensity ($kV\,cm^{-1}$)	Number of pulses	Temperature (°C)	Reference
	Whole egg	Egg yolk	Egg white				
Escherichia coli	–3.5 log cfu ml^{-1}	–3 log cfu ml^{-1}	–1 log cfu ml^{-1}	15	500	0	Amiali *et al.* (2004)
	–5 to 6 log cfu ml^{-1}			26		37	Martin-Belloso *et al.*
	–6 log cfu ml^{-1}	–5 log cfu ml^{-1}		48		41	(1997)
				30	105	40	Ma *et al.* (2000)
	–4 log cfu ml^{-1}			9–15		60	Amiali *et al.* (2007)
	–6 log cfu ml^{-1}			36	100–200		Bazhal *et al.* (2006)
							Ma *et al.* (1997)
Salmonella Enteritidis	–4.3 log cfu ml^{-1}	–5 log cfu ml^{-1}	–3.5 log cfu ml^{-1}	30	105	40	Amiali *et al.* (2007)
				25	118	55	Hermawan *et al.* (2004)
				35	9		Jeantet *et al.* (1999)
Salmonella Typhimurium	–4 log cfu ml^{-1}			45			Monfort *et al.* (2010)
Listeria innocua	–3.5 log cfu ml^{-1}			50	32	36	Calderon-Miranda *et al.* (1999)
Pseudomonas	–0.95 to –3.5 log cfu ml^{-1}			48			Gongora-Nieto *et al.* (2001)
Staphylococcus aureus	–3 log cfu ml^{-1}			40			Monfort *et al.* (2010)

resistance of food. This technique has many advantages: temperature is quickly achieved, there are low heat losses since thermal energy is generated directly within the food itself, and liquids are uniformly heated (Pereira and Vicente, 2010). This technology is particularly suitable for the processing of viscous liquid foods such as egg products (Icier and Ilicali, 2005). Reznik (1993, 1996) registered two patents for the application of ohmic heating in liquid egg product pasteurisation.

High pressure carbon dioxide (HPCD)

In the high pressure carbon dioxide (HPCD) technique, food is exposed to either (pressurised) sub- or supercritical CO_2, in a batch, semi-batch or continuous system. Sub- and supercritical CO_2 is defined as CO_2 at a temperature and pressure above or below its critical point values, respectively (T_c = 31.1 °C, P_c = 7.38 MPa). Besides the environmentally benign nature of the HPCD process (CO_2 is nontoxic), the CO_2 pressures applied for preservation purposes are much lower (generally < 20 MPa) than the hydrostatic pressures described in Section 23.4.5 (Spilimbergo *et al.*, 2002). The use of this technology for egg product preservation has been patented by Lehman and Juchem (1987). *Salmonella* Typhimurium was inactivated in egg white and yolk after 120 min at 13.7 MPa and 35 °C, whereas only 0.06 log cfu ml^{-1} reduction was obtained in whole egg (Wei *et al.*, 1991). However, Garcia-Gonzalez *et al.* (2009) extended the shelf-life of liquid whole egg up to 5 weeks at 4 °C after HPCD processing at 13 MPa and 45 °C for 10 min.

23.4.7 Pasteurisation of shell eggs

Shell surface contamination is an obvious risk for the internal contamination of eggs, either by penetration through the shell, or by contact when the shell is intentionally broken. This is why washing grade A eggs ('fresh' or 'table' eggs) is carried out in many countries such as the USA, Canada, Australia and Japan (Hierro *et al.*, 2009). However, the European Union does not consider washing of shell eggs intended for retail sale sufficient (OJEU, 2003). Additional techniques for decontamination of shell surfaces are required.

Hot water bath

Immersion in a hot water bath at 57 °C for 25 min is an efficient decontamination technique against *Salmonella* Enteritidis (–3 log cfu ml^{-1}), with no detrimental effect on the major egg properties (Hou *et al.*, 1996). Complete inactivation can be even obtained with more drastic treatments (50–57 min at 58 °C), but this is too long to be cost-effective and damages egg white quality (Schuman *et al.*, 1997). Higher temperature (100 °C, 3 s) causes numerous shell cracks (Himathongkham *et al.*, 1999).

Hot air
Hou *et al.* (1996) observed a 5 log reduction in *Salmonella* Enteritidis on shell eggs treated in a hot air oven at 55 °C for 180 min. James *et al.* (2002) identified 180 °C for 8 s as the best treatment, i.e. enabling the highest surface temperature that can be achieved without detrimental changes to internal egg quality. Pasquali *et al.* (2010) did not observe any quality changes after exposing eggs twice to a temperature of 600 °C for 8 s with an interval of 30 s of cold air, obtaining a 1.9 log cfu ml^{-1} reduction of *Salmonella* Enteritidis.

Pulsed light
Pulsed light is a non-thermal technology which applies short pulses (10^{-3} to 10^{-2} ms) of an intense broad-spectrum light (200–1000 nm) to a product. Dunn (1996) obtained 8 log cfu ml^{-1} reduction of *Salmonella* on eggs after eight flashes of 0.5 J cm^{-2}. Hierro *et al.* (2009) showed that the treatment is more efficient on unwashed eggs, and that it preserves the integrity of the cuticule. This suggests it is best carried out as soon as possible after laying.

