Poultry and pig nutrition

Challenges of the 21st century

edited by:
Wouter H. Hendriks
Martin W.A. Verstegen
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List of abbreviations

AA	amino acid
ADG	average daily gain
AFB1	aflatoxin B1
AGP	antibiotic growth promoters
AgRP	Agouti-related peptide
AHR	aryl hydrocarbon receptor
ALCA	attributional LCA
AMC	antimicrobial compounds
ANF	anti-nutritional factors
BALT	bronchus/lower airways-associated lymphoid tissues
BSF	black soldier fly
CART	cocaine and amphetamine-related transcript
CCK	cholecystokinin
CCR	C-C chemokine receptor
CH_4	methane
CLA	cluster analysis
CLCA	consequential LCA
CP	crude protein
CRH	corticotropin-releasing hormone
CrPic	chromium picolinate
DCs	dendritic cells
DDGS	dried distillers grains with solubles
DE	digestible energy
DGGE	denaturing gradient gel electrophoresis
DHA	dehydroascorbate (Chapter 8)
DHA	docosahexaenoic acid (Chapter 10)
DHAR	dehydroascorbate-reductase
DM	dry matter
DMN	dorsomedial nucleus
DON	deoxynivalenol
DP	degree of polymerisation
DVE	absorbed protein supply
ENS	enteric nervous system
ETEC	enterotoxigenic *E. coli*
EU	European Union
FB1	fumonisin B1
FC	fermentable carbohydrates
FCR	feed conversion ratio
FTU	phytase units

FUM	fumonisins
GALT	GIT-associated lymphoid tissues
GENALT	urogenital tract-associated lymphoid tissues
GHRH	growth hormone-releasing hormone
GI	gastrointestinal
GIT	gastro-intestinal tract
GMOs	genetically modified organisms
GPx	glutathione peroxidase
GR	glutathione-reductase
GSH	glutathione
GSSG	oxidised glutathione
HACCP	hazard analysis critical control point
HS	heat stress
HSF	heat shock factors
HSP	heat-shock protein
IL	interleukin
IPEC	intestinal porcine jejunum epithelial cells
LCA	life cycle assessment
LHA	lateral nucleus
LPS	lipopolysaccharide
m/z	mass to charge ratio
mAb	monoclonal antibody
MCF	methane conversion factor
MALT	mucosa-associated lymphoid tissues
MCH	melanin-concentrating hormone
MDHA	mono-(de) hydro-ascorbate-radical
MOR	μ-opioid receptor
MOS	mannan oligosaccharides
MP	metabolisable protein
α-MSH	α-melanocyte-stimulating hormone
N_2O	nitrous oxide
NADH	nicotinamide-adenine-dinucleotide
NADPH	nicotinamide-adenine-dinucleotide-phosphate
NALT	nasal cavity-associated lymphoid tissues
NARC	arcuate nucleus
NDC	non-digestible carbohydrates
NE	net energy
NH_3	ammonia
NIR	near infrared reflectance spectroscopy
NPY	neuropeptide Y
NSP	non-starch polysaccharides
NTS	nucleus tractus solitarius
OEB	degraded protein balance

OM	organic matter
OTA	ochratoxin A
PCA	principal component analysis
PCR	polymerase chain reaction
PLF	Precision livestock farming
POMC	proopiomelanocortin
PUFA	polyunsaturated fatty acids
PVN	paraventricular
ROS	reactive oxygen species
RS	resistant starch
SBM	soybean meal
SCFA	short chain fatty acids
SD-33	syndyphalin
SR-IMS	synchrotron radiation-based infrared microspectroscopy
T-2	T-2 toxin
TBARS	thiobarbituric acid reacting substances
TDP	total digestible protein
TJ	tight junctions
TLR	Toll-like receptor
TN	thermoneutral
Tregs	regulatory T cells
TRH	thyrotropin-releasing hormone
ZEA	zearalenone
ZFE	zebrafish embryos

Foreword

More pork and poultry meat is consumed globally than any other meat from terrestrial animals. On average, these two meat sources make up almost three-quarters of the total annual meat consumption. In addition, highly nutritious eggs provide the consumer with a versatile, tasty and affordable source of animal protein. World egg production has also dramatically increased during the past two decades and this trend is expected to continue, especially in developing countries. According to recent forecasts, the global human population will grow to nine billion by 2050 and 60% more food must be produced to meet the demand. It is estimated that the production of intensively reared animals (pigs and poultry) will at least need to be doubled to meet this increasing demand for animal derived foods. In this changing environment, the production systems must embrace established as well as potential advances in technology and innovations. Given the limited availability of global resources and the need to reduce pressure on the environment, the key challenge in the 21st century will be the sustainability of food and feed production systems. It is inevitable that future nutritional strategies will continually evolve to meet these challenges and, in this context, the publication of this book is a timely and valuable contribution.

'Poultry and pig nutrition – challenges of the 21st century' is focused on an array of emerging technology trends, which are multidisciplinary and interdisciplinary, with the aim of facing up to the complex problems and challenges we currently need to address. Containing contributions from world leading authorities in the field of non-ruminant nutrition, the 18 chapters in this book provide comprehensive summary on novel solutions to the issues faced in the nutrition of pigs and poultry.

In the past, animal nutrition research focused primarily on refining the nutritional needs of farm animals with respect to energy, protein and minerals. In recent decades, researchers have become cognizant of the many complexities relating to nutrition, especially the roles of physiology, microbiology, genetics and immunology to better interpret the science of animal nutrition. This realisation is opening up new and important possibilities. It is of vital importance that the global animal industry fully recognises the opportunities and initiate appropriate frameworks. The primary aim of the book is to bring together such new areas of science that are expected to revolutionise animal nutrition during the coming decades and an overview of potential stratagems is well summarised in Chapter 1. A range of relevant subject areas is covered and includes 'Physiological processes driving amino acid utilisation' (Chapter 2), 'Regulation of feed intake' (Chapter 3), 'Intestinal health management

in the post-antibiotic era' (Chapter 4), 'Development and functioning of immunity' (Chapter 5), 'Potential manipulation of intestinal microbiota by carbohydrates' (Chapter 6), 'Application of omics technologies' (Chapter 7), 'Alleviation of heat stress on animal productivity' (Chapter 8), 'Future of non-invasive bioanalytical techniques' (Chapter 9), 'Role of biotechnological tools' (Chapter 10), 'Co-products' (Chapter 11), 'Implications of mycotoxin contamination on animal productivity and feed security' (Chapter 12), 'Novel alternative protein sources' (Chapter 13), 'Life cycle assessment of the pig and poultry sectors' (Chapter 14), 'Nutrition and environmental sustainability' (Chapter 15), 'Nutrient utilisation models' (Chapter 16), 'Feed processing technology' (Chapter 17) and 'Precision animal farming' (Chapter 18). The discussion on all the above innovative approaches is carefully balanced and combined with established understanding of classical animal nutrition.

Our overriding conclusion is that this is a necessary and essential reference book providing a look into future technologies in the nutrition of pigs and poultry. The extensive bibliography, with up to date citations, is provided after each chapter for readers requiring further information on the areas covered. This book will be of immense value to researchers, nutritionists, university teachers and students of pig and poultry nutrition. Those involved in the commercial industry will find it highly valuable because of the wealth of information provided and the many insights shared by the various authors.

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Challenges in the 21st century in pig and poultry nutrition and the future of animal nutrition

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Summary points

- 21st century animal nutrition faces a number of challenges including animal welfare, environmental pollution minimisation, use of novel ingredients, use of ingredients not suitable for human consumption and more.
- New area of science are paramount for animal nutrition to meet these challenges such as molecular biology, nutrigenomics, molecular genetics, information technology.
- Precision animal nutrition will gain in importance as well as the validation of alternative feed ingredients and the increased use of food waste and co-products.
- There is an increasing need for better cooperation between medical and agricultural sciences on the basis of professional logic, as well as cooperation in R&DI programs and education.

Keywords: pig and poultry nutrition, 21st century challenges, future animal nutrition

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1.1 Introduction

The predicted increase in the human population and standards of living in developing countries by 2050are expected to create a high demand for animal-derived protein (Boland *et al.*, 2013). In many OECD countries, overconsumption of food has been increasing over the past 40 years. Currently, one in every 2 persons in these countries is overweight or obese with America having the highest obesity rate (38.2%) and Japan the lowest (3.7%) (OECD, 2017). Nearly 50% of deaths in the Europe Union are due to cardiovascular diseases, while 30% are caused by tumour diseases (Eurostat, 2018). It is without a doubt that nutrition is one of the key risk factors of these illnesses. By changing adverse nutritional habits and by consuming foods and food products which better conform to nutritional requirements, life expectancy of a larger number of people can be increased. In addition to the problems arising from lifestyle, eating habits and eating culture, food related allergies, the overreaction of the organism to certain foods or their ingredients, are increasing (Branum and Lukacs, 2009). Food intolerance – which also belongs to this range of problems – occurs when the abnormal symptoms caused by a food do not have an immunological origin. As a result of this oversensitivity, those affected often require special foods besides nutrition alternatives. As such, the challenge of 21st century animal agriculture is to sustainably produce foods and food products of animal origin in the proper quantity and quality which is safe to eat and can be traced within the production chain.

In order for the agricultural sector to be able to provide proper quantities of safe food materials to the food industry, there is an increasing need for better cooperation between medical and agricultural sciences on the basis of professional logic, as well as cooperation in R&DI programs and education. In addition to agricultural and medical science, nutrition biologists, genetic experts and other professionals dealing with nourishment will have important roles in the future. The food production chain approach and collaboration between various scientific disciplines should be instilled in current education programmes to develop young professionals, in order to produce high quality foods and improve human health. Efforts to such cooperation can already be observed e.g. in the USA, Canada, some EU-countries and in New-Zealand.

The present chapter focusses on the challenges in pig and poultry nutrition in the 21st century and provides a perspective of animal nutrition in the next decades.

1.2 Challenges in the 21st century

The agricultural and food production sectors of many countries have to face major challenges in the 21st century.

As depicted in Figure 1.1, these challenges include:

- Meeting the food demand of a rapidly growing world population. Currently, the human population is nearly 7.5 billion (Figure 1.2) but by the year 2050 the United Nations (2011) predict that there will be more than 9.0 billion people. In addition, living standards are also increasing.
- The increasing demand for high quality and safe food. FAO (2009) estimates the world will have to produce approximately 60-70% more food in the next 35 years. This organisation also predicts that animal protein production will increase at least three-times and meat production will double by 2050.
- Decreasing potential agricultural land area due to industrialisation, the building of new motorways, new city construction programs, urbanisation and natural soil erosion.
- Climate change, and its effects on animal production and elimination of these effects by genetic, nutritional and/or technical innovations (Pullar, 2011).
- Declining fresh water resources.
- Increasing severity of environmental load-related problems.

In addition, aspects such as animal welfare, food-feed-fuel competition and self-sufficiency level are additional challenges facing countries. It is also a known fact that the quality of food of animal origin is greatly determined by the nutrition of animals. Feeds in pig and poultry production systems can make up 50-80% of the costs of production and unutilised dietary components are a major contributor to pollution. As such, animal nutrition has a key role to play in solving many of the above-mentioned challenges. In 2017, nearly 1032 million tons of compound feed for farm animals was produced world-wide (Figure 1.2).

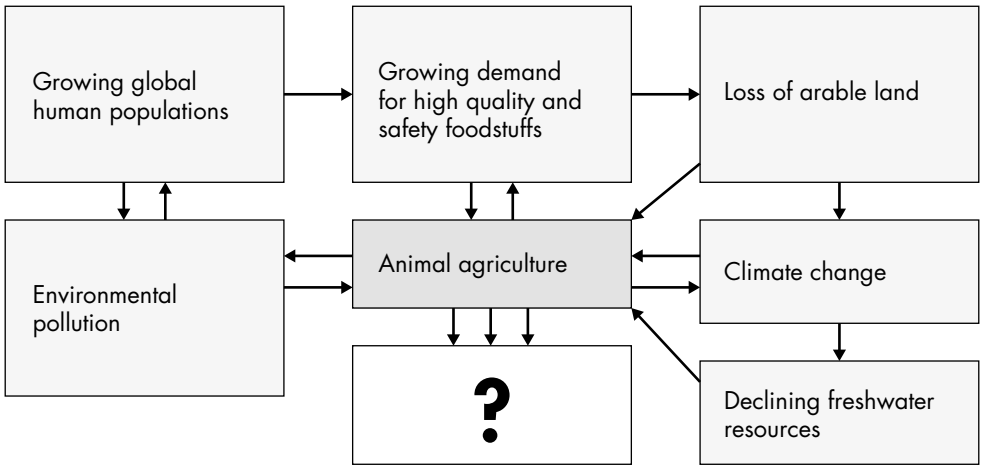

Figure 1.1. Main challenges in 21st century animal agriculture.

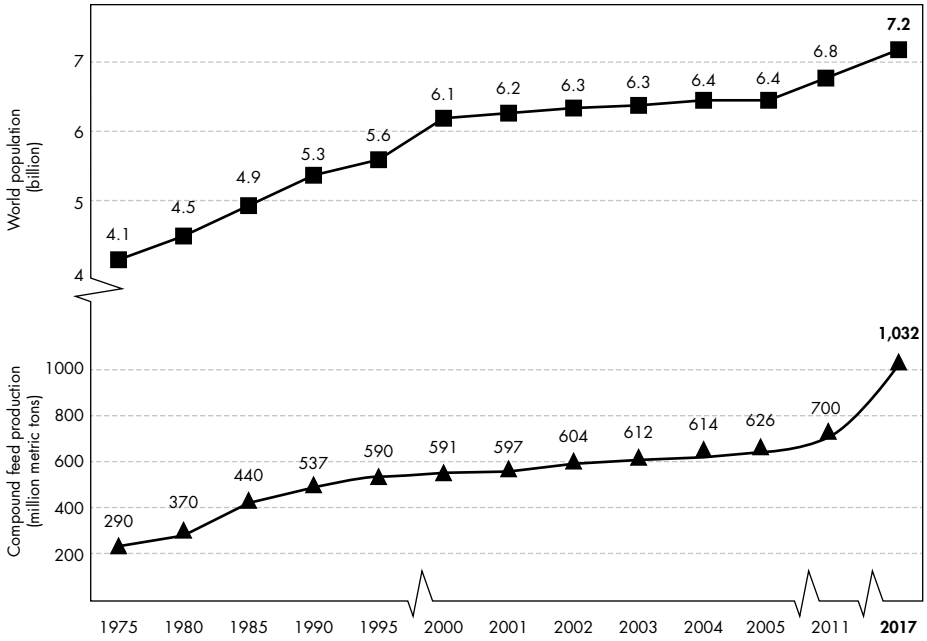

Figure 1.2. World population and compound feed production from 1975 to 2017 (based on Alltech, 2018; Gilbert, 2004; Gill, 2006; United Nations, 2011).

Today it is clear that the quality of the mixed feed can have a determinant effect on the quality of foods of animal origin. In addition, in many countries production animals are still fed diets containing ingredients with an uncertain origin, undermining feed safety and, therefore, food safety and health. It is obvious that livestock nutrition has a major responsibility for food production, not only in terms of quality and quantity of food but also safety and health. Livestock nutrition, therefore, can be considered to face the following important tasks in the 21st century (Babinszky and Halas, 2009):

- More active participation in animal production to supply safe food in sufficient quantities, in accordance with the requirements of society (Koerkamp *et al.*, 2007).
- Further improve the efficiency of animal nutrition (biological efficiency, technological efficiency and economic efficiency).
- Wider use of various co-products as well as further reduction of human edible ingredients in animal nutrition.
- Rethink the interrelation between animal nutrition, animal husbandry and environmental protection. The latter entails that good quality and safe food of animal origin should be produced using technologies which contribute to the increased sustainability of the system, i.e. environmental-friendly nutrition systems which lead to a reduction in nitrogen and phosphorus output.

Based on the above it can be stated, that the key issue will be to produce sustainable food via sustainable feed (and feeding) with year by year decreasing resources and with the need to reduce environmental pollution (Cortly, 2014). Already in 1996, Vavra wrote that if the rate of resource exploitation continues in the future, it will lead to a depleted earth. Most scientists agree that current production systems in animal agriculture are generally non-sustainable.

Sustainability of agriculture has been defined by many researchers but a uniform definition for it is still lacking (White, 2013). The most common definition used is: 'development which meets the needs of the present without compromising the ability of future generations to meet their own needs' (World Commission on Environment and Development, 1987), but there are many more definitions and interpretations. Rather than seeking or proposing another definition, White (2013) depicted sustainability as a Wordle derived from common elements in over 100 previously-published definitions. Sustainability is also often viewed to encompasses three pillars (Elkington, 1997). First, the planet (environment): in order for something to be sustainable, it must be environmentally viable. Second, people (social): sustainability must be socially viable, in relation to food affordability and changes that would directly impact the human population. Third, profit (economy): if something is not economically profitable, it is non-viable in the long run and will have detrimental effects on future generations. With the other words, this so called 'triple-bottom-line approach' includes three factors: environmental stewardship, social responsibility and economic viability (Capper, 2013). It should be noted that sustainability does not necessarily imply organic agriculture, although some components of this system might be needed to establish sustainable farming system (Tedeschi *et al.*, 2017).

In order to increase the efficiency of animal products, it is especially important to introduce the latest scientific findings into practice as quickly as possible. This means that the so-called innovation time (the time span between product idea and the actual production) has to be reduced as much as possible. However, the question is whether we can respond to the challenges of the 21st century with our classic animal nutrition knowledge. Probably not, and this is why it is important to involve new areas of nutrition (e.g. -omics, big data) but also new technologies (e.g. artificial intelligence, genetic fortune telling) into the innovation activities. Inter- and transdisciplinary research is needed into the mechanisms for achieving improved welfare standards within very different social and economic contexts (Buller *et al.*, 2018). In other words, to provide the right answers to new challenges, a more holistic approach is required involving state of the art technologies.

Babinszky and Halas (2009) presented other areas of natural science and/or technical science that are required besides classic animal nutrition knowledge in order to be able to adequately respond to the current challenges. Figure 1.3 summarises the envisaged relationship between natural, nutritional science and other related disciplines.

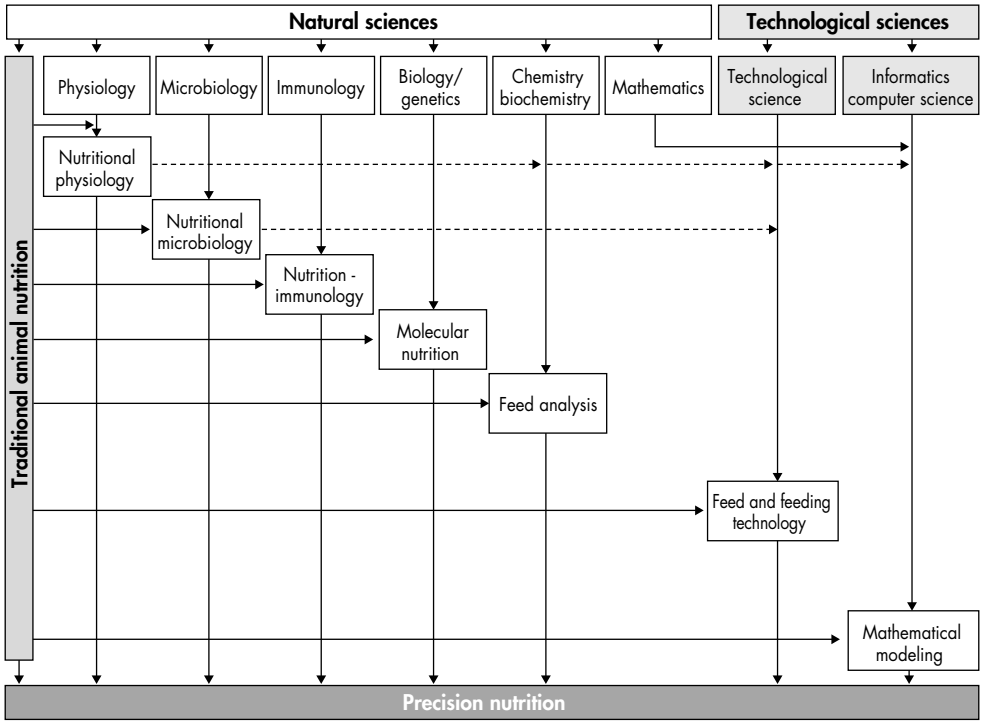

Figure 1.3. Relationship among traditional animal nutrition, natural and technological sciences (Babinszky and Halas, 2009). With permission of Taylor & Francis.

Precision animal nutrition applies the research findings of 'classical' nutrition as well as related areas to animal nutrition in order to meet the unique nutritional requirements of a specific group of animals kept under specific conditions with maximum accuracy. According to Nääs (2001), precision nutrition consists of meeting the nutrient requirements of animals as accurately as possible in the interest of safe, high-quality and efficient production, in addition to ensuring the lowest possible load on the environment. This concept is also in agreement with the principle of sustainability. Important areas of science which are currently being developed and are likely to make a direct impact on the nutrition of production animals in the near future are:

Molecular nutrition. This relatively new interdisciplinary research area has emerged from advances in molecular biology and requirements for explaining the organism's responses to nutrients at a molecular level including gene expression, signal transduction, and covalent modifications of proteins (Yan, 2015). The molecular biological methods developed in the last 20 years and new technologies made it possible to gain a better understanding of these biological processes. This knowledge will have great importance in the near future not only in the field of human nutrition,

but also in animal nutrition science. Among others, molecular nutrition examines how nutrients (glucose, fatty acids, amino-acids, vitamins, etc.) affect the signal transmission between cells and gene expression. Due to biochemical processes, micronutrients affect the information flow in cells and, thereby, they influence gene activity or suppression.

Nutritional immunology. Since the early ninety eighties, this has been a very intensively studied area of nutrition. Nutritional immunology investigates the role (mostly to improve) of dietary components (e.g. amino-acids, fatty acids, macro-, micro- and trace minerals, vitamins, etc.) and their interactions with other environmental factors and genes in the cellular and humoral immune responses of farm animals. The development of the so-called 'new type' growth promoters also belongs to this research/development field, as the use of these products results in enhanced performance through the improvement of the immune status of animals.

Nutritional microbiology (e.g. microbiological processes in the intestinal tract and the effect of these processes on animal production). This research area deals not only with the microbiological status of the feed ingredients or compound feeds but also with the impact of the diet on composition of the microbiome and moreover, with the relationships between various physiological processes and diseases in the animal body and the changing of the composition of microbiome. Based on the recent scientific findings it is evident that an optimally functioning gastrointestinal tract is of import to the overall metabolism, physiology, disease status and performance of pigs of all stages of growth and development (Pluske *et al.*, 2018). With the banning of the in-feed use of antibiotics in more and more countries, new methods for maintaining intestinal health and a commensal bacterial community is of the utmost importance. The recent finding that the microbiota affect the immune system of the gut as well as the systemic immune responses including lungs (Keely *et al.,* 2012; Molley *et al.,* 2012) opens new possibilities to improve the immune response to respiratory diseases.

Mathematical modelling of growth and production. One of the most important preconditions for economical production of high quality animal products (meat) is the prediction of the growth of animals, the determination of the nutrient requirements of the expected growth and – based on these – the provision of the necessary amount and quality of feed/nutrients. Mathematical modelling of the growth and nutrient requirements of animals has long been studied since the 1970s. In recent years, however, this area of science has develop in a spectacular way only as a result of the rapid development of information technology, the extended knowledge of physiology and the improvement of the accuracy of various animal testing methods. In a model, the biological processes of animals are described by mathematical equation systems that are built on the knowledge of genetics, biochemistry, physiological processes and environmental effects. The majority of the models used today are mechanistic, able to

predict the nutrient requirements of animals and estimate the production level which can be reached under given husbandry, feeding and management conditions.

Development of new in vitro *techniques* (e.g. in order to determine the digestibility of proteins, carbohydrates and other nutrients). Generally, *in vitro* methods are not a complete substitute for *in vivo* examinations. However, they can be of great help for feed analysis laboratories, where it is not possible to perform animal experiments, or for crop breeding institutions in scientific research projects if the large number of samples makes it necessary to perform a so-called preliminary selection before *in vivo* examinations. The significance of *in vitro* techniques will greatly increase in the future, because of new animal ethics laws that will provide less opportunity for *in vivo* examinations. It is usually referred to as the advantage of *in vitro* examinations that they are relatively cheap and quick, they do not call for the infrastructure needed for animal tests, the necessary number of replications can usually be increased more easily than in the case of *in vivo* experiments and it is possible to gain relatively large amounts of data even from a small quantity of investigated sample. However, the main disadvantage is that they are not always as accurate as animal tests and merely provide a ranking rather than an absolute value.

Development of environment preserving feeding technologies (e.g. feeding technologies to reduce N, P, Cu, Zn and methane emissions). Nutrients and other components in feeds not retained by the animal will be lost to the environment. In terms of environmental burden, two of the most critical polluting elements are nitrogen and phosphorus. Also copper and zinc as well as methane emissions are causing concerns. As such, in developing environment-friendly breeding and feeding technologies, the emission of primarily these elements needs to be reduced. In addition, more attention has to be paid to the reduction of unnecessarily high microelement emissions. Among our industrial farm animals, the highest nitrogen and phosphorus pollution is generated by the pig and poultry sectors. This can be traced back to the digestive characteristics of these two species, an inappropriate crude protein and amino acid supply, and the housing (keeping) technology as well as deficiencies in manure management. For copper and zinc, dietary oversupply and low bioavailability are important reasons for high concentrations in manure.

The large emission of P can be significantly reduced by means of expressing requirements in digestible P-content, further specifying the values of nutrient requirement, rational selection of feed ingredients and improving the digestibility of native P-content through phytase-enzyme addition. For example, in the case of pigs of different ages, the level of N emission can be significantly reduced by a more accurate specification of amino acid requirements, introduction and dissemination of modern protein evaluation systems (ileal digestible protein content of the diets) and the so-called ideal protein concept. There are further possibilities for reduction of N emission by improvement of amino acid digestibility of diet components, the further use of first

limiting, industrially produced amino acids and characterisation of bioavailability of dietary amino acids. These potentially available feeding tools can enable a 20-25% reduction in both N and P emission without decline in animal performance.

Precision animal (pig and poultry) nutrition, which is an integral part of precision livestock farming, contains several biological elements. Some of these are:

- Diet formulation should be based on available nutrients.
- Application of disposal cost for nutrients in diet formulation to find economical optimum for their inclusion, rather than nutritional optimum only (Van Kempen and Van Heugten, 2001). The consideration of nutrient disposal cost in diet formulation are especially actual nowadays, because large amounts of by-products are produced in the bio- fuels industry (Hadrich *et al.*, 2008). The term cost of disposal is used to describe the incremental expense that can be directly attributed to the disposal of an asset, contract, or cash-generating entity which can be regarded as a future liability.
- Application of phase feeding and split-sex feeding.
- Using mathematical modelling to predict animal performance.
- Reducing the harmful effect of heat-stress with different nutritional tools.
- Base nutrient and energy supply on genetic potential of livestock.
- Improve immune and health status of animals by macro- and micronutrient supply.
- Use industry and agriculture origin co-products based on actual nutrients and energy contents.
- Reduction of N, P, Cu, Zn and methane excretion by different nutritional tools.

Besides up-to-date nutritional knowledge, precision animal nutrition also requires the application of individual feeding based on computers and transponders. Observing the trends of today, it can be concluded that in future of animal feeding, the concept of sustainable precision livestock farming will become more and more important (Den Hartog and Sijtsma, 2011).

1.3 Future perspectives in animal nutrition

Many of the natural and technical sciences as well as interdisciplinary sciences such as animal nutrition, are developing rapidly. Nowadays it is not an easy task to predict the future of a given area of science. The demand by society on animal products and the way they are produced is likely to further increase in importance in the future, effectuated through increasing constraints on production systems by more stringent regulations. Public health issues will become increasingly important, such as concerns associated with the use of antibiotics, residues in food and diseases. New diseases have emerged, such as avian influenza H5N1, which have caused considerable global concern regarding the potential for a change in host species and emerging global pandemics (Thornton, 2010). Environmental issues related to animal production such

as methane mitigation, mineral pollution and ammonia emissions are also likely to increase. Stricter animal welfare concerns in many developed countries are likely to decrease production efficiency while the wide-scale use of human-edible ingredients in animal diets will re-ignite the food-feed-fuel competition debate in light of the future global protein shortage.

Animal nutrition science and its co-disciplines are based on biology, chemistry, biochemistry, microbiology, molecular biology, physiology, toxicology, immunology, molecular genetics, information technology, mathematics, physics, various areas of technical sciences, etc., as well as the development of the different examination methods (e.g. surgical techniques) applied during animal experiments. The past two decades has seen an unprecedented advancement in many of these areas of science. The advancement in genomics, transcriptomics, proteomics and metabolomics will continue and provide hitherto unknown opportunities to further fine tune the supply of nutrients through the diet and the demand of the animal for nutrients to grow or produce.

Today, it is self-evident that there is an increasing demand on behalf of societies for high quality, safe and transparently produced food products. However, in the case of foods of animal origin, this demand can only be fulfilled if modern animal nutrition knowledge is applied together with the latest findings of its co-disciplines. As a matter of course, this holistic approach to the problem requires strong teamwork, as even a highly skilled and constantly learning professional could face difficulties when exploring and expertly applying all new findings of a certain area of science.

Besides the demand for increasingly welfare friendly production systems, it is also an important and rightful expectation of society for animal experiments to fully comply with the actual ethical codes, legal stipulations and animal protection laws. Also these societal demands are expected to grow in the future and animal experiments and various examination methods can only be performed and applied in compliance with the increasingly strict prescriptions. The latter will have serious financial implications, which also need to be covered by society.

Based on the relevant literature data, information presented on various conferences and our own international observations, we present the main directions of changes using this non-exhaustive list of examples. We classified tendencies into two main groups: the next 5-10 years and subsequent decades.

1.3.1 Prognosis of the near future (the next 5-10 years)

This group includes the research areas and examination methods whose importance is most likely going to significantly increase in the near future, although they are important even today. Such research areas and examination methods include:

Research on alternative feed ingredients

Using new feed energy and protein sources is mainly aimed at the partial or total replacement of maize and soy. There can be several reasons for this shift, with the main goal being the fact that both feed ingredients are important in human nutrition or as food ingredients for humans. In many cases, feed ingredients are sold as a commodity to both the food and feed industry. The main priority is to supply the human population with high quality and safe foods. Several ingredients are not intended, less suitable or very unsuitable for human consumption. For example, genetically modified energy and protein sources of vegetable origin are banned in many countries. It could be a further argument for replacement that the actual stock exchange rate of these feed ingredients are not determined or not entirely determined solely by the demand in agriculture (livestock management), but also the industry (e.g. food industry, medicine industry, etc.) that generates more profit. On the contrary, this fact results in an increasing feed ingredient price (e.g. in the case of soy and maize), which may significantly increase the specific costs of producing food of animal origin, thereby deteriorating the market position of meat, egg and milk producers, among others.

In the case of protein sources of vegetable origin, further intensive research is expected in order to reduce or eliminate the harmful impact of anti-nutritive factors by means of crop breeding and/or various feed technology or management procedures, thereby improving the biological efficiency of animal nutrition and the production of high quality feed ingredients (Huisman and Tolman, 2010).

The novel protein sources (e.g. insects, algae, microalgae, seaweed, duckweed) are expected to enter the European feed and food market as partial replacement for conventional protein source or due to their potential beneficial effects above the nutrient content they contain (see Chapter 13, Van Krimpen and Hendriks, 2019). However, it should be emphasised that food safety aspects of these new protein sources are not well-known (Van der Spiegel *et al.*, 2013; Chapter 13, Van Krimpen and Hendriks, 2019). More systematic and thorough studies are needed to determine not only the digestible/available amino acid profile of these novel protein sources but also any adverse effects on animal and human health (e.g. possible viral infections), or any other detrimental effect on the consumer.

Determining nutrient requirement values more accurately

Meeting the nutrient requirements of production animals as accurately as possible based on examining the interactions between macro- and micronutrients remains a key issue for the coming years for the purpose of further improving efficiency of production. This topic also involves the effort to make the nutrient requirements of animals with high genetic capacity (e.g. so called 'improved pigs'), more accurate, i.e. to examine the correlations between genetics and animal nutrition (Close, 1994).

Currently the genetic potential of many pig breeds used in production systems are approximately 3-5 years ahead of the nutrient requirement estimates used for feed formulation as a result of continuous genetic improvement efforts and the difficult and time-consuming nature of determining nutrient requirements (Knap, personal communication).

Developing new alternatives for antibiotic growth promoters

The development of the alternatives for the effects commonly observed with the use of in-feed antibiotic growth promoters (Den Hartog *et al.*, 2015; Vallat *et al.*, 2005) is an ongoing effort. It is also a topical question whether antibiotics can be phased out totally (Leeson, 2012). According to some, it is possible to effectively use other alternative products instead of antibiotics in the production of pork and poultry meat (Den Hartog *et al.*, 2015). It is a just expectation on behalf of both the profession and society to obtain accurate knowledge of the exact mode(s) of action of such alternatives, as well as to reveal any possible risks. It is probable that further pro- and prebiotic-based products will be developed, but it is also possible that the key to the solution will be the use of the so-called combined or multifunctional feed additives (a mixture of symbiotic, antioxidants, immunomodulators, various vegetable extracts and new type toxin binders). However, it must be noted that one of the indispensable requirements of the safe use of supplement/additive is the accurate chemical identification of the active substance(s) (Christaki *et al.*, 2012), otherwise it is not possible to expertly use these additives or even to properly adjust the desired concentration of the active substance.

Reduction or total elimination of mycotoxins

Although mycotoxins and the protection against them is already an intensively researched area, it can be stated that it is not going to change in the coming years and decades. New findings in this important field will be greatly dependent on the development of new analytical methods, as well as their implementation in practical analytics and on how soon the obtained biological, biochemical, physiological and immunology research findings will be applied in animal nutrition. Technological advances and breeding for resistant varieties (Lehoczki-Krsjak *et al.*, 2010) are likely to contribute to reduced mycotoxin content of future feeds.

Increased use of co-products in animal nutrition

According to the relevant prognoses, the amount of agricultural, industrial and other co-products used in animal nutrition is going to increase in the coming years, mostly for economic and environmental reasons as well as the food-feed-fuel discussion. The proper and safe use of co-products is possible only if their chemical composition, digestible and/or usable nutrient content and energy content are known and if the

co-products used in animal nutrition are free from materials that are harmful to animal health. From an economic perspective, it is an important requirement that these products have a consistent quality and are cost-effective for inclusion in feeds. For this reason, the so-called shadow-price should also be known. In addition to co-products, food waste also has the potential to be used as an animal feed ingredient as is already actively promoted in Japan, South Korea, Taiwan, and Thailand (Chapter 13, Van Krimpen and Hendriks, 2019).

Examining the relationship among climate change, fodder crop production and animal nutrition

Climate change and the actions taken to mitigate its impacts are becoming increasingly topical in science. The findings of international research indicate that the impact of climate change will be more powerful worldwide in the future. In addition to comprehensive research, there is an increasing ##demanded for climate change and its impacts be included in education and in extension service (Babinszky *et al.*, 2011a). Since the climate continues to change and its future course is unknown, it is necessary both for meteorologists and users to constantly keep track of the process, as well as to monitor changes and their impacts.

The most frequently asked question regarding climate change is what impact it will have on agriculture (crop production, livestock management, production of food ingredients of vegetable and animal origin) and, in a broader sense, on food supply. This question has to be formulated at a regional, continental and global level and for this reason, it is more appropriate to provide the answers also at these levels. This means that the climate scenarios prepared by climatologists are necessary to be adapted to local circumstances and evaluated, as well as to connect them to regional production. Action programmes and the elements of the responses, prevention, adaptation, compensation and restoration can be built up mainly on the climatic change prognoses for a given region (Babinszky *et al.*, 2011a). However, it must also be noted that the food supply prognoses based on climate scenarios have certain margins of error, as agricultural production is significantly affected by not only climate, but also other factors as well (genetics, agrotechnics, adaptation ability, etc.). However, it needs to be emphasised that these research areas are still in their infancy. For this reason, it is highly probable that the analysis of climatic effects and, more specifically, the harmful effect of heat stress, its reduction and/or elimination will become a core research topic in the next decade (Babinszky and Halas, 2009; Babinszky *et al.*, 2011b). Currently it is one of the main topics of research to examine the harmful effect of heat stress on the anti- and pro-oxidant balance of animals and the possibility of mitigating this negative impact with various animal nutrition tools. In the near future, this topic is likely to be even more important (Chapter 8, Babinszky *et al.*, 2019).

Animal nutrition and immunology

Starting from the early 1980s, how nutrients (e.g. amino-acids, fatty acids, minerals, vitamins, etc.) and additives mixed into animal feed are capable of affecting the resistance, as well as cellular and humoral immune response of farm animals, has been a rather intensively researched area of animal nutrition. The findings of related research show that a slight decrease in protein supply compared to the recommended value does not compromise the immune system, but the partial shortage of certain amino acids results in a significant reduction of the defensive ability of the organism in case of an enhanced immunity. Knowing the role of nutrients in the immunity of the organism can contribute to maintaining health and reduce the amount of medication used in the course of producing foods of animal origin. Providing certain amino acids (e.g. methionine, threonine, arginine, glutamine or glutamic acid) above the amount necessary for maintenance and growth can potentially result in enhanced immunity of pigs and poultry. However, it must be noted that giving supraphysiological levels of certain nutrients (e.g. fatty acids) may result in immune suppression even before there is a deterioration in performance (Calder, 1998). Even though animal nutrition immunology is an intensively researched field, there are still many gaps in our knowledge on how to determine the amount of nutrients needed for an effective immunity of the organism when working out animal feed recipes (NRC, 2012). In addition to gaining knowledge of the role of nutrients, the development of so-called 'new type' growth promoters also belongs to this research/development field, as these products result in improved performance primarily through enhancing the immunity of the organism. Again, it is expected that the precise mode(s) of action of these products are provided, since this knowledge can provide explanations to the cause(s) of each potential interaction when using a given product.

Animal nutrition immunology will have a key role to play in practical animal nutrition in the near future and nutrient guidelines will need to be based in many cases on the results of animal nutrition immunology tests.

Animal nutrition and microbiology

This area involves the microbiological processes in the intestinal tract and the impact of nutrition on these processes as well as on the productivity of animals. Today, gut health is a well circumscribed field of animal nutrition research. This area is becoming more and more important in the nutrition of especially monogastric animals. With the ban in many countries of the use of in-feed antibiotics, maintaining intestinal health through the use of various additives has already received (Den Hartog *et al.,* 2015) and will receive more attention in the future. Also in ruminant nutrition, the modulation of rumen fermentation and digestion in order to reduce methane production is of paramount importance to develop more sustainable production systems. The latter is important for the drive of society to reduce green-house gas emissions from ruminant

production systems and can be to a large extent be solved by animal nutrition research (Cole, 2005; Forano and Flint, 2000; Verstegen and Tamminga, 2005).

Nutrigenomics

Nutrigenomics is a field of science focusing on the interaction between nutrition and genomics, combining the methods of nutrition science (animal nutrition science) and the so-called functional genomics. The aim of this area of science is to examine how bioactive ingredients or regular nutrients in foods or feeds affect gene expression and functioning. In essence, it encompasses the application of gene technologies in the field of nutrition and animal nutrition science. The investigation into the interaction between genes, nutrition (animal nutrition) and health is rapidly developing although it is complicated due to the fact often more than one gene is involved in the regulation of traits. Findings in this field are being used in practice at an increasing rate (Chapter 7, Vailati-Riboni *et al.*, 2019). In our opinions, nutrient supply will soon be based on genetic profiles (e.g. in pig nutrition).

Further research directions influencing the near future of animal nutrition

In addition to the above mentioned fields, there are currently many existing areas which are going to determine the short- and medium-term development of animal nutrition. However, due to the lack of space, these areas cannot be elaborated here. Examples are:

- *Mathematical modelling* of animal production, which is partially connected to bioinformatics (see below in this section),
- *Elaboration of new and more accurate* in vitro *and quick analysis methods*. The urgency for the development of *in vitro* protein, amino acid carbohydrate etc. digestion analyses methods is increasing, since the conduct of *in vivo* analyses is under increasing scrutiny. However, there are also other arguments for *in vitro* analyses to be developed, such as the relatively short duration of analysis and the lower costs than *in vivo* testing. Developing rapid analyses to estimate the chemical composition of animal feed and feed ingredients is also a key issue in the practice of precision animal nutrition.
- Even today, *nanotechnology* plays an important role in producing animal feed additives. An increasing number of micronutrients (e.g. vitamins, minerals, such as Se, etc.) is mixed into the animal feed in the form of nano-sized particles in order to improve absorption and, as a result, to increase nutrient dynamics, i.e. the efficiency of absorption. Based on the analysis of current trends, it can be concluded that nanotechnology will become more important in animal nutrition science.
- *Biotechnology*. The significance of this area of the green (agricultural) biotechnological industry in animal nutrition and the feed industry is already

apparent. Developments in the red (medical science and health industry), yellow (food industry and nutrition science), grey (classic fermentation industry) and white (industry and environmental protection) biotechnological industries contribute to various extents but are likely increase their contribution to the development of animal nutrition science and a more effective practical animal nutrition in the future.