Acidic electrolysed water
Acidic electrolysed water is generated by electrolysis of a dilute HCl solution; pH is 2.6, oxidation-reduction potential 1.150 mV, and free chlorine concentration between 50 and 80 ppm. It achieves complete elimination of *Salmonella* Typhimurium, *Staphylococcus aureus*, *Listeria monocytogenes* and *E. coli* from the surface of shell eggs (Russel, 2003). Acidic electrolysed water is as effective as detergent sanitiser treatment (Bialka *et al.*, 2004). To avoid a very acidic pH, Cao *et al.* (2009) have developed a slightly acidic electrolysed water treatment (pH 6 to 6.5), which has an equivalent bactericidal efficiency.

23.5 Egg product functionality and use as an ingredient

Baldwin (1986) has described the hen egg as a multifunctional ingredient since it can perform several technological functions simultaneously in formulated foodstuffs. Its foaming, emulsifying, gelling, thickening, colouring and aromatic properties make it a universal ingredient for food industry.

23.5.1 Foaming properties of egg

Properties of egg white proteins
Proteins represent more than 90% of the egg white dry matter. They are predominantly globular proteins. Some of them are very heat-sensitive and/or sensitive to surface denaturation, explaining their unique functional properties. The interfacial properties of egg white proteins are responsible for their excellent foaming properties. Table 23.2 summarises some data

Table 23.2 Parameters of the kinetic of diffusion toward the air–solution interface of three major egg white proteins

Parameters	Ovalbumin	Ovotransferrine	Lysozyme	Reference
Apparent diffusion coefficient (10^{-10} m^2 s^{-1})	0.5 ($C = 10^{-4}$% prot.) (in solution: 0.7)		0.2 ($C = 10^{-4}$% prot.) (in solution: 1)	De Feijter & Benjamins (1987)
			0.15 ($C = 1.5.10^{-4}$% prot.)	Xu & Damodaran (1993)
	0.5 to 1 ($C = 0.1$% prot.)			Pezennec *et al.* (2000)
Surface concentration (mg m^{-2})	1.6 ($C = 10^{-4}$% prot.)		2.4 ($C = 10^{-4}$% prot.)	De Feijter & Benjamins (1987)
	1.5 ($C = 5.4.10^{-4}$% prot.)	0.8 ($C = 1.2.10^{-4}$% prot.)	0.5 ($C = 0.35.10^{-4}$% prot.)	Damodaran *et al.* (1998)
	2.1 (native protein) to 2.9 (heat-treated protein) ($C = 0.01$% prot.)			Pezennec *et al.* (2000) Croguennec *et al.* (2007)
			1.4 (pH 4) to 3 (pH 11) ($C = 0.1$% prot.)	Perriman & White (2006)
Surface pressure (mN m^{-1})	1 ($C = 10^{-4}$% prot.)		3.5 ($C = 10^{-4}$% prot.)	De Feijter & Benjamins (1987)
	14 ($C = 5.4.10^{-4}$% prot.) 24 ($C = 0.01$% prot.)	2.5 ($C = 1.2.10^{-4}$% prot.)	2.5 ($C = 0.35.10^{-4}$% prot.)	Damodaran *et al.* (1998) Pezennec *et al.* (2000)
			8 (pH 5.6) to 14 (pH 11) ($C = 0.012$% prot.)	Roberts *et al.* (2005)
			9 ($C = 5.10^{-4}$% prot.) to 24.5 ($C = 0.1$% prot.)	Chang *et al.* (2005)
Lag phase	YES if $C < 0.01$% Not enough molecules at the interface to create an increase of π		YES if $C < 0.01$% Not enough molecules at the interface to create an increase of π	De Feijter & Benjamins (1987)
	NO	YES	YES	Damodaran *et al.* (1998)

on the interfacial properties of three major egg white proteins. Ovalbumin interfacial behaviour is well known, with a significant amount of available data describing its tensioactivity, adsorption kinetics, interfacial shear and dilatational rheology (de Feijter *et al.*, 1978; Benjamins and van Voorst Vader, 1992; Benjamins and Lucassen-Reynders, 1998; Lucassen-Reynders and Benjamins, 1999; Razumovsky and Damodaran, 2001). There are also many studies on its structure at the air–water interface (Renault *et al.*, 2002; Kudryashova *et al.*, 2003; Lechevalier *et al.*, 2003, 2005b).

Lysozyme interfacial behaviour has also been extensively studied (Razumovsky and Damodaran, 2001; Kim *et al.*, 2002; Postel *et al.*, 2003), as well as its structure at the air–water interface (Lechevalier *et al.*, 2003, 2005b). Lysozyme forms much thicker films than the ovalbumin monolayer, but generates a significantly smaller surface pressure (Le Floch-Fouéré *et al.*, 2009). These different behaviours at the two-dimensional interface result in different foaming properties; unlike ovalbumin, lysozyme is a very poor foaming agent (Townsend and Nakai, 1983). Moreover, ovalbumin–lysozyme mixtures form films that are much thicker than those of both proteins in single protein systems, suggesting synergy in interfacial adsorption between the two proteins (Le Floch-Fouéré *et al.*, 2009). Damodaran *et al.* (1998) have suggested that electrostatic complexes between positively charged lysozyme and other negatively charged egg white proteins could explain the specific properties of protein mixtures, compared to single protein systems.

Egg white is still considered as the reference for foaming properties, compared with other animal and plant protein ingredients (Vani and Zayas, 1995; Matringe *et al.*, 1999; Pernell *et al.*, 2002; Foegeding *et al.*, 2006; Davis and Foegeding, 2007). Egg white is a balanced mixture of efficient surfactants. Its proteins are amphiphilic and show a relatively high surface hydrophobicity, thus diffusing quite rapidly toward the air–water interface where they adsorb efficiently. Their molecular flexibility ensures conformational rearrangement at the interface, resulting in a great decrease of surface tension. Their ability to form a continuous intermolecular network, especially when a certain denaturation level has been obtained, enables them to form a viscoelastic interfacial film responsible for foam stability. However, egg white proteins are not all equivalent, and do not contribute in the same way to egg white foaming properties (Table 23.3).

However, despite many studies trying to rank the different egg white proteins (Nakamura, 1963; Johnson and Zabik, 1981; Mine, 1995), the complexity of this product and its potential synergies make it impossible to separate out the role of the different egg white proteins (Lechevalier *et al.*, 2005b). As a result, prediction of the foaming properties of any egg white protein mixture is difficult, since competition for the interface and possible exchange between proteins at the interface may occur (Le Floch-Fouéré *et al.*, 2010). Nevertheless, the foaming properties of isolated egg white proteins are always lower than those of egg white. Egg white foams are the main component of many foods such as meringue, nougat and angel food

Table 23.3 Interfacial characteristics of major egg white proteins (Mine, 1995)

Protein	Surface tension ($mN\,m^{-1}$)	Foam index ($cm^3\,g^{-1}\,min^{-1}$)
Ovalbumin	51.8	0.59
Ovotransferrin	42.4	0.34
Lysozyme	42	0.12
Ovomucoïde	39	0
Ovomucin	ND	0
Mixture in egg white ratio	46.7	3.08

cake. However, it is noticeable that foaming properties are not related to actual performance in the production of angel food cake (Foegeding *et al.*, 2006).

Effect of physicochemical conditions and of processes

In the specific case of egg white proteins, surface hydrophobicity (which determines protein adsorption at the interface), the number of disulphide bonds (which determines protein flexibility) and the number of free sulfydryl groups (which determines protein reactivity) are critical factors in the structural modifications that occur at the air–water interface. Moreover, the number and the nature of inter- and intramolecular interactions determine the rheological properties of the interfacial film, and so foam stability. These interactions are favored by some denaturation; however, too much denaturation weakens the interfacial film, resulting in foam collapse (Kinsella, 1976; Trziszka, 1993; Kato *et al.*, 1994; Van der Plancken *et al.*, 2007a).

Acidification increases egg white foaming capacity (Hammershøj and Larsen, 1999; Croguennec, 2000), while its effect on foam stability is still disputed (Nakamura and Sato, 1964; Tsai and Hudson, 1985; Hammershøj and Larsen, 1999). Liquid pH also influences foaming properties of the corresponding egg white powder; an optimal pH would be 6.5 (Kim *et al.*, 2006).

Egg white can be diluted up to 40% without modifying its foam stability (Yang and Baldwin, 1995). But research on the effects of concentration is inconsistent (Conrad *et al.*, 1993; Froning *et al.*, 1987; Tsai and Hudson, 1985; Hammershøj *et al.*, 2004; Lechevalier *et al.*, 2007).

Egg white foaming properties can be improved by the addition of sucrose (effect on foam stability) or salt (effect on foaming capacity) before pasteurisation (Raikos *et al.*, 2007a). However, sucrose reduces foaming capacity (Davis and Foegeding, 2007) while salt reduces foam stability (Croguennec, 2000; Lechevalier *et al.*, 2006). A protective effect on foam stability against damage from heat treatment was also obtained by adding cations such as copper in egg white (Sagis *et al.*, 2001).

Another special feature of egg white foams is their dependence on raw

material quality (Lechevalier *et al.*, 2005a). Because the foaming properties of thick and thin egg white are different (Nakamura and Sato, 1964; Nau *et al.*, 1996), every parameter modifying the ratio between these two parts (length of egg storage, hen age, hen breed) modifies egg white foaming properties (Sauveur *et al.*, 1979; Scholtyssek and El Bogdady, 1980; Knorr, 1992; Kreuzer *et al.*, 1995; Hammershøj and Qvist, 2001). Another extremely important parameter is egg white/yolk separation quality, since even small traces of yolk (0.022%) are seriously damaging to egg white foaming properties (Wang and Wang, 2009).

Heat treatment damages egg white foaming properties. Industrial pasteurisation can decrease foaming capacity and stability by 10% (Kline *et al.*, 1966; Lechevalier *et al.*, 2005a); up to a threefold increase in drainage was observed by Ferreira *et al.* (1995). Heating during egg white drying also decreases egg white foaming capacity by between 10% and 60%, depending on the drying pH (Hill *et al.*, 1965), and foam stability by 20%. Shear rates during spraying are not responsible for these decreases (Lechevalier *et al.*, 2007). However, these results are contradicted: Hammershøj *et al.* (2004) observed an increase of foaming capacity by 10% and no change in foam stability.

Dry heating is an efficient way to increase egg white foaming properties (Kato *et al.*, 1994; Berardinelli *et al.*, 2006). Water content and powder pH are as important as temperature in controlling foaming properties (Van der Plancken *et al.*, 2007b). Structural and chemical changes of proteins can occur during dry-heating (Kato *et al.*, 1990; Matsudomi *et al.*, 2001; Desfougères, 2009), but vary according to the protein in question (Kato *et al.*, 1990). Egg white freezing also increases its foaming properties, mainly by increasing viscosity (Janssen, 1971; Wootton *et al.*, 1981).