- *Using the findings of the above described research directions and those of classic animal nutrition*, we put together the so-called *precision food traceability chain* ('from farm to fork animal origin food production traceability chain'), which can be an important guarantee of the transparent and safe food production product path (Figure 1.4).
- As it can be seen in Figure 1.4, 'From farm to fork traceability food production chain' includes precision plant (crop) production, precision livestock farming and precision meat and/or milk industry. It also can be seen that the precision animal nutrition is an integrate part of precision livestock farming.
- The significance of these product paths will further increase in the future, mainly because of society's demand for healthy, high quality, safe and traceable foods. For this reason it can be safely stated that launching and implementing integrated research and innovation programs involving the whole product path will have much greater significance in the future.
- In connection with the previous point, it is highly likely that the legal and ethical issues associated with animal nutrition will be of greater importance to producers.

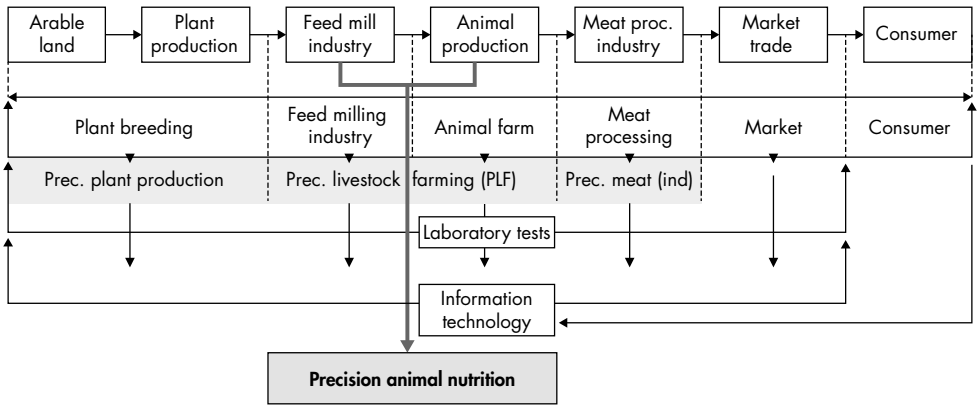

Figure 1.4. 'From farm to fork' precision food production and traceability chain.

1.3.2. Prognosis for the more distant future (the next 10-20 years)

Bioinformatics

Bioinformatics is a branch of science which uses information technology tools and methods in order to explore, model and affect biological processes. According to an early description, bioinformatics is interdisciplinary science which uses computer science in molecular biology (Luscombe *et al.*, 2001). Based on the definition used today, bioinformatics is the *in silico*, i.e. computerised application of all mathematical algorithms and methods which assist in providing solutions to biological problems based on experimental data. For example, from the aspect of animal nutrition, the mathematical modelling of various biological processes (e.g. animal growth, protein and fat incorporation or rumen fermentation). However, within bioinformatics there are entirely specialised areas. For example, structural bioinformatics focuses on the spatial structure of macromolecules. In addition to sequencing, there are several other data which are produced with the so-called 'high-throughput' method and can only be managed using bioinformatics. For example, gene expression, electrophoretic and mass spectrometry data and the genetic, metabolic, signal transmission and protein-protein interaction pathways and networks. Based on these data and the use of bioinformatics models, we will be able to explore the cause of several animal nutrition problems which are still unclear today (e.g. nutrient interactions and their targeted utilisation).

The relevant technical literature data so far led us to conclude that the findings of bioinformatics will not only be used in mathematical modelling, but also in the interpretation of processes connected to digestion physiology at an increasing frequency.

Molecular nutrition

Despite the fact that this area of science is already two decades old, it is obvious that this is only the beginning of the journey. In the coming decades, this field is going to develop, hitherto, unknown opportunities in both animal and human nutrition science. Among others, molecular nutrition focuses on how nutrients (glucose, fatty acids, amino acids, vitamins) affect signal transmission between cells and gene expression. Micronutrients affect the information flow within cells by means of biochemical processes, thereby, eliciting gene activation or suppression. The knowledge of all these processes is the basis for examining the transport mechanism of nutrients, as well as the correlation between micronutrients, cellular homeostasis, cell proliferation and apoptosis (Zhang, 2003). Since the functioning of all cells of the organism is basically under the control of genes, revealing these regulatory mechanisms greatly contribute to understanding vital processes (Babinszky and Halas, 2009).

Quantum biology and nutrition (animal nutrition) science

Using quantum biology, physicists and biologists attempt to interpret and explain complex physiological and biochemical processes at the subatomic level together. The question whether quantum mechanics can play a role in the interpretation of biological processes was raised only a few decades ago. According to numerous research findings, the answer is yes (Arndt *et al.*, 2009; Lambert *et al.*, 2013), as it seems that nearly all chemical processes are based on quantum mechanics. In their outstanding technical literature review, Arndt *et al.* (2009) came to the conclusion that the studying of quantum physics and biology in a coherent system (in quantum biology) in order to understand many biological processes is a very timely and important task. Scientific visions, theories and interdisciplinary research with a wide scientific background will be needed to develop in this rather new area of science. The question arises whether quantum biology can be used in the future in nutrition and animal nutrition science to provide subatomic interpretation of intermediary metabolism and the physiological processes of digestion, as well as the processes which are still unknown or only partially known today. The answer is rather hopeful than firm, since this scientific field is still in its infancy. However, as various physiological processes can be interpreted and understood at the level of electrons, protons and neutrons in 15-20 years, let us just consider molecular nutrition as similarly, 25 years ago, not many would have thought that biochemical processes could be examined and interpreted at the intracellular and molecular level (Sanders and Emery, 2003).

1.4 Conclusions

For the 21st century challenges in pig and poultry nutrition, it is necessary to involve into the innovation activity besides classical animal nutrition knowledge, newer areas of natural and technical sciences (e.g. nutrition physiology, nutrition immunology. molecular biology, molecular nutrition, molecular genetics, nutrigenomics, information technology, etc.). The importance of these disciplines will continue to grow in the near future, in the following 5-10 years. The use of precision animal nutrition in practice will greatly contribute to the implementation of the above written aims, as well as the improvement of the successfulness of innovation activity. It is highly probable that new areas of science will revolutionise animal nutrition sciences such as bioinformatics, molecular biology, molecular nutrition and nutrition immunology, as well as quantum biology. These areas of science will greatly contribute to the development of animal nutrition science and, as a result, to a more efficient animal nutrition, as well as to a better quality and safe foods based on products derived from animals.

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2

New facets to an understanding of dietary nutrient utilisation

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Summary points

- Dietary amino acids are used with a relatively low efficiency and there is considerable scope for improving the efficiency of utilisation of amino acids for protein synthesis.
- Several physiological processes (amino acid absorption, gut endogenous amino acid losses, inevitable catabolism of amino acids, protein/energy interaction and preferential amino acid catabolism) are central to dietary amino acid utilisation.
- Most progress in increasing the efficiency of future meat animal production will be based on a better, more mechanistic, understanding of these processes.
- New insights will be provided by a better understanding of the role of the microbiome and nutrient uptake kinetics and synchrony.
- A more mechanistic appreciation of the key physiological processes will be afforded by the tactical application of new methodologies from genomics, proteomics and metabolomics.
- The 'omics' techniques should not be applied haphazardly, but rather in a planned manner within an interpretive holistic framework of growth and development.

Keywords: amino acids, efficiency, metabolism, microbiome, omics

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2.1 Introduction

The world currently faces one of its greatest challenges: that of feeding, in a sustainable manner, nine billion people on our planet by the year 2050. It is estimated by the FAO[1] that meat consumption alone will double by 2050. Clearly, the pig and poultry industries, the most significant sources of global meat supply, will be crucial for meeting this challenge.

Considerable progress has been made over the last several decades in the improvement of the average daily gain and feed conversion efficiency of pigs and poultry and much of this improvement can be ascribed to gains from genetic selection and to advances in dietary formulation practice. Given this, it is perhaps surprising, that although genetic selection has led to animals with higher potential rates of whole-body protein deposition, lower body lipid to protein ratios and altered rates of food intake, the underlying efficiency of dietary protein utilisation has remained low. There is thus considerable scope for the further improvement of productive efficiency. This contribution will look at the underlying causes of inefficiency in the utilisation of dietary protein for body protein retention in pigs and poultry, and will discuss new frontiers in science that allow fresh insights into mechanism.

These new approaches will be briefly reviewed and their application couched in the context of the observed relatively low rates of efficiency of utilisation of dietary protein in animals, the improvement of which has proven to be quite intractable using conventional approaches.

2.2 The efficiency of utilisation of dietary protein

Dietary amino acids are not used particularly efficiently by the growing animal and this has implications not only for growth, but also for the environment. Simulated values (Moughan, 1989, 2008; Moughan and Verstegen, 1988) for the efficiency of utilisation of dietary crude protein for six commercial pig grower diets fed at two levels of intake to a 50 kg female pig are shown in Table 2.1.

The utilisation of dietary protein was predicted to be less than 50%, and on average around 70% of ingested nitrogen was excreted. Part of this inefficiency is due to dietary amino acid imbalance which may be purposeful and economically justified. It is thus informative to look at the inefficiency of utilisation of the first-limiting amino acid (in this case lysine), where the effect of imbalance on efficiency of utilisation is avoided (Table 2.2). Predicted lysine utilisation efficiency was higher than the comparable values for crude protein, but generally still over 50% of dietary lysine was not used for

[1] Available at: http://www.fao.org/ag/againfo/themes/en/meat/home.html.

Table 2.1. Efficiency of utilisation[1] of dietary crude protein (PE)[2] for six commercial pig grower diets given at two feeding levels to the 50 kg liveweight gilt.

Feeding level	Diet					
	1	2	3	4	5	6
1,710 g meal/day	20.4	30.0	23.1	33.8	32.3	42.1
2,270 g meal/day	22.4	23.7	24.6	27.4	34.4	31.7

[1] Predicted values from a pig growth simulation model.
[2] PE (%) = Body protein deposited / Diet protein intake × 100 / 1.

Table 2.2. Efficiency of utilisation[1] of lysine (LE)[2] (effect of amino acid imbalance removed) for six commercial pig grower diets given at two feeding levels to the 50 kg liveweight gilt.

Feeding level	Diet					
	1	2	3	4	5	6
1,710 g meal/day	37.2	38.3	38.5	43.5	54.0	59.0
2,270 g meal/day	40.9	30.2	41.1	35.3	59.0	45.0

[1] Predicted values from a pig growth simulation model.
[2] LE (%) = Body lysine deposited / Diet lysine intake × 100 / 1.

body protein deposition. A high range in values was observed (30-59%), indicating a considerable scope for improvement.

Analyses such as these, which are mirrored by *in vivo* data from pigs and poultry, highlight the importance of understanding the physiological processes leading to losses of amino acids from the body. The absorption and metabolism of amino acids is complex and highly integrated, with continuous flux within and between cells. It is useful, however, to visualise amino acid metabolism as discrete physiological processes (Moughan, 2003a). Such a framework for the processes underlying amino acid utilisation is shown in Table 2.3.

The integumental amino acid losses (though higher in birds than pigs and humans) are quantitatively minor, as are losses from the use of amino acids to synthesise 'other' compounds, irreversible amino acid modifications and urinary amino acid losses. But what is the relative quantitative importance of the remaining processes, and what is

Table 2.3. Framework for the processes underlying the utilisation[1] of ingested amino acids (AAs) by the growing animal.

- Faecal excretion of unabsorbed AAs
- Integumental AA loss
- Gut endogenous AA loss
- Body protein turnover
- Synthesis of non-protein nitrogen-containing compounds (e.g. tryptophan as precursor for serotonin)
- Irreversible changes to AAs (e.g. methylation of histidine, methylation, hydroxylation of lysine)
- Urinary AA loss
- Inevitable catabolism of absorbed AAs
- Catabolism of AAs supplied in excess of amount required for maximum rate of body protein synthesis.
- Preferential AA catabolism

[1] Efficiency of utilisation for whole-body protein deposition.

known about them? The simulated utilisation of dietary lysine (losses of lysine from the body) apportioned to different biological processes is shown in Table 2.4 and 2.5.

Losses of amino acids from the body due to faecal excretion or the uptake and urinary excretion of structurally altered (damaged) amino acids or losses of amino acids via

Table 2.4. Losses[1] of dietary lysine (g/d) by the 50 kg liveweight growing pig at two feeding levels of a commercial diet and two maximal rates of protein deposition (Pdmax, genotype).

Feeding level (g/day)[2]		1,505		2,633	
Pdmax (g/day)		100	160	100	160
Total lysine intake (g/day)		13.8	13.8	24.1	24.1
Losses (g/day)	Damaged lysine	0.7	0.7	1.2	1.2
	Unabsorbed available lysine	1.8	1.8	3.1	3.1
	Protein turnover	0.7	0.8	0.7	0.8
	Gut endogenous	1.6	1.6	2.1	2.1
	Inevitable catabolism	3.2	2.1	6	6
	Excess supply	0	0	4.4	0.3
	Preferential catabolism	1	1.8	0	0
	Total losses	9	8.8	17.5	13.5
Lysine deposited (g/day)		4.78	4.91	6.63	10.61

[1] Predicted values from a pig grown simulation model.
[2] Correspond to 8 and 14% of metabolic liveweight ($kg^{0.75}$).

Table 2.5. Utilisation[1] of dietary lysine (%) by the 50 kg liveweight growing pig at two feeding levels of a commercial diet and two maximal rates of protein deposition (Pdmax, genotype).

Feeding level (g/day)[2]		15,05[2]		26,33[2]	
Pdmax (g/day)		100	160	100	160
Losses (% of total loss)	Unabsorbed and damaged	28	28	25	32
	Protein turnover	8	9	4	6
	Gut endogenous	18	18	12	16
	Inevitable catabolism	36	24	34	44
	Excess supply	0	0	25	2
	Preferential catabolism	11	20	0	0

[1] Predicted values from a pig grown simulation model.
[2] Correspond to 8 and 14% of metabolic liveweight ($kg^{0.75}$).

inevitable catabolism are quantitatively significant losses occurring at all levels of feed intake. A loss of amino acid nitrogen in the urine arising from preferential catabolism, however, occurs only at lower levels of feed intake. Supplying balanced dietary protein in excess of the requirement for maximal rates of body protein deposition (set by the genotype, breed and strain) can under some dietary conditions lead to considerable inefficiency of utilisation, but this can be readily avoided by knowledge of the genotype of the animal and careful dietary formulation. Losses due to preferential amino acid catabolism, inevitable amino acid catabolism, gut endogenous amino acid excretion, and structurally altered (damaged) amino acids and amino acid excretion (the latter two losses being highly interdependent) are less easily avoided and are critical causes of inefficiency in the utilisation of the first-limiting amino acid. These losses are discussed in the following sections.

2.3 Unabsorbed and damaged lysine

Although this is a very significant source of inefficiency of utilisation of lysine (and other amino acids), considerable advances in understanding have been made in this area in recent years. It is now clear that ileal amino acid digestibility (rather than that based on faeces or total excreta) should be used in both pigs and poultry (Moughan and Smith, 1985; Moughan, 2003b; Moughan *et al.,* 2014). It is also well established that apparent digestibility coefficients can be inaccurate, as they are affected by the dietary amino acid concentration used in their estimation, and correction for ileal endogenous amino acids should be made to give 'true' (or standardised) digestibility coefficients (Boisen and Moughan, 1996; Stein *et al.,* 2007). These principles are well understood, but perhaps what is not so widely appreciated is that just because an

amino acid is absorbed it may not necessarily be able to be used for protein synthesis. Several amino acids (lysine being the most studied) can undergo structural changes during feed processing or storage, which although they may be absorbed renders them unavailable for body protein synthesis. The presence of these amino acids in the diet is not detected using conventional amino acid analysis. For material that has been heat processed or stored at ambient temperature, lysine, and other amino acids (e.g. methionine, arginine), will have undoubtedly undergone chemical reactions with various compounds (e.g. Maillard reaction), eliciting new chemical structures. During the strong acid hydrolysis step used in conventional amino acid analysis, an unpredictable proportion of this reacted (structurally altered) material reverts to lysine (or the corresponding parent amino acid). Thus total lysine overestimates available lysine (Fontaine *et al.,* 2007). Because of this, the 'actual' or 'reactive' lysine (e.g. fluorodinitrobenzene, O-methylisourea), may be determined, but it has been well established that not all of the 'actual' or 'reactive' lysine or amino acid is absorbed by the end of the ileum (Moughan, *et al.,* 1996). As amino acid derivatives revert in an unpredictable way to the parent amino acid in both ileal digesta and the feedstuff or diet, conventional digestibility values are also inaccurate. A bio-assay for lysine availability has been developed (Moughan and Rutherfurd, 1996) which circumvents the problems inherent in the conventional assay. Application of the assay highlights meaningful differences in the amount of dietary lysine deemed to be available for some feedstuffs (Rutherfurd and Moughan, 1997; Rutherfurd, *et al.,* 1997). The new technology allows significant improvements in dietary formulation and the efficiency of utilisation of dietary lysine. It has been applied widely in pig and human nutrition, but has not been so extensively exploited in poultry nutrition.

A number of important questions with regard to amino acid digestibility remain unanswered. Do methionine and cysteine behave like lysine with respect to availability? Considerable amounts of these amino acids are oxidised in some foods. Why is cystine in proteins so poorly digested? To what extent does gut bacterial amino acid catabolism and synthesis affect ileal amino acid digestibility coefficients?

2.4 Gut endogenous amino acid losses

The gut endogenous amino acid losses (Moughan *et al.,* 1998) are substantial (Moughan and Rutherfurd, 2012) and reflect a high metabolic cost (both in terms of protein and energy metabolism). They comprise losses due to digestive secretions, mucus, sloughed intestinal cells, proteins such as albumin and immunoglobulins, and bacteria (not strictly endogenous, but non-dietary). The endogenous loss of amino acids from the gut is influenced by the dietary dry matter intake, dietary protein concentration, to some extent the primary structure of the protein, amount and type of dietary fibre and the amounts and types of antinutritional factors (e.g. trypsin inhibitor, lectins, polyphenols). The need to replace the lost endogenous amino acids

by absorbed dietary amino acids, contributes to inefficiency of amino acid utilisation and should be a target for future research. Certain dietary treatments (e.g. enzymes, especially mucinases) may increase the recycling of endogenous amino acids, and more needs to be known concerning the effects of different types and structures of non-starch polysaccharides and plant secondary compounds. Seen through a different lens, the endogenous secretions may play a hitherto unappreciated metabolic role in the animal, as a consistent source of regulatory bioactive peptides (Moughan *et al.,* 2013), and this is a burgeoning and promising area of research.

2.5 Inevitable amino acid catabolism

Inevitable amino acid catabolism is an important source of inefficiency of amino acid utilisation. Not all of the absorbed amount of the first-limiting amino acid is used for body protein retention (above maintenance costs) even at relatively low amino acid and high energy (carbohydrate, fat) intakes. This is because an amount of the absorbed first-limiting amino acid is always catabolised (irrespective of ATP need) presumably because of the mere existence of active catabolic pathways. Such amino acid oxidation has been referred to as 'inevitable' catabolism. Part of the inevitable catabolism, may be related to 'first pass' gut metabolism which may be considerable (Stoll *et al.,* 1999). Published estimates of the inevitable catabolism of lysine in the growing pig range from 15 to 30% of absorbed lysine while values in growing broilers of 20 to 24% have been reported. The shape of the response of inevitable amino acid catabolism to amino acid intake in the individual animal (linear versus curvilinear) is contentious, but the bulk of evidence indicates that the percentage catabolism is relatively constant at lower levels of amino acid intake, but may increase at amino acid intakes approaching maximal protein deposition, and may vary among amino acids. The efficiency of utilisation of tryptophan appears to be particularly low. The underlying cause for, and mechanism of inevitable catabolism, remains largely unknown and must be another key target for future research.

2.6 Preferential catabolism of amino acids

The interdependency between amino acid and energy (non-protein) metabolism has long been recognised. This is because protein synthesis requires energy, and amino acids can either be deposited in protein or be used as a source of energy.

Preferential amino acid oxidation occurs whenever energy intake is liming for total tissue deposition (protein deposition becomes dependent upon metabolisable energy intake). It is the catabolism of amino acids above maintenance energy intakes for the express purpose of energy (ATP) supply. Preferential amino acid catabolism reflects the need for some level of lipid deposition to accompany protein deposition and

is affected by factors such as species, age, liveweight, gender, breed and strain. It is likely open to genetic manipulation but remains relatively unresearched and not well understood from a causal perspective. The rate of preferential catabolism of amino acids may also be related to some extent to the synchrony of supply of amino acids and non-protein energy (ATP generation) at the site of protein synthesis (see section below). If ATP generation is momentarily limiting for protein synthesis then amino acids will be catabolised.

2.7 New insights

The causative processes of gut endogenous protein metabolism, amino acid absorption, inevitable amino acid catabolism and preferential amino acid catabolism are well recognised as significant explanatory variables for the observed low rate of efficiency of utilisation of dietary protein. However, experimental approaches to date have not always cast sufficient light on the mechanistic basis of these processes and how they can be manipulated. How are we going to achieve new insights and fresh perspectives on these biological phenomena? Recently an appreciation of the role of the gut microbiome to gut health and body functions in general has developed, as too has an appreciation of the important role of the dynamics of nutrient uptake and the time-course of metabolic events. Fortunately a greatly expanded repertoire of tools from the field of molecular biology is also now at our disposal. Such approaches and techniques are being applied in animal science research and have the potential to unravel the mechanistic basis of key physiological processes. In the future, more research effort should be expended in developing an understanding of causal processes, rather than conducting yet more studies on the effects of specific diets and feedstuffs, where outcomes are already largely able to be predicted from the current knowledge base. If such investment is made and the new approaches and understandings are brought to bear, considerable progress can be made.

2.7.1 The microbiome

The mammalian intestinal tract is inhabited by 10^{14} microbes, over tenfold the number of cells comprising the body. The intestinal microbiota is dominated by bacteria and comprises from 400 to 500 different species, though up to 1000 species have been reported in some studies. Many of the bacterial species, particularly the anaerobes that have been difficult to culture, are yet to be described. The avian digestive tract (especially the crop and ceca) also contains a diverse microbial community. It can be estimated that the genomes of these bacterial species (collectively known as the microbiome), contain over 100 times the number of genes found in the animal genome. Clearly there is huge scope for bacterial gene expression to influence function (both positively and negatively) in the mammalian or avian host and it is becoming increasingly apparent that the microbiota play an important role in the

development of intestinal morphology and digestive function, as well as immune function and the processing of food materials and especially toxins. Indeed, the microbiota is often described as 'the newly discovered organ' or 'second genome', as it becomes increasingly clear that the complex microbial community may affect multiple body functions. This is enormously exciting and will open up completely new frontiers in animal and medical science. Activities of the microbiota, for instance, have been implicated in the aetiology of obesity and auto-immune diseases in humans such as diabetes, rheumatoid arthritis and multiple sclerosis and may be associated with some cancers and neuro-chemical imbalances associated with psychiatric and psychological disorders such as schizophrenia, depression, anxiety and bipolar disorder. Our understanding of the gut microbiota, and the complex interactions among its numerous members and with the enterocytes of the gut and the somatic cells, is in its infancy. One can predict an explosion of knowledge in this area over the next two decades. Of great interest to nutritionists, is that the composition of the microbiota is amenable to change by dietary intervention.

Bacteria in the intestine can adhere to the mucosal surface or remain unattached in the lumen. In either case, recent evidence makes it very clear that the intestinal microbiota is involved in molecular 'crosstalk' with the intestinal cells (Ulluwishewa, *et al*., 2011; Wells *et al.,* 2011). Either molecules secreted by the bacteria or present in the bacterial cell wall component mediate such crosstalk. This is likely the primary means by which the microbes affect the production of hormones and other compounds within the body, thus influencing regulatory and immune functions.

2.7.2 Dynamics of nutrient uptake and metabolism

Over the last decade, greater attention has been paid to the macro- and micro-structures of foods and feedstuffs and indeed the structures of the food molecules themselves, and how these influence the dynamics of stomach emptying, particle degradation and food polymer hydrolysis (Ferrua *et al.,* 2011). Collectively, differences in the time course of digestion can lead to differences in the dynamics of nutrient uptake and thus influence nutrient metabolism.

Advances in analytical techniques (especially mass spectrometry and nuclear magnetic resonance) are opening up new possibilities to allow the identification and quantitation of specific compounds, often present at low concentrations in complex mixtures. These have considerable application to studies of the dynamics of digestion at a molecular level. Mass spectrometry based analytical methods (Dyer and Grosvenor, 2014), for example, can be used to sensitively quantitate specific proteins and their fragments within complex mixtures such as digesta. Redox proteomics and quantitative peptide tracking offer powerful new tools for the qualitative and quantitative monitoring of protein digestion at a molecular level.

Considerable progress has been made in determining the digestibility of different dietary nutrients in different parts of the digestive tract (Coles *et al.,* 2005, 2010), and it is now possible to predict the uptake from the gut of a nutrient in a mixed diet with a reasonable degree of accuracy. It is also possible based on an understanding of biochemistry and metabolic pathways, to model the metabolism of nutrients post-absorption (Birkett and de Lange, 2001; Van Milgen, 2002; Coles *et al.,* 2013) and thus predict changes in body protein and lipid deposition, and bodyweight change. However, these conventional approaches to determining nutrient digestibility provide a sole digestibility value which describes the average digestibility of the nutrient over the time course of digestion. They provide no information as to the variation in the extent of digestion over time, and thus to the dynamics of nutrient uptake. Clearly, various nutrients from different foods are released and absorbed at different rates across the time-course of digestion. Further, body protein synthesis is an 'all or nothing' phenomenon, and an asynchrony of nutrient supply can lead to inefficiency in amino acid utilisation. Much more remains to be learned about the dynamics of the uptake from foods of amino acids, fatty-acids, glucose/sugars and volatile fatty acids, and how the pattern of uptake over time-of-digestion is affected by attributes of food and molecular structure (Donato-Capel *et al.,* 2014). The potential importance of the dynamics of nutrient uptake to metabolism is well illustrated by metabolic studies of the so-called slow and fast proteins (Boirie *et al.,* 1997). Whey proteins from milk are digested quickly leading to an elevated but more transient plasma amino acid concentration, while micellar casein from milk is more slowly digested resulting in a moderate but more prolonged increase in the peripheral plasma amino acid concentrations. The two proteins show quite different kinetics of absorption. Moreover, such differences in absorption kinetics influence post-prandial metabolism. Fast proteins induce higher rates of body protein synthesis and amino acid oxidation compared to slow proteins, while slow proteins support a greater and more prolonged inhibition of body protein degradation and thus result in a higher rate of body protein retention post-prandially and thus increased dietary protein utilisation (Boirie *et al.,* 1997; Dangin *et al.,* 2001). The situation is complex, however, in that different responses have been found when the fast protein has been included as part of a complete meal and also with different aged consumers (Dangin *et al.,* 2003). Further interesting observations come from the work of Tessari *et al.* (2007) who found different insulin and glycaemic responses to slow and fast proteins in type 2 diabetic patients and from Acheson *et al.* (2011) who showed that replacing 10% of the carbohydrate of a complete meal with protein promoted thermogenesis, with more pronounced effects for whey protein compared to soya or casein.

The mathematical simulation of food digestion at a mechanistic level offers promise as a means of predicting the kinetics of food particle breakdown, enzymatic hydrolysis and nutrient absorption. Several models of digestion have been developed (Bastianelli *et al.,* 1996; Rivest *et al.,* 2000), but more information on causality is needed (e.g. Kong and Singh, 2010, 2011; Ferrua *et al.,* 2011) to allow an even more detailed insight to

mechanism. Modelling allows the varying rates of flux of nutrients following a meal to be simulated, and potentially allows a description of factors, both food-related and animal, known to affect digestibility. This will lead to an enhanced accuracy of prediction of protein utilisation and animal growth.

2.7.3 Genomics, functional genomics and genetic engineering

Genomics applies recombinant DNA, DNA sequencing methods and bioinformatics to sequence, assemble and analyse the function and structure of genomes (the complete set of DNA within a single cell of an organism). Genomics also includes studies of intragenomic phenomena. The first complete genome sequence of a eukaryotic organelle, the human mitochondrion, was reported in 1981. The first complete genome sequence for a eukaryote organism (*Saccharomyces cerevisiae*) was reported in 1996. A landmark was the publication of the human genome (rough draft in 2001, finished draft 2007). Since then the genomes of several other mammals and eukaryotic organisms have been reported. A high quality draft genome sequence for the pig was published in Nature in 2012 and the first avian genome sequence (red jungle fowl) was announced in 2004. The gene (DNA) sequence of an organism provides the fundamental coding base for all of the cell functions.

While genomics is concerned with gene sequencing, functional genomics makes use of the very large quantities of data produced by gene sequencing projects to describe gene and protein interactions. Functional genomics focusses on gene transcription, translation and protein-protein interactions. Moreover, it is now well established that genes can be modified and gene activity changed, through changes in the genome not reflected in changes in the DNA sequence. Such changes which can be brought about by environmental influences may be heritable. Mechanisms, among others, that can produce such changes are DNA methylation and histone modification. The study of such alterations to gene function is referred to as epigenetics. A better understanding of epigenetics will allow in the future the programming of young animals for certain desirable traits (e.g. more efficient nutrient utilisation). The chicken is a prime target for such epigenetic modification, as the embryo can be 'programmed' readily during incubation of the egg. The fields of genomics, functional genomics and epigenetics are evolving rapidly and will explain the basis of phenomena such as developmental changes in animals, inter-individual variation in biological characteristics and diseases with an inheritable component. Two techniques to emerge from the field of molecular biology with great application to animal science are microarray analysis and the real time quantitative polymerase chain reaction.

A DNA microarray (or DNA chip) is a collection of microscopic DNA spots attached to a solid surface, and can be used to measure the expression levels (fluorescence) of large numbers of genes. DNA microarrays are commonly used to detect DNA or RNA

(usually as cDNA after reverse transcription). The measure via cDNA is commonly referred to as gene expression. In a single study, the expression of thousands of genes can be determined simultaneously to study the effect of experimental variables on gene expression. Differences in expression profiles and especially expression differences in pre-determined logical clusters of genes can usefully lead to the formation of new hypotheses concerning function.

Real-time quantitative polymerase chain reaction (PCR) is used to amplify and then quantify a targeted DNA molecule. PCR enables detection and quantification of one or several specific DNA sequences in a DNA sample. A common PCR method (reverse-transcription) quantifies messenger RNA and non-coding RNA in cells or tissues, by use of fluorescently-labelled sequence-specific DNA probes, that permit detection after hybridisation of the probe with its complementary RNA sequence.

The degree to which a gene is expressed in a cell can be determined by the number of copies of an mRNA transcript of that gene present in a sample. The technique allows quantitative measures of gene transcription. The expression of a particular gene or genes and how this changes over time in response to an experimental variable can be determined. The genome provides the base code for all biological function. An understanding of the genes present describes 'what can potentially happen', while knowledge of their expression levels describes 'what appears to be happening'.

Genetic engineering (genetic modification) refers to the direct manipulation of an organism's genome using various biotechnology techniques. Genes can be removed ('knocked out') or new DNA may be inserted in the host genome. The first genetically modified organisms (GMOs) were bacteria (early 1970's) and a GM mouse was bred in 1974. GMO's are now common and are of increasing importance in agriculture. One of the best known examples in agriculture is the introduction of glyphosate (Roundup) resistant corn and soybean. Genetically modified farm livestock are generally currently at the research stage, but will soon be commercialised (Forabosco *et al.*, 2013). The opportunities for animal production through GMO's are immense and this will be a defining feature of agriculture beyond 2020. GM has been used to produce faster growing pigs with higher feed conversion efficiencies and leaner carcasses. In poultry, disease resistance (e.g. birds unable to spread bird flu, H5N1, to one another) and the survival of newly hatched chicks have been improved. Of great interest to animal nutritionists is the GM improvement of cereal protein quality. For example, a transgenic lysine-rich maize cultivar has recently been produced in which the expression of a potato gene encoding a pollen-specific lysine-rich protein, results in higher levels of protein and lysine. From an experimentation perspective, knockout animals can now be readily sourced (e.g. knockout mice), enabling the effects of the presence and absence of single genes to be investigated.

2.7.4 Proteomics and metabolomics

Proteomics is the large scale study of cellular proteins, including their structures and functions. Gene expression measures cell RNA levels (transcription), but mRNA is not always translated into protein, with the amount of protein produced per unit mRNA being quite variable. Proteomics confirms the presence of a protein and provides a direct measure of the amount present in the cell or tissue. Further, a protein may be subjected to a wide variety of post-translational chemical modifications (e.g. phosphorylation, glycosylation, lipidation) which alter structure and function. Proteomics is a rapidly developing field, assisted greatly by the application of modern mass spectrometry based analytical systems (e.g. peptide mass fingerprinting using MALDI-TOF mass spectrometry).

Knowledge of the proteome is the critical bridge between known genome sequences and biological function, and studies at a proteomic level will assist to unravel cellular biological mechanisms for years to come.

Metabolomics is the study of small (<1 kDa) metabolites, such as amino acids, fatty acids and carbohydrates, which are the products and intermediates of metabolism in a cell, tissue, organ or whole organism. Metabolomics (sometimes referred to as metabonomics) determines the metabolites as a total set. The interest is in investigating if and how the metabolite pattern changes due to treatment, age, genetic makeup or some other condition. Specific statistical procedures (e.g. Principal Component Analysis) are used to identify the metabolites that differ between groups and to correlate these with particular biochemical pathways. The ultimate goal is to link metabolic changes to phenotype. Metabolomics is a rapidly emerging field of study, behind genomics and proteomics (Figure 2.1). The challenge with metabolomics is to measure an adequate number of metabolites in a biological sample, as with today's techniques only part of the metabolome can be analysed. There is no single analytical method available for determining all metabolites collectively. Metabolomics rests heavily on the methods of liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry and nuclear magnetic resonance spectroscopy. Metabolomics can be very useful for discovering differences that may not always be obvious, then from an analysis of such differences and potential biochemistries new hypotheses can be formulated and tested.

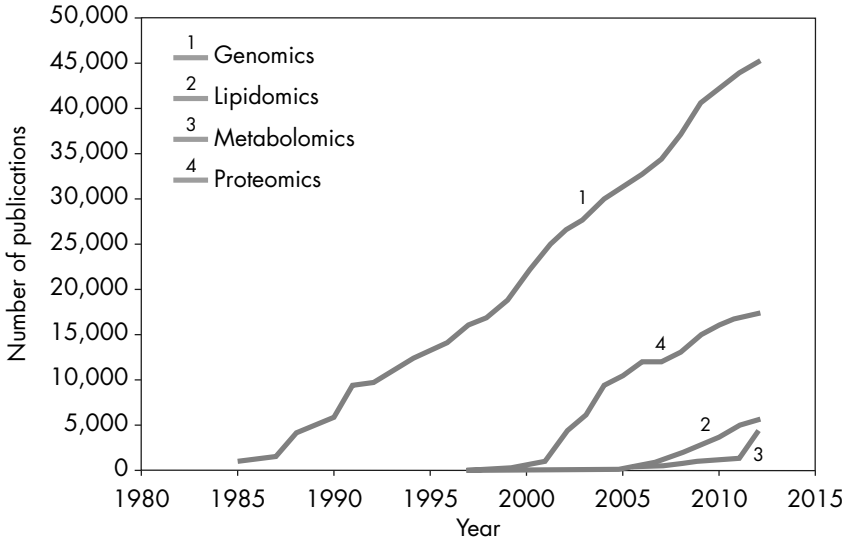

Figure 2.1. Number of peer-reviewed papers published in the fields of genomics, lipidomics, proteomics and metabolomics (1985-2013).

2.8 Some examples of a planned application of the new approaches

The following are examples from my own team's work of how the new technologies, when applied judiciously, can greatly assist in leading to a clearer mechanistic understanding of experimental observations.

2.8.1 DGGE/gene sequencing

The work of Balan (2011) sought to elucidate the mechanisms underlying the known positive effects on mammalian and avian growth and body protein retention of the oral administration of plasma immunoglobulins. In one of the studies, denaturing gradient gel electrophoresis (DGGE), which is a method for separating out fragments of DNA and RNA, showed up a significant enhancement of certain intestinal bacteria with the immunoglobulin treatment. The bacterial bands were excised from the gel (DGGE) and sequenced to identify the bacteria based on genotype. The bacteria, that were more prolific with the immunoglobulin treatment, were all found to be probiotic bacterial species and showed significant immune enhancing activity. Thus a completely new research angle developed whereby plasma immunoglobulins were hypothesised to have prebiotic-like activity.

2.8.2 Reverse transcription polymerase chain reaction

Reverse transcription PCR can be applied to study the expression (RNA levels) of specific genes regulating specific processes. In a recent study (Han *et al.,* 2008), we were interested in elucidating the mechanisms around gut endogenous protein secretions, and specifically the production of gastric and intestinal mucin protein and how it may be influenced by dietary peptides. We used reverse transcription PCR to measure the expression of specific genes coding for mucin protein synthesis in the rat. Primers and probes for specific mucin genes were designed, with the β-actin gene as an internal reference. The small intestinal expression of the gene Muc3 was greatly enhanced by the presence of dietary peptides (as compared to a protein-free diet or synthetic amino acids) (Figure 2.2) as was the colonic expression of the Muc4 gene. These gene expression results are consistent with the results of a study by Claustre *et al.* (2002) showing that peptides from casein induce mucin secretion in the rat jejunum.

2.8.3 Microarray

In studies into gut bacteria-enterocyte messaging (Ulluwishewa *et al.,* 2011; Sengupta *et al.,* 2013), two AgResearch (New Zealand) strains of *Lactobacillus fermentum* (AGR 1485 and AGR 1487), known to differ only by a single gene locus, have been shown to have contrasting effects on intestinal epithelial barrier integrity. AGR 1487 has a

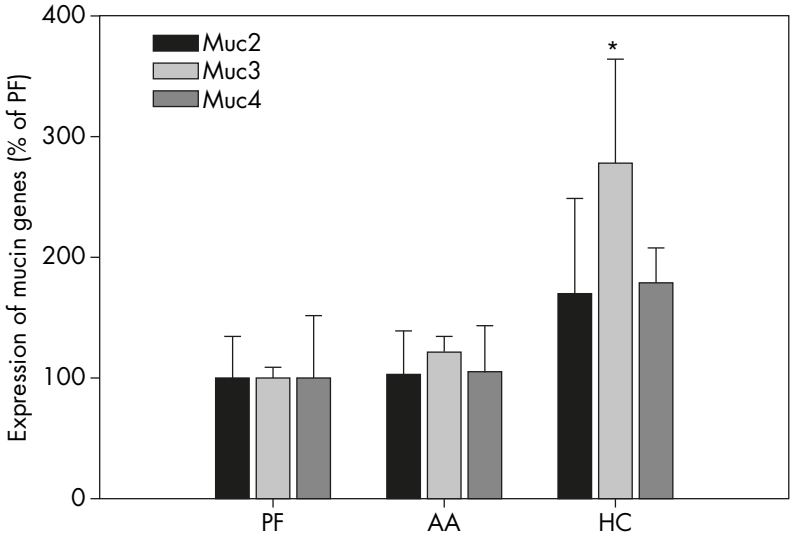

Figure 2.2. Effect of diet protein source on the mRNA levels of three mucin genes (Muc2, Muc3, and Muc4) in the rat small intestine. The results are expressed as percentage increases as compared with the protein-free (PF) diet. Values are means ± SEM; **P*≤0.05 vs PF diet (Han *et al*., 2008).

negative effect on intestinal barrier integrity, loosening the tight junctions leading to increased permeability, while AGR1485 has a positive effect. The maintenance of tight junction integrity is necessary for healthy gut function. It is remarkable that such small genetic differences at the bacterial level can have such effects on intestinal function. In other work (Ulluwishewa, 2013), we have used a novel apical anaerobic culture model to examine molecular crosstalk between the obligate anaerobe *Faecalibacterium prausnitzii* and a model intestinal cell line. The facultative anaerobe *Lactobacillus rhamnosus* was used as a comparator. *F. prausnitzii* has a lower prevalence in the mucosa-associated microbiota of patients with irritable bowel syndrome, a condition where intestinal barrier integrity is known to be compromised. The aim of the study was to determine whether *F. prausnitzii* affects intestinal barrier integrity (measured by mannitol permeability) and whether such changes in permeability could be related to altered gene expression of this enterocyte. *L. rhamnosus* markedly increased permeability of the cell monolayer (i.e. reduced barrier integrity), but barrier integrity was not influenced by *F. prausnitzii*. Global gene expression analysis (microarray) was used to determine differential gene expression in the mucosal cells. The presence of *F. prausnitzii* in the growth medium altered the expression of a large number of genes, inferring significant messaging between the bacterium and gut cells, and the number and type of genes differentially expressed also differed between the two species of bacteria. The differentially expressed genes were examined for networks of biological interaction. This analysis indicated that the differential gene expression related to the presence of *F. prausnitzii* was associated with the functions of cell signalling, humoral immune response and protein synthesis.