Foaming properties are improved after low to mild shear rates ($<1000\ s^{-1}$) (Forsythe and Bergquist, 1951; Thapon, 1981; Lechevalier *et al.*, 2005a, 2005c, 2006). However, higher shear rates decrease foam stability (Thapon, 1981; Lechevalier *et al.*, 2005c). In industry, pumping and filtration steps during the processing of egg white powder are bad for foaming properties (Lechevalier *et al.*, 2007).

Many chemical modifications can improve egg white foaming properties: protein-polysaccharide complexes (Kato, 1995), proteolysis (Regenstein *et al.*, 1978; Lee and Chen, 2002; Hammershøj *et al.*, 2008), protein carboxylation (Ma *et al.*, 1986), protein succinylation and acylation, or deamidation (Ball *et al.*, 1982; Kato, 1995).

Industrial pasteurised liquid egg white always has worse foaming properties than egg white from manually broken eggs (Lechevalier *et al.*, 2005a), because of the damage caused by these industrial processes. The addition of hydrocolloids (such as guar gum, xanthan, carrageenan, pectins, etc.) before pasteurisation offsets the loss of foam stability. However, foam volume generally decreases when using these ingredients.

23.5.2 Emulsifying properties of egg yolk

Emulsifying activity is related to the capacity of surface-active molecules to cover the oil–water interface created by mechanical homogenisation, thus reducing interfacial tension. Emulsion stability involves the capacity to avoid flocculation, creaming and/or coalescence of oil droplets. Creaming and flocculation are reversible phenomena, which can be avoided by a simple agitation of the emulsion. Coalescence is the irreversible fusion of oil droplets due to the rupture of the interfacial film created by emulsifying agents. This phenomenon leads to a complete destruction of the emulsion. This underlines the importance of the structure and viscoelasticity of the interfacial film. Usually food emulsions are oil-in-water emulsions such as mayonnaise, sauces, custards, etc.

The main components of egg yolk are lipids (about 65% of the dry matter) with a lipid to protein ratio of about 2:1. Lipids are exclusively associated with lipoprotein assemblies, whereas proteins are present as free proteins or apoproteins included in lipoprotein assemblies. The interactions between lipids and proteins result in lipoproteins (low and high density), which represent the main constituents of yolk. Yolk is a complex system consisting in aggregates (granules) in suspension in a clear yellow fluid (plasma) (Chang *et al.*, 1977).

Yolk and plasma are very similar in their emulsifying properties, whilst emulsions made with granules show very distinct behaviour (Dyer-Hurdon and Nnanna, 1993; Anton and Gandemer, 1997; Le Denmat *et al.*, 2000). The latter are coarser (higher oil droplet size), especially at an acidic pH where granules are not soluble and their creaming rate (stability) is also distinct (Fig. 23.4) (Le Denmat *et al.*, 2000). These studies have demonstrated that yolk emulsifying power is predominantly due to plasma. Among plasma constituents, many studies have established the major role of low density lipoproteins (LDL) (Aluko *et al.*, 1998; Mine and Keeratiurai, 2000; Anton *et al.*, 2003; Martinet *et al.*, 2003), even if, in some conditions, high density lipoproteins (HDL) are more efficient than LDL in forming and stabilising oil–water emulsions (Hatta *et al.*, 1997; Mine, 1998). Martinet *et al.* (2003) established that LDL made finer emulsions than HDL, depending on different pH and ionic strength conditions. The interactions between apoproteins and lipids in assembling LDL particles are essential to transport the surfactants in a soluble form to the vicinity of the interface, and then to release them at the interface itself. Using Langmuir film balance (air–water interface), Martinet *et al.* (2003) showed that LDL break down when they come into contact with the interface, releasing neutral lipids, phospholipids and apoproteins from the lipoprotein core, which allow them to spread. LDL should thus serve as vectors to the interface for surfactant constituents (apoproteins and phospholipids) which are not soluble in water (Dauphas *et al.*, 2006). The apoproteins situated on the LDL surface start the LDL disruption mechanism by their initial anchorage at the interface, which provokes unfolding of the protein, leading to the destabilisation of the external layer of LDL (Fig. 23.5).