2.8.4 Metabolomics

In a study using a metabolomics approach (Hindmarsh *et al.,* 2012), we fed two groups of pigs the same amount of a semi-synthetic diet which differed only in the source of nitrogen, either intact or hydrolysed casein (the same parent casein). Metabolic differences may be anticipated with such a feeding scenario but would be difficult to predict. A metabolomics study of urine pointed to statistically significant differences in the urinary concentrations of five metabolites. There was a much higher urinary excretion of taurine with the hydrolysed casein which would not be anticipated from first principles, and would not likely be measured in a conventional study. It is known that the hydrolysed casein would be emptied more rapidly from the stomach and that the kinetics of uptake of the dietary amino acids would differ from casein. Potentially there are effects on pancreatic taurine synthesis and, or bile salt turnover. Or perhaps the more rapid uptake and metabolism of amino acids affects osmoregulation. The nature of the observed differences leads to testable hypotheses concerning function.

2.9 Future perspectives

As the world's population and middle class continue to grow, the demand for food and food protein will increase. It is believed that by the year 2050 the world will need 70% more food than it does today. Moreover, and consistent with the strong international trend towards healthy foods, lifestyle and disease prevention, the demand for specialty functional foods and high protein foods will also increase. This will all occur against a backdrop of scarce resources. The intensification of animal and poultry production will not decline but will increase, as the efficiency of production will be paramount. Indeed all animal industries will come under increased scrutiny for their efficiency of production. It behoves industry to foresee the new order and instigate research programmes now, to achieve step changes in productive efficiency.

The future for animal science beyond 2020 is a bright one. There is considerable potential for the advancement of knowledge. Most progress, however, will be made not by the use of traditional methodologies, but rather by application of the 'new biology'. This should not occur haphazardly, however, but rather in a planned manner within an integrative holistic framework of growth and development, such as the paradigm described here for the efficiency of utilisation of dietary protein.

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3

Feed intake and regulation

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Summary points

- Several centres in the brain are responsible for the capture and integration of internal and external signals that regulate feed intake.
- Orexigenic neuropeptides stimulate feed intake upon secretion while anorexigenic neuropeptides suppress appetite.
- Input signals are diverse: mechanoreceptors from the digestive system, gut hormones, the pancreatic hormones and adipokines all communicate the energy status of the body to the brain.
- In pigs, understanding feed intake regulation is of importance especially in the weaning period when feed intake is usually decreased.
- During pregnancy and lactation: sows might also suffer from hyper- or hypophagia.
- In pigs, injection of neuropeptides or opioid therapy for weaning piglets, or certain feeding strategies (fibre composition) for sows might be new strategies to better understand and control feed intake.
- Although chickens have many similarities in their appetite-regulatory mechanisms with mammals, some peculiarities exist such as ghrelin, known to stimulate food intake in mammals, while reducing feed intake in chickens.

Keywords: neuropeptides, hormones, pig, chicken, brain

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3.1 Introduction

The energy balance is the net result between energy intake on the one hand and energy expenditure on the other hand. Maintaining a proper energy balance (at least within a certain time period or physiological status) implies a strict homeostatic regulation. Most homeostatic regulatory mechanisms are complex and rely on multiple physiological pathways involving several organ systems. Such homeostatic mechanisms require a controller (sensors), messengers (e.g. hormones), receptors, actors (cells, organs) and feedback mechanisms. With respect to the regulation of food (human)/feed (animal) intake, the situation is even more complex as feed intake regulation is much more than only energy intake regulation as there are also specific appetites for macronutrients, minerals, etc.

Until some decades ago, the so-called 'set-point' hypothesis assumed that an organism has a predetermined set-point for ideal body weight, called a 'ponderostat' in the brain. This ponderostat would work according to the mechanism of a thermostat, in which negative feedback signals from the body would be sent to the ponderostat when the body weight increased above the predetermined weight. The ponderostat would then trigger a number of physiological responses that has to restore the body weight to its original, pre-set level.

However, it became quickly clear that such a 'simplistic' view was not tenable and that the systems controlling body weight and energy balance were much more complex (Berthoud, 2002). Furthermore, it was also recognised that not only one, but several overlapping neural and endocrine pathways are involved in negative as well as positive feedback mechanisms, which together provide a long-term stable energy status.

This chapter provides a generalised overview on the current knowledge of appetite regulation in humans and animals. Some peculiarities in pigs and poultry are highlighted separately.

3.2 The hypothalamus as central integrator of input signals

3.2.1 Hypothalamic nuclei

Several centres in the brain (e.g. nucleus tractus solitarius (NTS) in the brain stem, hypothalamus, brain cortex) are responsible for the capture, integration and relay of a vast array of internal (classified as pregastric, gastric, postgastric) and external (e.g. smell, sight) signals. In addition, emotions, learning processes and social influences all have an effect on food selection and ingestive behaviour. The hypothalamus is responsible for the final integration of all these signals (King, 2005).

The hypothalamus is a paired structure and is situated at the third brain ventricle. It consists of many nuclei located in different zones of the hypothalamus. With respect to food intake regulation, the arcuate nucleus (NARC) consisting of a medial and lateral part, the paraventricular (PVN) and the lateral nucleus (LHA) are considered to be the most important nuclei (Figure 3.1), although other nuclei such as periventricular, dorsomedial (DMN) and ventromedial nuclei are also involved in the control of food intake. When triggered, these nuclei will (co-)express specific neuropeptides and receptors, which will stimulate or temper food intake (for excellent reviews, see Schwartz *et al.,* 2000; Broberger, 2005; Ahima and Antwi, 2008):

- medial part of arcuate nucleus: neuropeptide Y (NPY); Agouti-related peptide (AgRP);
- lateral part of arcuate nucleus: proopiomelanocortin (POMC) as precursor of α-melanocyte-stimulating hormone (α-MSH); cocaine and amphetamine-related transcript (CART);
- paraventricular nucleus: thyrotropin-releasing hormone (TRH); corticotropin-releasing hormone (CRH);
- lateral nucleus: melanin-concentrating hormone (MCH); orexins A and B; dynorphin.

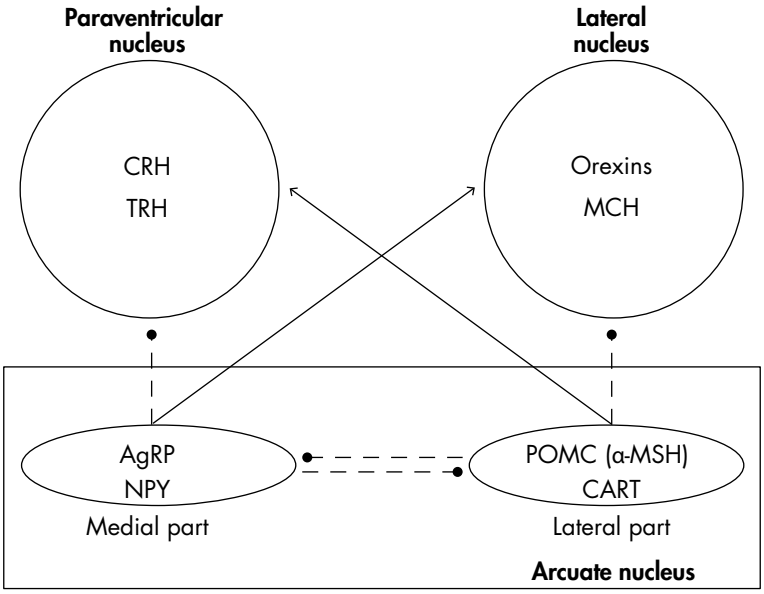

Figure 3.1. Schematic illustration of the interactions between hypothalamic nuclei involved in food intake regulation. ⟶ = stimulatory; —● = inhibitory; AgRP = Agouti-related peptide; CART = cocaine and amphetamine-regulated transcript; CRT = corticotropin-releasing hormone; MCH = melanin-concentrating hormone; NPY = neuropeptide Y; POMC = proopiomelanocortin; TRH = thyrotropin-releasing hormone; α-MSH = α-melanocyte-stimulating hormone.

3.2.2 Hypothalamic orexigenic neuropeptides

NPY is a polypeptide belonging to the pancreatic polypeptide group and is produced by 15-20% of the neurons in the NARC from which it is projected to cells in the PVN and LHA. Furthermore, NPY is also produced in the DMN, especially when there is a high demand for energy (e.g. during lactation).

The synthesis and release of NPY by the hypothalamus is controlled by various factors. NPY production is reduced by α-MSH, leptin, insulin, polypeptide YY, glucose and oestrogens whereas ghrelin, orexins and glucocorticoids are known to stimulate its production. In humans and rats, at least five subtypes of NPY receptors (Y1-Y5) are described.

NPY (co-expression with AgRP) stimulates energy balance in two ways:
1. Increasing appetite by:
- stimulating the orexigenic MCH and orexin-producing cells in the LHA after binding on the Y5 receptor;
- suppression of anorexigenic POMC cells in NARC (via Y1 receptor);
- blockade (together with AgRP) of the inhibitory effect of α-MSH on the orexigenic MCH-producing cells of the LHA (via MC4-R).
2. NPY and AgRP reduce energy expenditure by direct and indirect (AgRP blockade of PVN MC4-R) inhibition of PVN neurons that produce TRH and CRH.

AgRP is a peptide that is predominantly expressed in NARC neurons, together with NPY. AgRP is an antagonist of the MC4-R of MCH-producing cells in the lateral hypothalamus. In the absence of AgRP, the anorectic α-MSH (>POMC) normally binds to this MC4-R and prevents the expression of the orexigenic MCH. However, when AgRP is present, it prevents the anorectic effect of α-MSH as the orexigenic MCH is fully expressed.

The orexins A and B (also known as hypocretins) are mainly produced by neurons in the lateral nucleus of the hypothalamus (Li *et al.,* 2014). The orexin-producing neurons project to the PVN, NARC, and NTS in the brain stem. Orexins stimulate appetite, but their effect is of short duration. They are less potent orexigenic molecules than NPY. Orexin A is about 10 times more potent than orexin B. Blocking the orexin receptors reduces feed intake. Their orexigenic effect would occur partially through a stimulation of the NPY production and an inhibition of the POMC-system.

The MCH-producing cells of the LHA are stimulated by NPY (via Y5 receptor), and inhibited by α-MSH (via MC4-R). These neurons are inhibited by leptin and insulin and stimulated by glucose. AgRP blocks the MC4-R, thus preventing the suppressive effect of α-MSH. MCH stimulates food intake.

3.2.3 Hypothalamic anorexigenic neuropeptides

In the hypothalamus, melanocortin hormones (primarily α-MSH) are generated from the common precursor POMC in neurons within the lateral NARC (Ellacot and Cone, 2004). These neurons project to various zones in the hypothalamus such as the PVN, LHA and DMN. α-MSH suppresses appetite by inhibiting the expression of the orexigenic MCH in the LHA via MC4-R. However, this effect of α-MSH is blocked by AgRP. In the PVN, melanocortins stimulate hypophysiotropic hormone-producing neurons to express TRH and CRH via MC4-R. CRH has an anorexigenic effect and stimulates the release of adrenocorticotroph hormone. TRH is also anorexigenic and stimulates the release of thyroid-stimulating hormone, which increases energy expenditure after activation of the thyroid gland to produce thyroid hormones. NPY-producing cells in the medial NARC are inhibited by α-MSH (via MC3-R) whereas POMC neurons in lateral NARC are inactivated by NPY (via Y1-R), illustrating the mutual interaction between these distinct neural populations within the NARC.

In 1981, CART was discovered in the brain of rats and humans (Leu and Herzorg, 2014). Its production is stimulated by psychogenic stimulants such as cocaine and amphetamine. The pro-CART protein gives rise to a long and short active peptide. These neuropeptides are mainly produced in neurons of the hypothalamic NARC, PVN, DMN, LHA and in the NTS. It works anorexigenic and induces TRH release from the hypothalamic PVN, leading to an increase in energy expenditure. When injected into the brain, CART prevents the fasting-induced increase in NPY concentrations and hence blocks food intake when it becomes available.

The hypothalamic CART-concentrations decrease during fasting, are suppressed in leptin-deficient ('ob/ob' mice) and are restored by leptin administration. CART expression is also modulated by glucocorticoids. The CART cells in the NTS also possess cholecystokinin (CCK)-A receptors, suggesting that CART may play a role in the satiety effects of CCK.

Other anorexigenic neuropeptides are serotonin, urocortin and neurotensin but are not discussed here. For a review on the mechanisms through which urocortin, or neurotensin regulate food intake, the reader is referred to Stengel and Taché (2014) and Schroeder and Leinninger (2018), respectively.

3.3 Input signals from the digestive system

3.3.1 Mechano- and chemoreceptors

Feedback signals from the gastrointestinal, portal and hepatic region play an important role in the control of food intake. The flow of information from the intestines to the brain is primarily regulated by the vagus nerve (nervus X).

The filling of the stomach (gastric distention) is detected by specialised mechanoreceptors on the vagal ends in the myenteric plexus and in the outer smooth muscle cells of the stomach and acts as an important satiety signal.

Chemical signals from the small intestine and the hepatic region also inform the brain about the amount of food, energy, or specific nutrients that have been absorbed from the gastrointestinal tract. This information is also largely transmitted by the vagus nerve (for excellent reviews, see Cummings and Overduin, 2007; Wren and Bloom, 2007; Moran, 2009; Dockray, 2014). Information related to chemical stimulation by nutrients in the gastrointestinal tract comes mainly from studies applying direct intra-intestinal infusions of iso-osmotic solutions (iso-osmotic to plasma in order exclude pure osmotic effects on satiety). The reduction in food intake following infusion is directly proportional to the energy content of the infusate, and this may in turn affect the rate of gastric emptying in a dose-dependent way (entero-gastric reflex). However, different nutrients with similar isocaloric value do not necessarily have the same effect on food intake e.g. triglycerides are much less effective than long chain fatty acids, and unsaturated fatty acids are more effective than saturated fatty acids.

3.3.2 Cholecystokinin

One of the important local gut hormones involved in satiety feedback signals from the intestines is CCK. This hormone reduces food intake in a dose-dependent manner, and vagotomy abolishes the satiety-inducing effects of CCK (Dockray, 2009).

This gut peptide is present in endocrine (I) cells of the stomach and small intestine and in some neurons of the neural network of the intestinal wall. Several types of this peptide are found in the blood, but the sulphated C-terminal octapeptide is needed for its anorexigenic properties. Its production and release is stimulated by some feed ingredients, especially fats and digested peptides, and to a lesser degree by carbohydrates. Trypsin inhibitors also trigger the release of CCK. It was also demonstrated that the adipokine leptin can induce the release of gut CCK.

Two types of CCK receptors have been described so far: high and low affinity CCK-A receptors typically found in the gastrointestinal tract ('Alimentary') and

CCK-B receptors usually found in the brain ('Brain'). However, both receptors can be simultaneously present in the same tissue (e.g. CCK-A receptors in rat brain). Besides its role in satiety, CCK has also effects on the gastrointestinal tract. CCK stimulates the secretion of protease and lipase from the pancreas, delays gastric emptying by reducing pylorus contractions, stimulates gallbladder contractions, causes vasodilation and increased motility in the intestine. These effects are mediated via high affinity CCK-A receptors. CCK works synergistically with the gut hormone secretin for the production of water and bicarbonate by the pancreas.

3.3.3 Ghrelin

This so-called 'hunger' hormone ghrelin is mainly produced by specialised enterochromaffin cells of the stomach and proximal duodenum, and to a much lesser extent in the pancreas, kidneys, thyroid gland, placenta and hypothalamus (Cummings, 2006; Cummings and Overduin, 2007). Once the peptide is produced after posttranslational splicing of its precursor molecule preproghrelin, it needs to be acylated with octanoic acid before it becomes biologically active. Ghrelin can cross the blood-brain barrier after binding to the ghrelin receptor which are abundantly present in the brain (NARC, brainstem, NTS) and periphery. Ghrelin was first known as a powerful growth hormone-releasing hormone (GHRH). Nowadays, ghrelin is also characterised as an appetite-regulating hormone but it has several other effects such as modulation of gastrointestinal motility, gastric acid secretion, pancreatic functioning, cardiovascular functioning, a role in immunity and inflammation, reproduction and sleep. With respect to its orexigenic properties in mammals, ghrelin inhibits the POMC cells, presumably after stimulation of the NPY/AgRP neurons. Similarly, ghrelin also induces the release of CRH in the PVN, after first acting on NPY/AgRP neurons. For age-related effects of ghrelin on food intake and age-associated changes of other appetite-regulating peptides, we refer to the review of Akimoto and Miyasaka (2010).

3.3.4 Obestatin

Obestatin was first isolated and purified from the rat stomach. This 23 amino acid amidated peptide is derived from the same precursor protein preproghrelin as ghrelin. Obestatin was first claimed to decrease appetite and body weight gain in rodents (Zhang *et al.,* 2005). Furthermore, obestatin had apparently opposing actions to ghrelin, which is known to increase gastric emptying and intestinal motility in humans and rodents. However, subsequent studies failed to confirm the originally observed effects of obestatin on food intake and body weight and gastrointestinal motility and contractility in rodents (e.g. De Smet *et al.,* 2007). The physiological function of obestatin in mammals remains unresolved and needs further investigation.

3.3.5. Insulin

Insulin is produced and secreted by the beta cells of the pancreas. All cells except neurons require insulin for glucose uptake. The release of insulin is controlled by various systems. Already during the cephalic phase, insulin release is secreted by means of parasympathetic stimulation. As a result, the glucose content decreases slightly in the blood. This drop, in turn, is detected by the NPY/AgRP-producing neurons of the arcuate nucleus (directly and indirectly via glucose-sensitive and orexin-producing neurons, respectively). Once activated, these neurons stimulate food intake. During the gastric phase, insulin production is further stimulated by the release of CCK. The largest increase in insulin evidently occurs during the absorption phase because of the elevated plasma glucose concentrations. These postprandial high insulin levels now reduce appetite via an inhibitory effect on the NPY/AgRP neurons and a stimulatory effect on the POMC/CART neurons in the NARC. These neurons have indeed insulin receptors.

Insulin thus has an effect similar to that of leptin, but it is fast-acting and of short duration. There is intense crosstalk between the leptin and insulin pathways but a profound discussion on this topic is beyond the scope of this chapter.

3.3.6 Other signals

Food intake is not solely regulated by chemical and metabolic signals. Indeed, other factors such as cognitive, social and emotional factors are also involved (Singh, 2014). Besides the hypothalamus, other brain centres such as the prefrontal brain cortex, amygdalia and nucleus accumbens play a role in these processes. These aspects are well studied in humans as they are important determinants of the ultimate amount of food consumed and are reputed to contribute to obesity. However, data in farm animals is scarce and, therefore, not dealt with in this chapter.

3.4 Input signals from adipose tissue: leptin

Leptin is mainly produced by adipocytes, although small amounts are also produced by the hypothalamus, pituitary, placenta, skeletal muscle, and epithelium of the stomach and of the mammary glands (Ahima and Flier, 2000; Ahima *et al.*, 2000). This adipokine belongs to the group of cytokines having a common structure, and its sequence is strongly preserved in all mammalian species. Leptin appears free in the circulation or is bound to specific proteins, of which the soluble extracellular portion of the long leptin receptor is the most important.

The leptin receptor was characterised in 1995. Two leptin receptor ('obese receptor' or OB-R) forms have been described so far: a long (OB-Rb) and a short form (OB-

Ra) leptin receptor. OB-Rb is predominantly present in the brain (mainly in the hypothalamus), but also in the ovaries, testes and pituitary. OB-Ra is found mainly in the kidneys where it binds leptin in order to excrete the hormone from the body. The short form is also present in the epithelium of the choroid plexus and brain capillaries, where it plays a role in the (saturable) transport of leptin across the blood-brain barrier.

The levels of circulating leptin increase exponentially with the fat mass of the body. Subcutaneous fat cells produce more leptin than visceral fat cells. The amount of leptin produced by adipocytes increases exponentially with the filling of the adipocytes (hypertrophy). Insulin, glucocorticoids and oestrogens all stimulate leptin secretion. Hyperinsulinemia and high glucocorticoid levels, therefore, are associated with high levels of circulating leptin.

Animals that lack leptin (e.g. 'ob/ob' mice) or having a deficient leptin receptor (e.g. 'db/db' mice, and 'fa/fa' Zucker rats) are extremely obese. Injecting leptin is only effective reducing appetite and inducing weight loss in ob/ob mice.

In mammals, leptin is a major anorexigenic hormone that regulates the energy balance by a direct action on specific neurons in the hypothalamus. Indeed, leptin in the hypothalamus suppresses the production of NPY/AgRP and stimulates the POMC/CART production, resulting in a reduction of feed intake. The leptin-induced alterations in neuronal activity in the NARC elevate the TRH and CRH expression in the PVN, leading to an increase in energy expenditure. Leptin would also modulate the CCK-sensitive vagal afferent neurons through leptin receptors on the vagus nerve.

Given the fact that the leptin level in the blood does not change during or after a meal suggests that leptin does not really work as a direct short term anorexigenic agent, but rather as a signal substance that is communicating the energy status of the body to the brain.

Besides leptin, adipocytes also produce other adipokines such as adiponectin (lowers insulin resistance), resistin (increases insulin resistance in rodents) and visfatin, which is an adipokine from visceral fat and is known to be able to stimulate insulin receptors (Ahima and Lazar, 2008).

3.5 Peculiarities in pigs

3.5.1 Weaning piglets

At weaning, pigs are exposed to several stress factors: maternal separation, dietary changes from liquid milk to solid feed, new housing and new social interactions, resulting in a decreased feed intake and growth and an increased susceptibility to disease. Therefore, several researchers investigated hormones that control feed intake, shortly after weaning in an attempt to find a way to make the decreased feed intake less severe. Some results are now further discussed.

Kojima *et al.* (2007) investigated the effect of weaning weight (small vs large) and weaning diet (spray-dried plasma, control weaning diet or cross-fostered pigs) on neuroendocrine regulators of feed intake in pigs. Weaning diet did not affect expression of adipose leptin or hypothalamic leptin receptor, which supports the concept that leptin expression may be regulated independently of adiposity or feed intake during early development (Ahima *et al.,* 1998). Weaning diet did not affect expression of appetite-regulating genes. However, an effect of weaning weight was seen on hypothalamic NPY, AgRP, orexin and type 2 orexin receptor gene expression, i.e. large pigs expressed greater amounts of these transcripts. Therefore, these authors speculated that there exist individual predispositions, either genetically or environmentally induced, that make the animal eat and grow. However, this hypothesis should be further investigated.

In weaned pigs, during experimental feed deprivation, a brief suppression of serum ghrelin at 12 h is observed followed by an increase up to a plateau at 36 h and 48 h (Salfen *et al.,* 2003), which is consistent with a study in rodents (Tchop *et al.,* 2000). This was further confirmed in prepuberal gilts by Govoni *et al.* (2005). When weaned piglets received exogenous ghrelin three times daily for 5 days, a positive effect on weight gain was observed with a concomitant increase of growth hormone, insulin and cortisol secretion, while no effect on feed intake was reported, possibly as ghrelin was metabolised from one injection to the next so no systemic ghrelin accumulation could occur (Salfen *et al.,* 2004). Measurements of ghrelin in pre- and postprandial conditions during several feeding regimens (*ad libitum*, feeding twice or once a day) suggested that plasma ghrelin concentrations in the pig appear to be influenced by chronic changes in energy balance rather than the feeding pattern per se (Scrimgeour *et al.,* 2008). Indeed, there was neither a preprandial rise nor postprandial fall in circulating ghrelin concentrations in response to feeding (Scrimgeour *et al.,* 2008), which is different from observations in humans (Cummings *et al.,* 2001). The importance of ghrelin in controlling feed intake has also been shown by Vizcarra *et al.* (2007), by an active immunisation against ghrelin with a concomitant decrease in

feed intake and slower growth. Ghrelin and its biological effects on pigs have been reviewed by Dong *et al.* (2009).

Injections of other neuropeptides around weaning age have also been shown to stimulate feed intake. Dyer *et al.* (1999) has shown that an intramuscular injection of synthetic porcine orexin in weanling pigs stimulated feed intake at 12 h after treatment. An intramuscular injection of syndyphalin (SD-33), an opioid molecule, before weaning, increased feed intake in pigs from 9 days after weaning (Kojima *et al.,* 2009). In control pigs, an increase in the expression of the hypothalamic μ-opioid receptor (MOR) was observed because of weaning, which was not found in SD-33 injected pigs (Cooper *et al.,* 2011). The down regulation of hypothalamic MOR expression in SD-33 injected pigs supported the notion that SD-33 binds to the μ-opioid receptors, down-regulating its expression. Weaning increased hypothalamic PC4R expression in control pigs, but treatment with SD-33 appeared to abrogate this effect. The strong positive correlation in the expression of MC4R and MOR across all their measured times (before weaning, 1, 4, 7 d post-weaning) and treatments supports the concept that the melanocortinergic pathway is influenced by opioid tone at all time. Expression of AgRP was not significantly altered by weaning or SD-33 treatment, at 1 d after weaning. At 4 d after weaning, expression of AgRP was greater in SD-33 injected pigs than in control pigs, but at 7 d expression was less in SD-33 pigs than in control pigs. The authors speculated that the temporary increase in AgRP occurred earlier in SD-33 treated pigs, initiating eating behaviour earlier in these pigs compared to untreated pigs (Cooper *et al.,* 2011). Therefore, novel opioid therapies may represent an ideal strategy to improve well-being in animals during periods of stress such as weaning, castration, or transport (Cooper *et al.,* 2011).

3.5.2 Sows

During pregnancy, hyperphagia occurs before the increased demand of energy intake (Trujillo *et al.,* 2011). Therefore, dietary strategies are set up to control feed intake of pregnant sows. Saleri *et al.* (2015) showed that in restrictively fed pregnant sows, plasma leptin levels increased from the end of the 2nd stage of pregnancy to reach the highest levels at the 3rd stage and at delivery. Their findings suggest that lactogenic hormones (prolactin, progesterone) are involved in altering leptin signal during different stages of pregnancy in sows, contributing to modulate the leptin signal and allowing increased nutrient availability for the foetus.

Highly prolific sows often experience a decreased feed intake in the peripartum period, with a decreased production rate as a consequence (Edwards, 2002). To better understand the causes of this hypophagia, Cools *et al.* (2013) investigated the peripartum profile of feed intake-regulating hormones. Plasma leptin and serum resistin levels increased gradually throughout the peripartum period (from 8 days before farrowing until 5 days post-partum), whereas plasma ghrelin peaked on day

109 of gestation compared with day 107 of gestation and day 1 and day 3 of lactation, with other time points being intermediate. Only levels of leptin differed between fat (>22 mm back fat thickness) and moderate (between 18-22 mm) or lean (<18 mm back fat thickness) sows. Feeding strategies (restricted or *ad libitum* feeding) did not affect plasma levels of leptin or ghrelin, nor serum levels of resistin (Cools *et al.,* 2013). Moreover, body condition and late gestation feed intake did not affect peripartum hypophagia.

More research on the understanding of the control of feed intake, and interplaying factors (such as body condition, feeding strategy) might help to adapt management strategies. As a feeding strategy, fibres might be increased in the diet of pregnant sows to dilute the feed. Resistant starch (RS) seems to prolong the duration of satiety in pigs, likely because of its slow rate of fermentation (Souza da Silva *et al.,* 2013). Underlying mechanisms for the satiating effect of RS are the increased plasma short chain fatty acids and triglyceride levels throughout the day and decreased postprandial glucose and insulin responses (Souza da Silva *et al.,* 2014). Moreover, plasma serotonin levels in RS-fed pigs were lower, which might have affected colonic motility and overall transit time of digesta and, thereby, potentially affected satiety (Sleeth *et al.,* 2010). Therefore, putative mechanisms of the satiating effects of RS merit further research, as RS may potentially be used for improving welfare in restrictedly fed sows that may experience hunger (Souza da Silva *et al.,* 2014).

3.6 Peculiarities in poultry

Mammalian and avian species commonly utilise similar signalling molecules but this does not necessarily mean that these compounds also have the same function. Examples are the orexins A and B, ghrelin, GHRH and obestatin (Table 3.1). Since leptin is still controversial in avian species, we will not discuss this issue in this chapter. For a review on leptin, the reader is referred to the review of Ohkubo and Adachi (2008), although more recent publications on the leptin gene can be found (e.g. Seroussi *et al.,* 2017). Orexins A and B are potent orexigenic agents in mammals but are apparently without any significant on appetite in chickens. Indeed, Furuse *et al.* (1999) reported that intracerebroventricular injection of mammalian orexins did not affect feed intake of neonatal chicks.

As outlined above, the gut hormone ghrelin is known to stimulate food intake in mammalian species. Avian ghrelin is a 26-amino acid peptide, sharing 54% amino acid sequence identity with rat and human ghrelin. Surprisingly, central rat ghrelin injection decreased appetite of neonatal chicks (Saito *et al.,* 2002). We also observed a reduction (although of short duration of <1 h) in feed intake after intravenous injection of chicken ghrelin in broiler chickens (Geelissen *et al.,* 2006; Buyse *et al.,* 2009). In addition, a single intravenous chicken ghrelin administration significantly

Table 3.1. Effect of (an)orexigenic peptides in avian species (adapted from Song *et al.*, 2013).[1]

Compound[1]	Administration site[2]	Response in feed intake in avian species[3]
Ghrelin	icv, iv	↓ (mammals ↑)[4]
Obestatin	iv, ip	↓
α-MSH	icv	↓
CRF	icv	↓
Urocortin	icv	↓
NPY	icv	↑
AgRP	icv	↑
GHRH	icv	↓ (mammals ↑)
MC3/4R agonist	icv, iv	↓
CART	icv	↓
μ-Opioid agonists	icv	↑
Bombesin	icv	↓
Leptin?	icv, iv	↓
Insulin	icv	↓
Peptide YY	icv	↑
Pancreatic polypeptide	icv	↑
Orexins A&B	icv	» (mammals ↑)
GLP-1	icv	↓

[1] AgRP = Agouti-related peptide; CART = cocaine and amphetamine-related transcript; GHRH = growth hormone-releasing hormone; NPY = neuropeptide Y; α-MSH = α-melanocyte-stimulating hormone.

[2] icv = intracerebroventricular; ip = intraperitoneal; iv = intravenous;

[3] » = no clear effect; ↑ = increase; ↓ = decrease.

[4] Effect in mammals.

decreased the respiratory quotient for up to 16 h, but was without effect on heat production (Geelissen *et al.,* 2006). It was suggested that ghrelin might be involved in the preferential use of substrates for oxidation and storage (e.g. decreased lipogenesis).

In mammals, GHRH is also known to be involved in the regulation of feed intake, but its effect seems to be dose-dependent. Indeed, icv administration of GHRH in the picomole range stimulated food intake but nanomole doses of GHRH inhibited feeding in young Wistar male rats (Veyrat-Durebex *et al.,* 2001). On the other hand, icv injection of GHRP-2, a synthetic growth hormone, secretagogue inhibited feed intake of neonatal chicks (Saito *et al.,* 2002).

An obestatin-like sequence was also found in the chicken ghrelin precursor protein, suggesting the possible existence of a 'chicken' obestatin peptide (Song *et al.,* 2012). In view of the opposite effects of ghrelin on food/feed intake and gut contractility between

rodents and broiler chicks, it was initially hypothesised that 'chicken' obestatin might also have opposite effects on appetite and gut contractility in avian species: stimulating appetite and decreasing muscle contractility of gut segments. However, after several attempts, we could not find any significant effect of 'chicken' obestatin on feed intake nor on *in vitro* contractility in broiler and layer chicks, doubting a physiological role for obestatin in feed intake regulation in chickens (Song *et al.,* 2012).

3.7. Conclusions

Feed intake regulation involves a whole array of signals, hormones, and receptors. Diverse signals, from the gut or other tissues, provide information to the brain, where the hypothalamus will integrate all these signals. Hence, the triggering of specific nuclei in the hypothalamus will (co-)express specific neuropeptides and receptors, which will stimulate or temper food intake. Although chickens have many similarities in their appetite-regulatory mechanisms with mammals, some peculiarities exist. Understanding the mechanisms that regulate feed intake is necessary for the optimisation and efficiency of animal production. Fundamental research on this topic will help to develop new treatments and feed strategies to have an optimal feed intake and consequent production performance.

3.8 Future perspectives

Further fundamental research on the control of feed intake, and the interplaying factors such as body condition, age, gender will allow to develop strategies that may improve health and welfare status of the animals. Indeed, if we can develop strategies that stimulate feed intake in weaned piglets, less severe problems of post-weaning diarrhoea could be expected. Understanding how to reduce appetite or induce satiety, could improve the welfare status of sows and broiler breeders, maybe by dietary strategies such as RS, meriting further research.

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4

Gut nutrition and health in pigs and poultry

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Summary points

- 'Gut health' is a term being used in the pig and poultry industries to describe the structure and function of the gastrointestinal (GI) tract and its relationships to nutrition, immunology, microbiology, and the environment.
- This term has originated in the pig and poultry industries predominately in response to production practices where some antimicrobial compounds have been banned or curtailed in their use.
- The term 'gut health' essentially relates to effective digestion and absorption of food, the absence of GI tract illness, a normal and stable intestinal microbiome, absence of zoopathogens, an effective immune status, and a status of animal well-being.
- The GI tract microbiome and GI barrier function are critical components to a healthy and robust GI tract.
- These aspects of 'gut health' can be influenced by a plethora of internal and external challenges that disrupt homeostasis and (or) the homeorhetic function of the GI tract.
- Future research efforts should focus on the rapid and comprehensive diagnosis of 'gut health' and on the mechanistic description of the impact of specific dietary factors.

Keywords: pig, poultry, gastrointestinal tract, health, disease

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4.1 Introduction

Domestic animals continue to make important contributions to global food supply and, consequently, animal feeds and animal feeding have become increasingly critical components of the integrated global food supply chain. Worldwide, livestock products account for about 30% of the global value of agriculture and 19% of the value of food production, and provide 34% of the protein and 16% of the energy consumed in human diets (Thornton, 2010). Meeting growing consumer demand, particularly in the world's developing countries, for more meat, milk, eggs and other livestock products will depend largely on the availability of regular supplies of appropriate, cost-effective and safe animal feeds. In the past, increases in livestock productivity have been driven mostly by animal science and technology, and scientific and technological developments in breeding, nutrition and animal health will continue to contribute to increasing potential production and further efficiency and genetic gains. However, future demand for livestock products is likely to be moderated by socio-economic factors such as food habits and trends, human health concerns (e.g. antibiotic resistance, food safety), and changing socio-cultural values such as welfare attitudes to animal farming (Thornton, 2010).

In this context, the pig and poultry industries make major global contributions to animal protein supply (OECD-FAO, 2011) and are well positioned to take advantage of the growing worldwide demand for meat (Herrero and Thornton, 2013). Nevertheless, there are challenges to both industries, some of which are familial but others that are unique to either, that limit continued improvements to the efficiency in which feedstuffs, including by-products from food processing, are converted to foods available for human consumption. In this regard, consideration and management of the gastrointestinal (GI) tract, especially in view of increasing limitations in the way that some additives such as antimicrobial compounds (AMC) can be used in the respective industries, has become a considerable focus for those involved in the production of pig and poultry products. It is acknowledged that there are major differences in the global situation with regard to the use of AMC, but a more restrictive view of the use of such substances can be noted in many parts of the world.

There is a vast body of literature pertaining to this topic and hence it is simply beyond the scope of this chapter to discuss fully the many influences and factors and the resultant production, physiological, immunological, and microbial and welfare consequences and outcomes related to gut health (whether it be the *status quo*, compromised, or enhanced). Rather, an attempt has been made to feature some aspects of gut health that are perhaps less frequently reported but which are nonetheless still germane to the overall topic and relevant to modern-day pig and poultry farming. With this in mind, the aims of this review are to: (1) attempt to define gut health as it pertains to pigs and poultry; (2) to describe approaches for influencing GI tract structure and function; and (3) to give an outlook for future developments.

The chapter concludes by summarising the key aspects of the review. We contend that whilst gut health might be the term used to discuss virtually any aspect associated with influences on GI tract structure and function, and any consequences thereof, it is the management of the GI tract during a period (or periods) of insult and how the GI tract then responds that is most interesting, and should form the basis for future investigations in this sizable field of interest.

4.2 Is there a unifying definition of 'gut health'?

The term 'gut health' generally lacks a clear and unifying definition in the literature, although it has been used in both human medicine (see review by Bischoff, 2011) and appears in peer-reviewed articles associated with the interface between pig and poultry health and production (e.g. Lallès *et al*., 2007; Lallès, 2008; Pluske, 2008; Yegani and Korver, 2008; Choct, 2009; De Lange *et al*., 2010; Pluske, 2013; Lindberg, 2014; Celi *et al*., 2017; Moeser *et al*., 2017; Pluske *et al*., 2018a; Celi *et al*., 2019). The term is also regularly used in the popular press and in on-line articles and reports (e.g. Collett, 2013), and is a common point of discussion and debate at meetings, forums and workshops related to pig and poultry nutrition, enteric challenges and production. Consequently, the term is used in many different contexts, which makes it difficult for a consensus to be formed regarding its precise use, application and assessment in the pig and poultry industries. Nevertheless and in accordance with the World Health Organisation definition of 'health' from 1948 (cited by Bischoff, 2011), which proposed a positive definition instead of 'the absence of diseases', Bischoff (2011) commented that gut health can be defined as 'a state of physical and mental well-being in the absence of GI complaints that require the consultation of a doctor, in the absence of indications of or risks for bowel disease and in the absence of confirmed bowel disease'. Bischoff (2011) argued that although the World Health Organisation defined health as being more than just the absence of disease, prevention or avoidance of GI disease forms an integral part of our understanding of the overall issue. In this regard and in the pig and poultry industries, there is sometimes a tendency to associate gut health with bacterial and (or) viral pathogens that cause, either clinically or subclinically, illness to the animals. However, and in pigs for example, and in agreement with Bischoff's (2011) definition, gut health can be compromised in the absence of any disease in the GI tract. For instance, the low feed intake after weaning of piglets means an absence of proper luminal nutrition (Diamond and Karasov, 1983). The immediate post-weaning period in pigs not only causes marked structural and functional changes to the small intestine (Kelly *et al*., 1991a,b; Pluske *et al*., 1996a,b), but contributes to an intestinal inflammatory status that in turn compromises villous-crypt architecture (McCracken *et al*., 1999; Spreeuwenberg *et al*., 2001) and GI tract barrier function (Wijtten *et al*., 2011; Kim *et al*., 2012, 2013; Moeser, 2013).

Bischoff (2011) defined five major criteria that could form the basis of an overarching definition of gut health, being: (1) effective digestion and absorption of food; (2) absence of GI illness; (3) normal and stable intestinal microbiome; (4) effective immune status; and (5) status of well-being. These criteria mirror closely those applicable to the pig and poultry industries, and indeed represents a foundation for future assessment, evaluation and investigations in this field. Nevertheless, it is recognised that the functions of the GI tract extend well beyond the processes associated with feed intake, digestion, and the subsequent active or passive absorption of nutrients and fluid along the GI tract. Though not strictly reflecting the term 'gut health', reduction of zoonotic bacteria is a specific goal for animal nutritionists and many concepts have been developed, for example, *Salmonella* reduction in pigs using particle size and feed processing differences (Mikkelsen *et al*., 2004). Many studies in both animals and humans have demonstrated that the GI tract communicates with the endogenous microbiome that supports digestion, and that the GI tract plays a major role in regulating epithelial and immune functions of vital importance for normal biological functioning and homeostasis in both the GI tract and the body in general. The association between the enteric nervous system (ENS) and the higher centres via the parasympathetic nervous system and (or) endocrine system also plays a key role in animal well-being, health, and structure and function of the GI tract. For example, a study in germ-free mice reported that the GI tract microbiome directly influenced not only GI tract functions but also the development of behaviour and corresponding neurochemical changes in the brain (Neufeld *et al*., 2011). The precise mechanisms of how the GI tract individual microbiome contributes to gut health, however, are less clear, as are the likely ramifications for nutrition, feeding and (or) feed processing in the pig and poultry industries.

4.3 Underlying biological mechanisms associated with a healthy gastrointestinal tract

The GI tract of a pig or chicken is a very complex, dynamic and ever-changing organ, with for example the GI tract of young pigs at weaning undergoing rapid changes in size, protein turnover rates, microbiome mass and composition, and quick and marked alterations in digestive, absorptive, barrier and immune functions (e.g. Hampson, 1986a,b; Cranwell, 1995; Pluske *et al*., 1996a,b, 1997, 2003; Boudry *et al*., 2004; Lallès *et al*., 2004; Pluske, 2013, 2016). Similar changes occur in the chicken post-hatch (Nitsan *et al*., 1991; Wijtten *et al*., 2012). Whilst there has been a very large body of research conducted in increasing understanding of the various factors and influences on morphological, anatomical, enzymatic and immunological changes occurring at key stages during the development of a pig or chicken, less emphasis has been placed on more functional characteristics of the GI tract in regard to gut health [and arguably the management of the GI tract at critical life stages and (or)

during critical production impositions] and how this may be affected, for example by nutrition and feeding (Choct, 2009). Dietary factors that modulate the immune system and gut microbiota should therefore be considered when formulating diets and managing feeding practices.