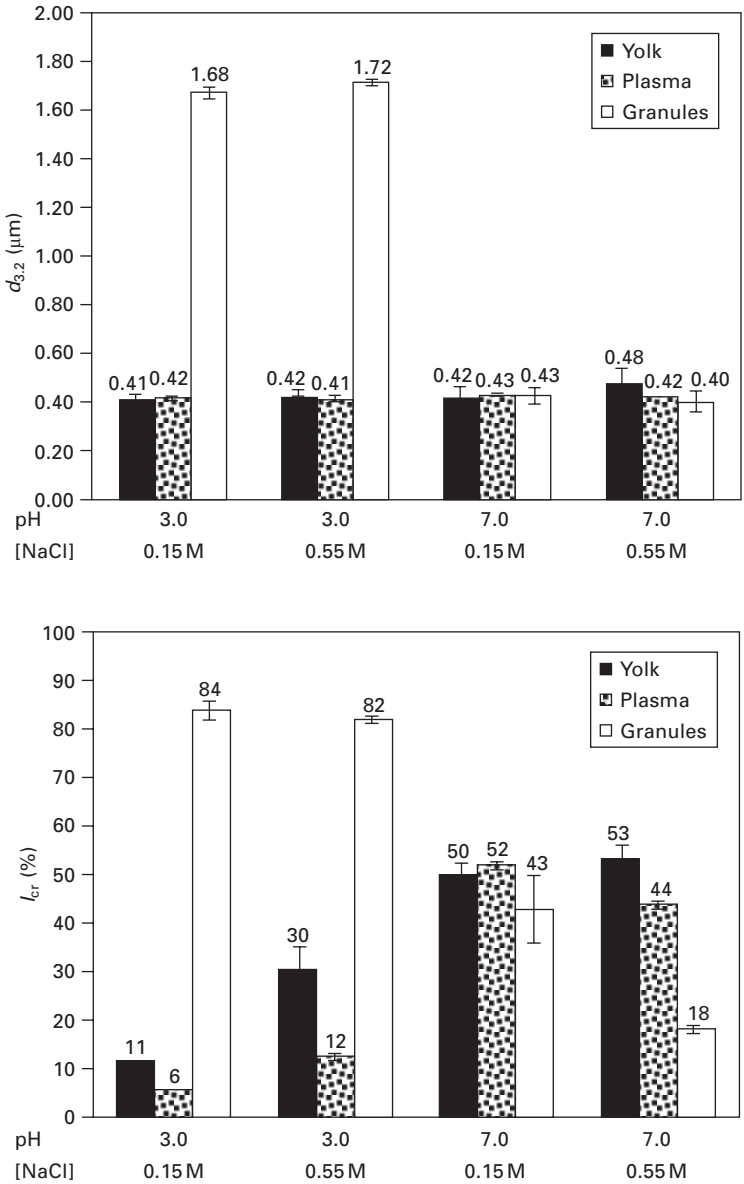

Fig. 23.4 Mean droplet diameter ($d_{3,2}$) and creaming index (I_{cr}) in oil–water emulsions (30 :70) prepared with yolk, plasma and granules, protein concentration: 25 mg/ml, homogenisation pressure: 200 bar, $n = 3$.

23.5.3 Gelling properties of egg white and yolk

Thermogelation of proteins

Proteins are responsible for gelling properties in whole egg as well as in white and yolk. Gelation occurs when the protein stability in solution is modified,

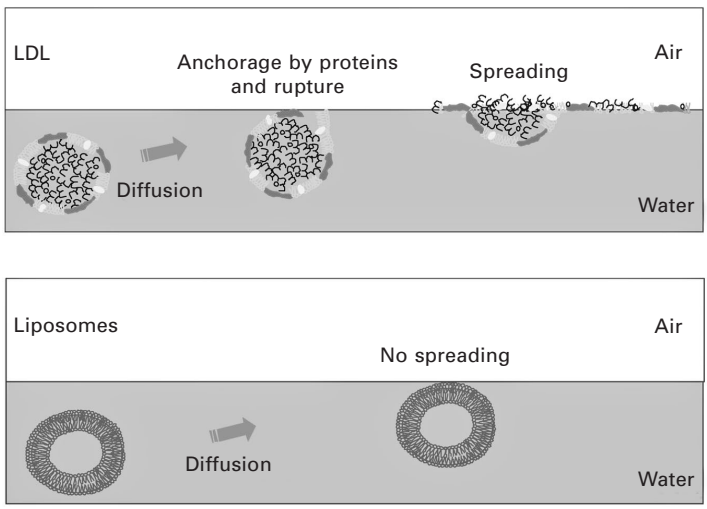

Fig. 23.5 Hypothetical mechanism of LDL adsorption at an oil–water interface as compared with liposome behaviour.

i.e. when the equilibrium between attractive (van der Waals) and repulsive (electrostatic, steric) forces is disrupted. Some treatments can modify protein structure with resulting effects on these repulsive and attractive forces. This is especially the case during heat treatment which is widely used in the food industry for egg white and yolk gelation. Heat-induced gelation of egg is consistent with the model of globular proteins heat gelation. It is a two-step phenomenon: first, unfolding of native proteins occurs, producing denatured proteins exposing their hydrophobic internal structure; second, the denatured proteins interact to form high molecular weight aggregates that can further interact with each other to result in a three-dimensional gel (Clark *et al.*, 2001). Unfolding and aggregation steps depend on many factors (protein concentration, ionic strength, pH, presence of sucrose, etc.) that can modify the number and/or the kind of interactions, with consequences for gel rheology. In the heat-induced gels of egg proteins, the interactions involved are predominantly hydrophobic and electrostatic, but some highly energetic interactions can be observed (disulphide links); thiol and amine groups are indeed very reactive, especially in alkaline conditions.

Egg yolk gels

Yolk undergoes gelation when it is subjected to heat treatment, but also to freeze–thaw processing. LDL, the major components of plasma, are involved in both cases (Kojima and Nakamura, 1985; Kurisaki *et al.*, 1981; Nakamura *et al.*, 1982; Tsutsui, 1988; Wakamatu *et al.*, 1982; Le Denmat *et al.*, 1999). Freezing–thawing gelation of yolk appears at temperatures below –6 °C (Lopez *et al.*, 1954). Rapid freezing and thawing results in less gelation than a slow freeze–thaw process (Kiosseoglou, 1989). Freeze–thaw

gelation is partially reversible by heating. Freezing acts by 'dehydration' (A_w decrease) of LDL apoproteins, favouring their rearrangement, interactions and aggregation, which finally leads to gelation. LDL are also involved in heat-induced gelation of yolk (Saari *et al.*, 1964). LDL denaturation starts at 70 °C (Tsutsui, 1988), and stable gels are obtained when heated at 80 °C for 5 min (Kojima and Nakamura, 1985). Heat-induced gelation of LDL occurs within a large pH range (4–9) (Nakamura *et al.*, 1982). Between pH 6 and 9, gels are opaque (coagulum) but translucent at extreme pH levels (4–6 and 8–9) (Kojima and Nakamura, 1985; Nakamura *et al.*, 1982). On the other hand, granules are very resistant to heat treatments, suggesting their use for other specific applications (Le Denmat *et al.*, 2000).

The primary stage of both freeze–thawing and heat-induced gelation is the disruption of the LDL structure (Kurisaki *et al.*, 1981). It is assisted by dehydration in the case of freeze–thawing, or by unfolding for heating. Lipid–protein interactions are disrupted and non-polar protein interactions are increased (Mahadevan *et al.*, 1969).

Egg white gels

With the exceptions of ovomucin and ovomucoid, all the egg white proteins coagulate when heated (Johnson and Zabik, 1981), but the temperature of denaturation significantly varies from one protein to another: 84.5, 74 and 65 °C for ovalbumin, lysozyme and ovotransferrin, respectively, at pH 7 in egg white (Donovan *et al.*, 1975). Ovalbumin and ovotransferrin are the main contributors to egg white thermal gelation. Ovalbumin can establish covalent bonds with other egg white proteins through thiol/disulphide bond exchanges or thiol oxydation. Ovotransferrin is usually considered both as the gelation initiator and, finally, also as a limiting factor in egg white pasteurisation. Ovotransferrin elimination has then been suggested as a way of improving egg white gelling properties (Kusama *et al.*, 1990). Moreover, because ovotransferrin is more stable at an alkaline pH, at high ionic strength, and when metal ions are bound to it, the gelation temperature of egg white can be significantly increased by modification of these parameters (Cunningham and Lineweaver, 1965). Gelation occurs at a critical protein concentration. Over this critical gelation concentration, gel firmness increases with protein concentration. Gel firmness also increases with heat treatment intensity up to an optimum, which is 85.2 °C for egg white at pH 9.0 and an ionic strength of 80 mM (Holt *et al.*, 1984). Beyond this optimum, gels are coarser with poorer rheological properties.

The extent and the kind of the interactions between the denatured proteins depend on the protein structure and, in particular, the extent of unfolding at the end of the denaturation step. These interactions also depend on the overall physico-chemical conditions that can be either limiting or favouring, resulting in an increase or a decrease of aggregation rate respectively, and then in a decrease or an increase of the denaturation extent before interactions take place (Totosaus *et al.*, 2002). These mechanisms have been extensively

studied for egg white and ovalbumin heat-gelation, with a focus on the effect of ionic strength on the structure and characteristics of the gels (Holt *et al.*, 1984; Woodward and Cotterill, 1986; Woodward, 1990; Croguennec *et al.*, 2002; Raikos *et al.*, 2007b). When heating occurs at high ionic strength, protein charges are screened, favouring hydrophobic interactions (Doi, 1993). In these conditions, random aggregates of slightly denatured proteins appear, corresponding to opaque gels with low rigidity, elasticity and water retention capacity. On the other hand, at low ionic strength, high electrostatic repulsions delay aggregation (Raikos *et al.*, 2007b), favouring denaturation. Finally, further aggregation involves hydrophobic regions and induces linear polymeric aggregates (Fig. 23.6). In higher ionic strength conditions, these aggregates can interact to form gels with the right functionality.

A two-step heating process has been proposed to produce translucent gels from egg white which are very firm and elastic, and with an exceptional water retention capacity (Kitabatake *et al.*, 1988a). pH is another major parameter for egg white gelation control. Close to their *p*I (around 5), proteins tend to form random aggregates, similar to those obtained at high ionic strength. In contrast, at alkaline pH, egg white offers the best gelling properties (Fig. 23.7) (Ma and Holme, 1982; Kitabatake *et al.*, 1988b). On the other hand, at acidic pH (2.0), limited protein solubility is responsible for low gelation temperature and the low rheological properties observed (Raikos *et al.*, 2007b). Egg white gelling properties also depend on the nature of ions: iron, copper, aluminium, calcium, magnesium but also sulphate, phosphate and citrate are among the most influential (Nakamura *et al.*, 1979; Oe *et al.*, 1989; Lin *et al.*, 1994; Croguennec *et al.*, 2001a, 2001b). Their effects are due to specific binding on egg white proteins, either stabilising ovotransferrin, thus delaying egg white thermal gelation, or destabilising ovalbumin, by modifying its surface charge.

Sugar protects egg white proteins from heating, but coarser aggregates

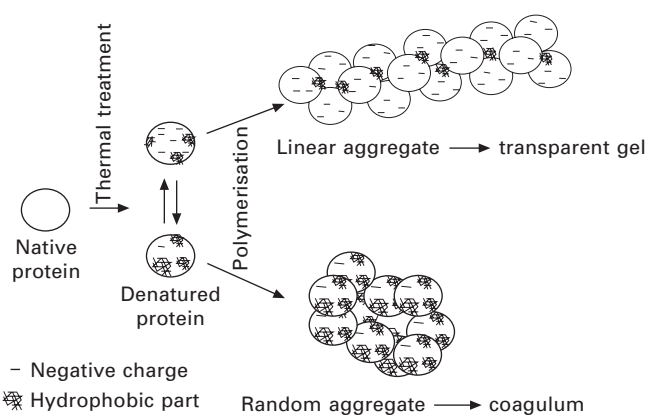

Fig. 23.6 Effect of protein charge on thermal gelation of egg white (according to Doi, 1993).