Bischoff (2011) commented that two functional entities key to achieving a healthy GI tract ecosystem are the GI tract microbiome (e.g. Clemente *et al*., 2012) and the function of the GI tract barrier (e.g. Camilleri *et al*., 2012), and the interaction between the two. In pig and poultry nutrition, our attention, understanding and appreciation of these two factors has increased considerably in the last two decades due predominately to changes in the respective industries aimed at reducing reliance on the use of AMC, especially antibiotic growth promoters (AGP; substances that affect intestinal bacteria and digestive function that are administered at a low, sub-therapeutic dose) and (or) minerals such as zinc and copper. Banning AGP use in food animals is intended to reduce pools of resistance genes, with the risk being that resistance genes can disseminate via the food chain into the intestinal microbiome of humans and compromise the use of antibiotics for human disease control (Cogliani *et al*., 2011). In 1986, the use of AGP was discontinued in Sweden, and in Denmark, avoparcin use was banned in 1995 and virginiamycin in 1998, with a comprehensive ban on AGP implemented by 2000. In 1997, the European Union (EU) banned avoparcin for all uses in agriculture. In 1999, EU officials discontinued further use of AGP from drug classes also used in human medicine, imposing a ban on tylosin, spiramycin, virginiamycin, and bacitracin. Other antimicrobials were phased out in the EU in 2006. Bans/restrictions on the use of AMC have occurred in other parts of the world also, e.g. Taiwan and South Korea, with numerous other countries having semi-restricted use of AGP (Maron *et al*., 2013). In contrast, there has been relatively little regulatory activity regarding AGP use in the United States (Dibner and Richards, 2005), although from 2017, the introduction of the Veterinary Feed Directive made the use of many feed-grade antibiotics for growth promotion and increased feed efficiency illegal. Antibiotics that contain ingredients closely linked to human medicine, like penicillin and tetracycline, now require a Veterinary Feed Directive prior to their use. Drugs like cephalosporins and fluoroquinolones are considered as important reserve antibiotics as well and require a Veterinary Feed Directive, and are only available for uses of prevention and treatment of illness.

It is not our intention to discuss the various arguments posed for and against such bans/restrictions on AMC and (or) minerals, nor to discuss the many and varied purported modes of action of AGP (see reviews in pigs and poultry by for example Anderson *et al*., 1999; Gaskins *et al*., 2002; Dibner and Richards, 2005; Huyghebaert *et al*., 2011; Sugiharto, 2016), but merely to highlight that such changes have caused a marked shift in the nature and volume of the research being conducted in pigs and poultry pertaining to gut health. In this regard, discussion relating to the GI tract

microbiome and GI tract barrier function and the gut associated immune system is highly germane to this topic.

4.4 The gastrointestinal tract microbiome

There is a plethora of information available for both pigs and poultry relating to the enumeration, composition, temporal changes during growth and development (e.g. Thompson *et al*., 2008 in pigs, and Stanley *et al*., 2013 in poultry), and function of the GI tract microbiome, and more relevant for this chapter, their myriad interactions with nutrition and feeding (e.g. Gaskins, 2001; Pluske *et al*., 2002; Pluske *et al*., 2004; Yang *et al*., 2009; Zentek *et al*., 2013). The multiple functions of the GI tract microbiome have, therefore, been summarised extensively in previous publications, but in essence, the GI tract microbiome prevents colonisation by potentially pathogenic microorganisms, provides energy for the enterocytes (as well as for the indigenous bacteria) from ingested nutrients, and regulates the mucosal immune system, not only educating the naive infant immune system but also serving as an important source of immune stimulation throughout life (Bar-Shira and Friedman, 2006; Lewis *et al*., 2010). The GI microbiome hence contributes to energy homeostasis, helps to prevent localised mucosal infections, and likely mitigates immune system hyper-reactivity and allergic reactions (Gaskins, 2001; Pluske, 2008; Bischoff, 2011). Furthermore, and possibly most importantly, the GI tract microbiome contributes to the maintenance of an intact GI barrier, which intersects with infectious, inflammatory and (or) allergic/toxic challenges. In essence and as commented by Gaskins (2001), the word *détente* aptly describes the relationship between the host and its microbiome in the GI tract.

Bischoff (2011) stated that a normal GI microbiome of rich diversity, as well as an intact GI barrier that counteracts the bacteria and cooperates with the commensal microbiome, is needed to maintain gut health. This description, in our view, is overly simplistic given the deep complexity of interactions between the GI microbiome, the host, and what the animal consumes, because it is very difficult to characterise what is 'normal' with respect to the microbiome and how this might interrelate with GI tract health, and indeed the management of the GI tract. Any impairment of the GI microbiome, for example, by administration of antibiotics (Collier *et al*., 2003) or by feeding different substrates such as different fibre types (Lindberg, 2014), will influence the functionality of the host's local defence systems. Certain bacterial families (e.g. *Lachnospiraceae*), genera (e.g. *Faecalibacterium, Propionibacterium*, and *Ruminococcus*), or species (e.g. *Faecalibacterium prausnitzii, Bacteroides fragilis*, and some *Lactobacillus* spp.) have been reported to increase with AGP use, are associated with improved growth performance, and show benefit across species, which may be related to their production of short-chain fatty acids (Broom, 2018).

On the other hand, any malfunction of the epithelium, the immune cells or the ENS will likely influence microbial diversity and functionality. In particular, GI tract integrity (mediated in part by the GI tract's barrier functions) will be directly altered not only by GI tract-derived disturbances (such as increased epithelial permeability due to infection, or stress, or any loss of function of particular immune cells and their mediators; Moeser, 2013) but also by any systemic burden. In this respect, Hillman (2004) suggested that emphasis should be placed on an 'optimal' GI tract microbiome rather than a 'normal' microbiome being present, precisely because it is very difficult to define what is 'normal' given the wide array of conditions pigs and poultry are grown and kept under throughout the world. There is also growing evidence that animals have a very individual microbiome, similar to humans, which may help to explain different reactions to external stressors in a herd or flock.

A desired goal of studying the GI tract microbiome is a better understanding of how the microbial communities develop and are temporally altered during host growth, and develop and resist important intestinal pathogens that can potentially be attributed to the presence of specific beneficial microbial species, as well as by an earlier establishment of a more stable and diverse adult bacterial community. As a case in point, studies comparing indoor- and outdoor-reared sows and their piglets (e.g. Mulder *et al*., 2011; Schmidt *et al*., 2011; Lewis *et al*., 2012) have revealed exciting information and established that both the pre-farrowing and post-farrowing environment in which pigs are raised, as well as the use of antibiotics, can have a profound influence on the composition and enumeration of the microbiome and the expression (up-regulation, down-regulation) of genes along the GI tract. These findings support other studies demonstrating that the composition of the microbiome is strongly influenced by the mother or the environment (Pieper *et al*., 2012; Starke *et al*., 2013; Pieper *et al*., 2014). Moreover, and apart from the composition of the intestinal microbiome, it is also of interest to consider the metabolic activity of intestinal bacteria. It has been shown for instance that the protein supply and the supply of fermentable carbohydrates have a lasting impact on the spectrum of microbial metabolites in the GI tract. High protein diets can lead to negative effects on gut health, for example, by excessive ammonia or histamine production in pigs (Pieper *et al*., 2012) or, especially animal proteins, in poultry (Kaldhusdal *et al.,* 2016, Prescott *et al.,* 2016).

Lessons gleaned from such studies of the microbiome point towards further and rational justification for the selection and use of probiotics, an additive capable of influencing gut health in pigs and poultry, for future application in the pig and poultry industries. In this regard, it seems necessary to consider probiotics on a case-by-case basis as the impact on gut health varies considerably between different strains and under different conditions (e.g. Szabo *et al*., 2009; Mafamane *et al*., 2011; Scharek-Tedin *et al*., 2013; Stensland and Pluske, 2017). In the future, further refinement and more rapid (diagnostic) means to assess the GI microbiome composition and its functionality will allow not only greater description and clarity concerning the multiple

relationships between the GI tract microbiome and gut health, but also a more robust and phenotypic assessment of the impacts of interventions (nutritional, management, health) intended to restore a healthy microbiome when appropriate. Indeed, recent methodological developments such as next generation sequencing allow for a detailed insight into the diversity of the intestinal microbiome.

4.4.1 Diseases of the gastrointestinal tract and effects on gut health

Pigs and poultry can succumb to a myriad of bacterial, protozoal and (or) viral pathogens that invariably cause mortality and (or) morbidity commensurate with economic loss and production penalty under commercial conditions of farming. Descriptions for enteric diseases in pigs for example can be found in Hopwood *et al*. (2005) and Pluske and Hampson (2009) and, in poultry, refer for example to Hafez (2011). In a review by Kim *et al*. (2013), the impacts of the systemic responses to sub-clinical and clinical infections on intestinal barrier function and growth in pigs were summarised where the negative impacts that can occur were enunciated. In this sense, Pastorelli *et al*. (2012) conducted a meta-analysis using 122 publications examining the effects of pathogen and disease/sanitary/feed-related environmental challenges on production responses, and reported that digestive bacterial infections, poor housing conditions, mycotoxicoses, parasitic infections and respiratory disease challenges reduced growth by 40, 16, 30, 8 and 25%, respectively. Even sub-clinical infection of the GI tract in the absence of clinical disease, in this case by *Lawsonia intracellularis*, was shown to diminish daily gain, feed intake and feed efficiency by 37%, 21% and 21%, respectively (Paradis *et al*., 2012). Studies display the adverse and sometimes long-lasting effects that a variety of pathogens, often considered as normal constituents of the microbiome of both pigs and poultry in the GI tract, can have on GI tract structure and function, to impact upon gut health.

4.4.2 Effects of bacterial endotoxins on gut health

The GI tract of both pigs and poultry is a large reservoir of both Gram-positive and Gram-negative bacteria, which may have the capacity to produce enterotoxins or in the case of Gram-negative bacteria, endotoxins (Mani *et al*., 2012). As alluded to above, the production efficiency of pigs and poultry in commercial settings is affected by viruses, live bacteria and dead bacteria that contain cell wall compounds such as lipopolysaccharides (LPS). The importance of the endotoxin LPS to pig and poultry production is that chronic activation of the immune system, through for example pathogenic overgrowth, can antagonise growth and performance because nutrients are partitioned toward production of cytokines, acute phase proteins, and other immune modulators rather than toward the anabolic processes that support protein synthesis (Johnson, 1997; Spurlock, 1997). Furthermore, LPS has been shown to activate heterophils and up-regulate pro-inflammatory cytokine and chemokine expression in

poultry (Kogut *et al*., 2005). The immune activation and associated inflammation make endotoxins an important factor that is commonly overlooked in livestock production (Mani *et al*., 2012), and therefore warrants mention in this chapter given its potential detrimental effects on gut health.

Endotoxins can stimulate localised or systemic inflammation via the activation of pattern recognition receptors including Toll-like receptor (TLR)-4 and other proteins including LPS binding protein, cluster of differentiation 14, and myeloid differential protein 2 (Mani *et al*., 2012). Additionally, endotoxicosis and inflammation can negatively affect intestinal epithelial function by altering integrity, nutrient transport, and utilisation. Luminal endotoxins can enter the circulation via two routes: (1) nonspecific paracellular transport through epithelial cell tight junctions; and (2) transcellular transport through lipid raft membrane domains involving receptor-mediated endocytosis. Paracellular transport of endotoxins occurs through dissociation of tight junction protein complexes causing reduced intestinal barrier integrity, which can be a result of enteric disease, inflammation, and (or) environmental and metabolic stress. Transcellular transport, via specialised membrane regions rich in glycolipids, sphingolipids, cholesterol, and saturated fatty acids, is a result of raft recruitment of endotoxin-related signalling proteins leading to endotoxin signalling and endocytosis. Both transport routes and sensitivity to endotoxins may be altered by diet and environmental and metabolic stressors. Intestinal-derived endotoxaemia and inflammatory responses can cause suppressed appetite, activation of the immune system, and partitioning of energy and nutrients away from growth toward supporting the immune system requirements (Figure 4.1; from Mani *et al*., 2012).

More specifically and in pigs, Aschenbach *et al.* (2003) reported that in pigs given or not given LPS (30 mg/day of *Salmonella* Typhimurium DT-104 orally for 14 days), endotoxin feeding increased plasma C-reactive protein and histamine levels (but without evoking clinical signs), decreased colonic ion conductance, increased bacterial translocation to proximal jejunal lymph nodes, and generally elicited an acute phase response and affected intestinal electrolyte transport and mast cell function. Earlier, Schrauwen *et al.* (1988) infused intravenously live *Escherichia coli* (7×10^8/kg), the equivalent amount of endotoxin (20 μg/kg), or a high dose of endotoxin (2.5 mg/kg), and studied haemodynamic, clinical and pathological parameters and survival rate. Infusion with *E. coli* and endotoxin caused pulmonary hypertension, systemic arterial hypotension, a decrease in cardiac output and an increase in heart rate. Clinical signs were characterised by respiratory and nervous disturbances, whereas necropsy revealed haemorrhages and oedema in several organs. Infusion of *E. coli* or the high dose of endotoxin caused a significant mortality whereas all pigs survived infusion of the low dose of endotoxin, suggesting that lethal pathophysiological mechanisms may only become activated when a sufficient amount of endotoxin is released into the circulation. The implications of such findings, therefore, for GI tract structure and function may be significant especially under a considerable *Enterobacteriaceae* challenge of the GI tract.

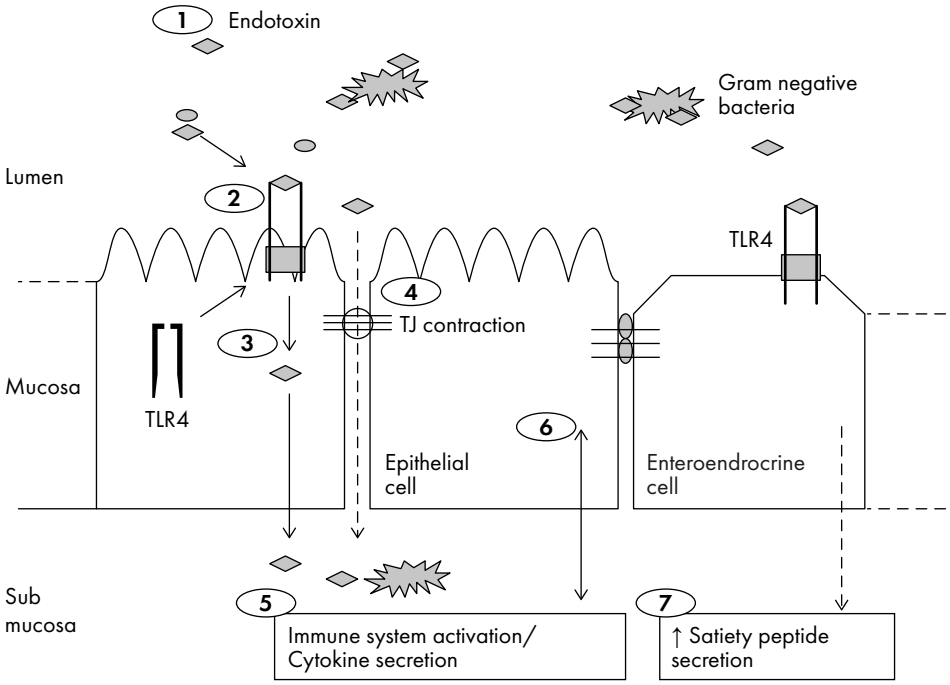

Figure 4.1. A summary of the effects of intestinal endotoxin transport and inflammation on gut integrity and function. Gram-negative bacteria in the intestine release endotoxin during growth, division, and death (1). Endotoxin may be free or bound to proteins such as lipopolysaccharide binding protein in the lumen. Recruitment of Toll-like receptor 4 (TLR4) and associated proteins to membrane lipid raft regions allows receptor-mediated endocytosis of bacteria and endotoxin in cells (2). Intracellular endotoxin may be transported bound to organelles (i.e. golgi) or albumin proteins in the cytosol (3). Opening of tight junctions (TJ) and increased paracellular transport of endotoxin (4) can occur because of intestinal inflammation or stress. Increased proinflammatory cytokine secretion and activation of innate and adaptive immune cells and intestinal inflammation occurs from endotoxin transported across the intestinal barrier (5). Secreted cytokines may enter the intestinal epithelial cells through the basolateral side, resulting in increased inflammation and the activation of myosin light chain kinase and phosphorylate-myosin light chain. Together, this causes the disruption of TJ complexes (6) and increased paracellular endotoxin transport. After sensing of endotoxin via TLR4, suppression of nutrient transport and enteroendocrine cell signalling (7) can reduce appetite via the depolarisation and secretion of appetite regulating neuropeptides such as cholecystokinin and glucagon-like peptide 1 (from Mani *et al.*, 2012).

In this regard, the effects of a short-term, high-dose intramuscular injection of *d*-α-tocopherol were studied in pigs given a challenge dose of LPS (Webel *et al.*, 1998). Pigs received either 0 or 600 mg *d*-α-tocopherol by intramuscular injection for 3 days before receiving an intraperitoneal injection of saline containing either 0 or 5 μg/kg body weight *E. coli* LPS. Plasma α-tocopherol levels were 13-fold greater ($P<0.01$) at time 0 in pigs pre-treated with 600 mg *d*-α-tocopherol (9.9±1.3 mg/l) than in those

not treated with *d*-α-tocopherol (0.74±0.09 mg/l). Injection of LPS increased ($P<0.05$) plasma levels of interleukin (IL)-6 and cortisol at 2-h post-injection, regardless of vitamin E treatment. However, pigs that received α-tocopherol before the LPS challenge had substantially lower ($P<0.05$) peak levels of IL-6 and cortisol than pigs not receiving α-tocopherol. These results suggest that supplementation with a surfeit level of vitamin E reduced the response of pigs to endotoxin. Kim and Pluske (2014) reported that supplementation of 200 IU vitamin E maintained plasma vitamin E levels >2 mg/l following enterotoxigenic *E. coli* (ETEC) infection, and enabled plasma vitamin E levels to return to pre-infection level by d 21 after weaning. Supplemental vitamin E also reduced the acute-phase infection response but had no effects on preventing/mitigating the inflammatory cascade via modulation of cyclooxygenase activity (i.e. no effect on prostaglandin E_2 production).

4.5 Gastrointestinal tract barrier function

Barrier function of the GI tract is inextricably linked to both the microbiome of the GI tract and mucosal immune function, and is under the influence of a range of external and internal factors. Examination of the underlying mechanisms and the various factors affecting GI tract barrier function in pigs and poultry is a relatively new phenomenon, even though the implications of damage to barrier function have been recognised for many years and mainly with respect to pathogenic challenge in the GI tract (see review by Kim *et al.*, 2012). Lallès *et al.* (2004), Kim *et al.* (2012, 2013), Campbell (2013) and Moeser (2013) in pigs, and Wu *et al.* (2013) and Broom (2018) in chickens, have provided reviews describing the mechanisms and impacts of various production factors, including nutrition and feeding, on GI tract barrier function. Wijtten *et al.* (2011) also reviewed intestinal barrier function and absorption in pigs after weaning and concluded that, based on the literature, the four major factors affecting barrier function were weaning age, weaning stress, feed intake and diet composition. In addition, barrier function is differentially affected after weaning in the proximal and mid jejunum compared to the ileum. These relationships after weaning are illustrated in Figure 4.2, although these interactions occur also in the colon. Based on their review, Wijtten *et al.* (2011) commented that three different approaches can be followed to improve GI tract barrier function after weaning in pigs by ways of dietary composition or manipulation: (1) improve the palatability of the diet to increase feed intake after weaning, which despite its relative simplicity as a management tool still remains a major issue and cause of production setback in the industry worldwide; (2) identify crucial nutrients (e.g. specific amino acids) that may be supplied to pigs with low feed intake in a concentrated form or through the drinking water to prevent the loss of barrier function; and (3) add specific biologically active components to the diet to modulate the stress response or the subsequent immune response, to prevent the loss of barrier function. With this last approach,

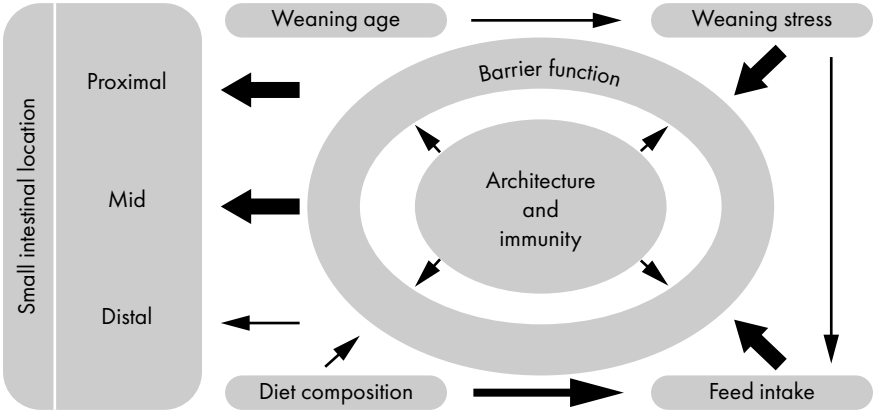

Figure 4.2. Schematic representation of the interactions between small intestinal barrier function, small-intestinal location and factors (age, stress, feed intake or diet composition) that affect the barrier function (from Wijtten *et al.*, 2011).

the authors stipulated that it is essential the diet should be eaten, otherwise the active component needs to be supplied through the drinking water.