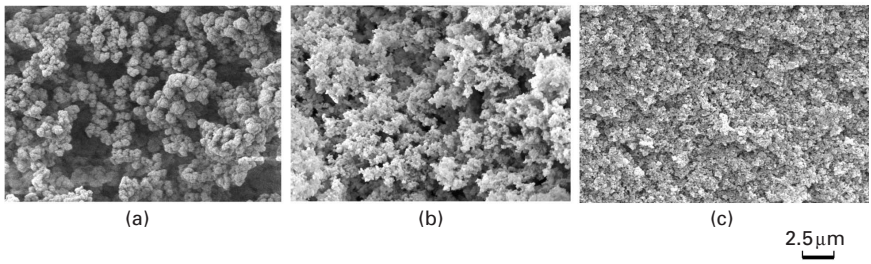

Fig. 23.7 Effect of pH on egg white gels microstructure observed by electronic transmission microscopy (× 5000): (a) pH 5, (b) pH 7, (c) pH 9 (Croguennec *et al.*, 2002).

are formed, slightly decreasing rheological properties of gels (Raikos *et al.*, 2007b). Egg white exposure to air decreases its gelling properties, whereas shearing has a positive effect (Lechevalier *et al.*, 2005a). Egg white cooling has little effect on its gelling ability (Xu *et al.*, 1997). However, freeze–thaw cycles decrease gel strength and elasticity (Dill *et al.*, 1991), probably because of protein aggregation; the addition of sugar limits this damage. If industrial pasteurisation conditions do not affect egg white gelling properties, drying may be harmful; pH adjustment before drying limits this problem. Hill *et al.* (1965) advised a pH of 8.5, whereas Kim *et al.* (2006) obtained the best gel firmness at pH 6.5.

Finally, dry-heating at high temperatures (80 °C) for a long period (up to 10 days) is an efficient way of improving the gelling properties of egg white (Kato *et al.*, 1989); pH control is an important parameter (Mine, 1996, 1997). This treatment increases protein flexibility and availability of reactive groups that can further interact to strengthen the gel formed when the egg white is solubilised and heated in solution.

23.5.4 Industrial applications

Egg yolk for sauces

Egg yolk including 8–12% salt, and possibly 2–10% sugar, has long been used in cold emulsified sauces such as mayonnaise. Salted egg yolk is still the reference emulsifier for sauce applications since its high viscosity favours sauce stability by limiting coalescence. Moreover, the yolk can be adjusted to improve mayonnaise texture by decreasing oil droplets and size dispersion. As an example, the proportion of yolk can be increased (Campbell *et al.*, 2005), or strongly pasteurised yolk (68 °C, 11 min) can be used (Guilmineau and Kulozik, 2007). Enzymatic treatment of yolk with A2-phospholipase is another technology (Dulith and Groger, 1981) with the texture of sauces produced by structural modifications of granules and LDL (Daimer and Kulozik, 2009).

Egg yolk for sweetened products

Egg yolk is also a traditional ingredient for desserts such as cakes, ice creams, etc. Proportions from 2 to 10% of the ingredient mix give colour and creaminess or limit sugar crystallisation in products such as custard and pastry cream. It is sometimes used in higher concentrations in some speciality products. For all these applications, natural egg yolk is used often with 10% added sugar. Because sugar preserves the organoleptic properties of yolk during heat treatment, egg yolk with 20–50% added sugar has also been suggested for some speciality products. In addition, 50% added sugar significantly reduces yolk A_w (0.85), improving microbiological stability after pasteurisation. Although more unusual, 10% sugared egg yolk can be sold frozen: sugar is a good cryoprotective agent, limiting freeze–thaw gelation (Telis and Kieckbusch, 1998).

Egg white for foams

Foams made with egg white, sometimes with 10–30% added sugar, are blended into sweetened sauces (e.g. chocolate mousse) or batter (e.g. sponge cake) to lighten them. In cakes, egg white also contributes to product firmness after cooking, because of its gelling properties. Other recipes use egg white foams with 40–80% added sugar: for example, macaroons, Italian or French meringues or nougat. In this case, egg white is foamed and caster sugar or hot sugar syrup is gradually added. Egg white foam is also used increasingly to lighten salted products such as fish or vegetables foams. But it is still a real challenge to select egg white with the right properties for such complex formulas. Many different processes (various time–temperature combinations for liquid pasteurisation and dry-heating) and formulations (e.g. hydrocolloids and/or sugar) have been developed to create the right type of egg white for particular user applications and processes.

Whole egg uses

Around two-thirds of pasteurised egg products in more traditional sectors of the food industry are whole egg. Most of these applications are cooked products. Whole egg is used as a multifunctional ingredient. In batter (genoeses) whole egg with sugar is a foaming ingredient, whereas in dough (brioches, cakes), emulsifying and film-forming properties contribute to the alveolar structure. Whole egg also has a binding function in shortcrust pastries and is also used to glaze Viennese pastries after cooking. In dried pasta, whole egg is nowadays limited to regional specialities to add colour and texture. However, egg is still a major ingredient in fresh pasta, since it gives excellent heat stability during pasteurisation. The egg white/yolk ratio can be modified: egg white increases pasta firmness, whereas egg yolk favours pasta blowing during cooking (Alemprese *et al.*, 2009). Some specific formulas such as whole egg powders or sweetened concentrated whole egg may provide interesting technical solutions.

23.6 Speciality egg products

These products result from cooking of either shell eggs or egg products. The hygienic requirements of these two products are noticeably different: on the one hand raw material is a non-processed farm product, whereas on the other hand it has been previously stabilised. Thus, liquid egg products require cold storage, whereas it is not obligatory for shell eggs, and factories using both raw materials have to organise specific product flows to avoid cross-contamination.

23.6.1 Hard-cooked eggs

Processing

Hard-cooked eggs are mainly designed for mass catering, but also for industrial caterers, sandwich makers, and even the retail market in some countries. Eggs are candled in preparation units, to eliminate cracked eggs that may blow up during cooking. This is indeed the main difficulty in the cooking step, with economic and hygienic consequences. To prevent cracking, the shell is weakened before cooking to enable some volume increase. Moreover, shell weakening eases peeling. To do this, metal points are used to pierce shells, or eggs are gently dropped over a flat area. Shell eggs are then cooked either in a steam room, or in a hot water bath. Cooking conditions usually vary between 95 and 100 °C for 13–20 min. The inner temperature has to be around 85 °C to make sure that yolk is completely coagulated (Buay *et al.*, 2006), without discoloration of the yolk surface. After cooking, eggs are cooled by immersion in cold water baths to bring the inner temperature under 30 °C before peeling which is mechanically performed by egg friction, e.g. passing eggs between two metal plates for example. After peeling, eggs may stay for many hours in a refrigerated acidification bath to finish egg cooling and prevent the discoloration (black edging) of the yolk surface, which is due to the formation of ferrous sulphide. Eggs are then packaged either in brine, or under modified atmosphere. Pickling in brine is a simple preservation method, with the acid ($3 < pH < 4$) and salted brine limiting bacteria growth; antifungal agents (sodium sorbate or benzoate) may also be added. Eggs remain preserved as long as they are covered by brine. Modified atmosphere packaging (MAP) is used for small packs (10 to 30 eggs). MAP involves the injection of a gas mixture (CO_2/N_2) into the packaging, thus depriving bacteria of oxygen. MAP leads to better organoleptic quality than brine, but it favours anaerobic species growth. Moreover, packaging opening requires the complete consumption of the product.

Quality criteria and factors affecting them

The fresher the egg is, the harder it is to peel. Ease of peeling depends on egg white pH, which has to be over 8.5 to prevent any difficulty (Britton and Fletcher, 1987). Egg freshness and shell egg storage are thus specifically controlled.

The microbiological quality of hard-cooked eggs depends on hygienic practices during cooling, peeling and packaging. Cooling water quality is a critical point, since cooling water flows in a closed circuit for reasons of cost. To limit bacterial growth, the counter-flow has to be high enough to limit its heating. Water filtration and chemical or physical treatments are also carried out to disinfect water and keep it clean. A good setting of the peeling machine is also essential for hard-cooked egg quality. It must neither crush the eggs, which can be ensured by using graded eggs, nor push shell fragments in eggs.

23.6.2 Other speciality egg products

Poached eggs and fried eggs

Poached eggs are cooked either directly in hot water, or in pans. Cooking has to be controlled in order to coagulate the white only. After cooling, eggs are packaged under modified atmosphere or vacuum-wrapped, or pickled in brine. Cooking in final packaging also exists.

Fried egg cooking is performed in flat pans, in such a way that yolk remains liquid, but coagulated on its surface to prevent its further bursting. This product is exclusively packaged under modified atmosphere.

Omelettes, tubes of hard-cooked eggs, stiffly beaten egg whites

Omelettes are prepared with liquid whole egg, possibly added with milk or egg white. More or less sophisticated garnishes (cheese, bacon cubes…) can be added, either in the liquid preparation, or just after cooking. Hydrocolloids may be added to control texture, and to limit syneresis when omelettes are frozen. Citric acid can be added to prevent discoloration due to ferrous sulphide. Cooking is carried out on hot plates in pans, or using infrared or vacuum ovens. Immediately after cooling, omelettes are packaged, either under modified atmosphere (CO_2/N_2) for a use-by date of 3 to 4 weeks at 4 °C, or frozen for a use-by date of 12 to 18 months.

Few manufacturers have the equipment to produce tubes of hard-cooked eggs. Liquid egg white and egg yolk are separately cooked in cylindrical and coaxial tubes which are heated by warm water flow. First egg white is cooked in the external ring, then the central cylinder is removed and yolk is dropped and cooked until coagulation. Tubes of hard-cooked egg are then vacuum-wrapped and cooled or frozen. The major critical point consists in egg white and yolk adhesion that have to be perfect to ensure satisfactory appearance of slices.

Whipped egg whites are one of the rare sweetened speciality egg products, exclusively sold refrigerated. Sugared (around 20%) pasteurised liquid egg white is foamed, by air or gas injection. The sweetened egg white foam is then dosed in plastic containers, and cooked slightly before cooling.

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Index