4.5.1 Interactions between stress and barrier function to influence gut health

The 'little brain', the ENS, is a complex control and defence system that monitors luminal conditions via a host of sensory receptors, with that information then communicated to four major effector systems: the entero-endocrine hormonal signalling system; the innervation of the GI tract, both intrinsic and extrinsic; the immune system of the GI tract; and the local tissue defence system (Furness *et al.*, 2013). Additionally, conditions are monitored by primary afferent neurons activated by secretagogues from enterochromaffin cells or mast cells, such as biogenic amines (serotonin and histamine) or proteases (Bischoff, 2011). The ENS is independent from the central nervous system yet regulates (or contributes to the regulation of) almost all major functions of the GI tract, such as epithelial secretion, absorption and permeability, immune functions, and the GI microbiome (Collins and Bercik, 2009). Studies suggest that luminal conditions and signals, as well as the GI tract microbiome, are integrated into a gut-brain axis that responds to both local and external stimuli. For example, Sudo *et al.* (2004) reported that the stress-induced adrenocorticotropin hormone response in animals is significantly more pronounced in germ-free mice than in colonised animals.

In pig production, a major insult of both the central nervous system and ENS occurs at the time of weaning where multiple stressors are abruptly and simultaneously imposed

(Funderburke and Seerley, 1990; Pluske *et al*., 1997). Although stress at weaning, particularly at younger ages, has long been recognised as a major impediment to vitality, health and improved production in the immediate post-weaning period, it has only been relatively recently that the underlying physiology contributing to comprised GI tract barrier function has been elucidated. In pigs subject to stressful events such as weaning, Moeser and colleagues (summarised in Moeser *et al*., 2017) have shown that GI tract barrier function is compromised concomitant with elevated levels of stress-related hormones (cortisol, corticotropin releasing factor), increased mucosal expression of corticotropin releasing factor (Figure 4.3), and aberrant mast cell physiology. Furthermore, these data have shown that a stressful event early in life can have deleterious consequences for GI tract structure and function at latter stages of the production cycle, collectively indicating that stress-signalling pathways activated by weaning, particularly at younger ages, mediate both short-term and

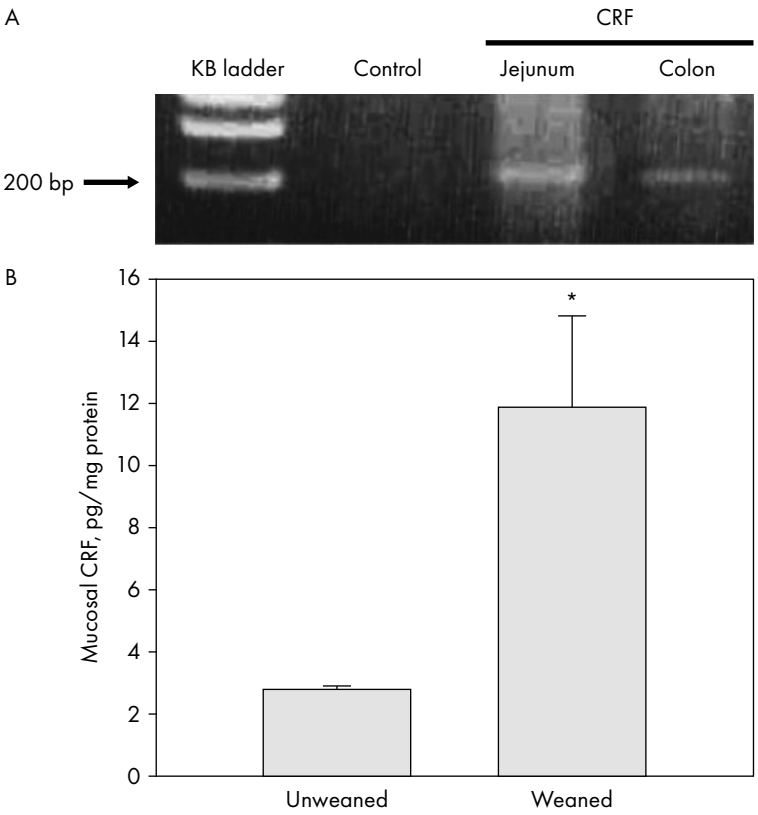

Figure 4.3. Expression of corticotropin releasing factor (CRF) in the weaned pig intestinal mucosa. (A) mRNA encoding CRF was detected in the porcine jejunum (lane 2) and colonic mucosa (lane 3). The control sample (lane 1) contained RLT buffer only. (B) CRF ELISA experiments showed a significant increase (*P<0.05) in CRF levels in the weaned colonic mucosa compared with unweaned tissues (from Moeser *et al*., 2007).

longer-term intestinal dysfunction in the pig. In a broader context, this may help to, at least partly, explain the observations of Main *et al.* (2004) showing that in a multisite production system, increasing weaning age from 12 to 21.5 days reduced grow-finish pig mortality and increased weight sold per pig weaned by 1.80±0.12 kg for each day increase in weaning age. Moreover, and from a behavioural perspective, belly-nosing behaviour and umbilical lesions were less frequent ($P<0.05$) as weaning age increased and although the incidence of belly-nosing behaviour gradually decreased as weaning age increased to 21 days, nosing activity and umbilical lesion scores nearly doubled as weaning age decreased from 15 to 12 days of age (Figure 4.4; Main *et al.*, 2005).

The term gut health in pig production is sometimes thought of only in the context of the post-weaning scenario, where most negative impacts on GI tract structure and function are likely to occur. Consequently, the feed industry has directed the majority of its efforts to assist in ameliorating the post-weaning 'growth check' during this time. However, it is evident that stress can occur at different stages of the pork production cycle, for example mixing and (or) moving pigs between the nursery, grower and (or) finisher stages (Davis *et al.*, 2006), out-of-feed events (Brumm *et*

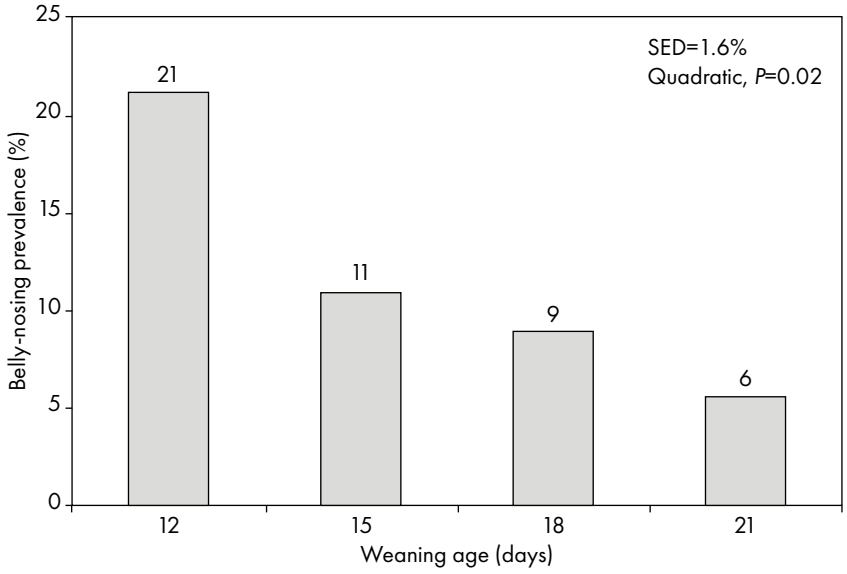

Figure 4.4. The effects of weaning age (12, 15, 18, or 21 days; 2,272 pigs in 64 pens) on the prevalence of belly-nosing behaviour in the nursery of a commercial multi-site production system. The study was conducted in four randomised complete blocks, with all pigs within each block weaned on a single day into the same off-site nursery and four replicate pens per weaning-age treatment per block. Prevalence was calculated as the mean percentage of the population in a pen observed demonstrating sustained belly-nosing activity for ≥10 seconds during a 15-minute observation period, as measured on days 7, 14, and 21 post-weaning (from Main *et al.*, 2005).

al., 2008), transportation (Saco *et al.*, 2003; Sutherland *et al.*, 2014), and aggression associated with group housing of sows (Arey and Edwards, 1998). If feed intake is limited during these events, such as an out-of-feed event, then the absence of adequate luminal nutrition (Diamond and Karasov, 1983) will likely exacerbate the stress-induced changes to GI tract structure and function (e.g. barrier function) causing an even greater detrimental effect on gut health.

Gut barrier function also has important impacts on broiler and layer health and several nutrients including amino acids and vitamins have been identified to affect barrier function of the GI tract (Moran, 2017). An increasingly important issue for the broiler industry is mycotoxin-related changes of intestinal barrier function, for instance by deoxynivalenol, a *Fusarium* toxin belonging to the trichothecenes, that inhibits protein synthesis and can be found in many feedstuffs (Awad and Zentek, 2015).

4.5.2 Effects of heat stress on gut health

A more insidious form of stress that impacts negatively on the GI tract in pigs and poultry that can go undetected is heat stress (HS). Pigs and poultry are generally more sensitive to phases of hot weather than other livestock species because they do not sweat and their lungs are relatively small compared to their body size. In addition, a review of pig heat and moisture production by Brown-Brandl *et al.* (2003) suggested that new genetic lines of pigs produce nearly 20% more heat than their counterparts of the early 1980s. Prolonged HS can lead to excessive water absorption that causes changes in electrolytes and the acid-base balance, resulting in possible diarrhoea. It is recognised that HS influences feed intake, body weight, physiology and cellular immune function (Brown-Brandl *et al.*, 2003) and, in some instances, pigs may die from heart failure when environmental temperature rises above 30 °C and body temperature increases to 43 °C. St-Pierre *et al.* (2003) documented that the economic cost of HS in the US swine industry was approximately $300 million, however, the more chronic impacts of HS on GI tract and gut health, and dietary methods to mitigate the problem, are less well known and understood. The following section discusses aspects of GI tract structure and function and HS in different classes of pigs, although it is well recognised that similar impacts can occur in poultry, especially with a pathogenic challenge (Quinteiro-Filho *et al.*, 2012).

Work by Pearce *et al.* (2012), who examined how acute HS alters growing pig intestinal integrity and metabolism, demonstrated that pigs exposed to HS (35 °C, 24-43% humidity, for 24 h) had expected increases in rectal temperature (40.9 vs 39.3 °C; $P<0.01$) and respiration rate (119 vs 52 breaths per minute; $P<0.05$) relative to thermoneutral (TN) control pigs (21 °C, 35-50% humidity, for 24 h). Physiologically and compared to TN pigs, mucosal heat shock protein 70 increased in HS pigs (101%; $P<0.05$) concomitant with compromised intestinal integrity (as measured by

transepithelial resistance) in the ileum and colon, which decreased by 52 and 24%, respectively ($P<0.05$). Furthermore, serum endotoxin concentrations increased 200% due to HS ($P=0.05$) and intestinal glucose transport and blood glucose were elevated due to HS ($P<0.05$). In a subsequent study, only 2-6 h of HS was required to elicit these negative physiological effects on gut health (Pearce *et al*., 2014).

In gilts, Boddicker *et al*. (2014) reported that the offspring from dams subjected to HS during the first half of gestation had a smaller *longissimus dorsi* cross-sectional area following chronic postnatal HS compared to those offspring from dams gestated in TN conditions during the first half of gestation. Additionally, pigs from dams heat-stressed for the first half of gestation had increased subcutaneous fat thickness and circulating insulin concentrations compared to pigs produced from sows exposed to TN conditions for the first half of gestation. Moreover, postnatal feed intake was altered by gestational treatment and positively correlated to subcutaneous fat thickness. These phenotypic changes occurred as a direct result of environmental HS as maternal nutrient intake (during gestation) was similar between thermal environments, suggesting that programming of piglets may occur *in utero* during the first half of gestation resulting in an altered metabolic hormone profile and body composition during subsequent growth and development.

In conjunction with the findings in growing pigs discussed previously, such a report has potentially enormous ramifications for the ways that pigs are managed during periods of hot weather. In this regard, and in terms of how HS may be managed nutritionally, it is recognised for example that dietary Zn improves a variety of bowel diseases and conditions. Given the markedly compromised GI tract that occurs with only a short duration of HS, a study from the same group at Iowa State University (Sans Fernandez *et al*., 2014) evaluated the effects of supplemental Zn amino acid complex (ZnAA) on intestinal integrity in heat-stressed growing pigs. Crossbred gilts were *ad libitum*-fed one of three diets: (1) control (ZnC; 120 mg/kg Zn as $ZnSO_4$), (2) control+100 mg/kg Zn as ZnAA (Zn220; containing a total of 220 mg/kg Zn), and (3) control+200 mg/kg Zn as ZnAA (Zn320; containing a total of 320 mg/kg Zn). After 25 days on their respective diets, all pigs were exposed to constant HS conditions (36 °C, ~50% humidity) for either 1 or 7 days. As anticipated, HS increased rectal temperature ($P<0.01$; 40.23 vs 38.93 °C) and respiratory rate ($P<0.01$; 113 vs 36 bpm). Pigs receiving ZnAA tended to have increased rectal temperature ($P=0.07$; +0.27 °C) compared with ZnC-fed pigs. The HS markedly reduced feed intake ($P<0.01$; 59%) and caused body weight loss (2.10 kg), but neither variable was affected by dietary treatment. As HS progressed from days 1 to 7 both ileal and colonic transepithelial resistance decreased ($P<0.05$; 34 and 22%, respectively), which was mirrored by an increase in ileal and colonic permeability to the macromolecule dextran ($P<0.01$; 13- and 56-fold, respectively), and increased colonic LPS permeability ($P<0.05$; threefold) with time. There was a quadratic response ($P<0.05$) to increasing ZnAA on ileal transepithelial resistance, as it was improved ($P<0.05$; 56%) in Zn220-fed pigs

compared with ZnC. Consequently, supplementing ZnAA at an appropriate dose can improve aspects of small intestinal integrity during severe HS.

The effects of HS in broilers are well understood and also impact on aspects of gut health and production, and have been summarised in reviews by Cronje (2007), Sahin *et al.* (2009), and Lara and Rostagno (2013). Consistent with the aforementioned work in pigs with Zn, the review by Sahin *et al.* (2009) suggested that supplementation of Zn would also appear to be beneficial in broilers, although other nutritional modifications have also been suggested (e.g. Das *et al.*, 2011). Rhoads *et al.* (2013) commented that animals experiencing environmental hyperthermia exhibit a shift toward carbohydrate use coincident with increased circulating basal and stimulated plasma insulin concentrations. Limited data cited by the authors in a number of species, including dairy cows, indicate that proper insulin action is necessary to effectively mount a response to HS and minimise heat-induced damage. Hence, nutritional interventions that enhance insulin sensitivity may improve tolerance and productivity during periods of HS to improve the likelihood of surviving an otherwise lethal heat load. HS has also been described to have negative impacts on GI tract barrier function in chickens and may induce higher susceptibility to *Salmonella* invasion (Quinteiro-Filho *et al.,* 2012; Santos *et al.,* 2015).

4.6 Interaction between the mucosal immune system and the gastrointestinal tract

Discussion of the GI tract microbiome and barrier function and their roles in determining gut health cannot occur without a description of the mucosal immune system. Extensive descriptions of the porcine and chicken mucosal immune systems have been published previously (e.g. Stokes *et al.*, 1994, 2001; Brisbin *et al.,* 2008; Burkey *et al.*, 2009), but nonetheless, the normal growth, development and function of the mucosal immune system is predicated on the basis of the permanent challenge of bacterial antigens (Gaskins, 2001; Kelly and King, 2001). It is, therefore, not surprising that the GI tract immune system contains cells capable of recognising bacterial antigens by specific receptors, such as T-cell receptors, and B cell-derived, surface-bound antibodies of the adaptive immune system, as well as TLR and other pattern recognition receptors of the innate immune system. Dendritic cells (via TLR), lymphocytes (via T-cell receptors and antibodies) and innate immune cells such as macrophages and mast cells (via TLR and other pattern recognition receptors) are also involved in communication between the GI tract microbiome and the GI tract immune system so that dangers from pathogens can be recognised and dealt with appropriately.

To support the animal against luminal bacteria and other potentially harmful substances, such as those that might be ingested, the GI tract immune system has an array of mechanisms including plasma cell-dependent immunoglobulin A, goblet cell-derived mucus production and the synthesis of antimicrobial peptides cells (Sang and Blecha, 2008). These mechanisms play a crucial role in regulating the composition and metabolism of the GI microbiome and protecting the host against invasion of luminal bacteria through the epithelium. Under normal conditions, these mechanisms also prevent direct contact between commensal bacteria and the GI epithelium (Swidsinki *et al*., 2007; Pluske *et al*., 2018b). Moreover, the GI immune system allows regulation of inflammatory responses to harmless antigens, such as food antigens or bacterial antigens derived from commensals, by mechanisms that together result in mucosal tolerance (Bischoff 2011). This illustrates the complex balance and orchestration of interactions between the GI microbiome and the GI immune system that protect the host and contribute to the maintenance of gut health in pigs and poultry.

4.7 Oxidative stress in pigs and poultry: impacts on 'gut health'

Reactive oxygen species (ROS) are chemical compounds that contain oxygen and are highly reactive because they have, or can be easily converted to, compounds that have unpaired electrons. Common ROS in biological systems include superoxide, hydroxyl radical, hydrogen peroxide, and fatty acid peroxides. The ROS are produced via normal oxidative metabolism and certain ROS are essential for cell signalling and other functions. However, and due to their reactive properties, concentrations of ROS must be controlled, and sophisticated physiological antioxidant systems have been developed by animals to keep ROS in check. Nevertheless, oxidative stress occurs when the antioxidant system is overwhelmed by the production of ROS that in turn can lead to increased prevalence of infectious diseases via impaired immune function, longer-term health disorders, and perhaps various sudden death syndromes (Weiss and Mahan, 2008). Under such conditions, for example the peri-weaning period, gut health is likely negatively affected.

Oxidative stress commonly occurs during an infection or other challenge to the immune system. Indeed, the massive production of ROS is essential to kill invading bacteria and trigger various immune responses. These ROS can also cause tissue damage and prolong the disease state; therefore, antioxidants are extremely important to certain types of immune cells (Weiss and Mahan, 2008). Naturally, nutritional efforts have been made to try and ameliorate such oxidative states, with a variety of nutritional interventions tested in pigs and poultry. In pigs, Lu *et al*. (2014) determined the effects of a dietary antioxidant blend (ethoxyquin and propyl gallate) and vitamin E on growth performance, liver function, and oxidative status in pigs fed diets high in oxidants,

and found that in the oxidative stress model used in this study (5% oxidised soybean oil and 10% polyunsaturated fatty acids source), dietary addition of the antioxidant blend or the antioxidant blend plus vitamin E was effective in improving growth, liver function, and plasma markers of oxidative stress, but vitamin E alone was not. There is also evidence that ZnO may reduce systemic oxidation and improve the antioxidant status in the jejunal and ileal mucosae after weaning (Bergeron *et al*., 2014), whilst Zhu *et al*. (2012) reported that an antioxidant blend fed after weaning (comprising 6.75 g/kg, including 200 mg vitamin C, 100 mg vitamin E, 450 mg tea polyphenols, 1 g lipoic acid, and 5 g microbial antioxidants fermented by *Bacillus*, *Lactobacillus*, photosynthetic bacteria, and beer yeast; microbial antioxidants were inactivated after fermentation) had the potential to prevent free radical-induced damage of the GI tract and suppress oxidative stress by modulating the expressions of tumour protein 53 and PGC-1α genes. Moreover, Gessner *et al*. (2013) showed that dietary supplementation of a polyphenol-rich grape seed and grape marc extract suppressed the activity of transcription factor nuclear factor kappa B in the duodenal mucosa of pigs and thus might provide a useful dietary strategy to inhibit inflammation in the GI tract that can occur. Feeding grape seed and grape marc extract did not influence vitamin E status and the antioxidant system of the pigs, however, but improved the gain:feed ratio. Finally, and as an alternative nutritional strategy, N-acetylcysteine (a precursor for the antioxidant glutathione that undergoes rapid metabolism within the small intestine to produce glutathione and aids neutralisation of ROS) was found to alleviate mucosal damage, improve absorptive function of the small intestine and maintain performance in *E. coli*-LPS-challenged pigs (Hou *et al*., 2012). Several conditions have also been associated with increased oxidative stress in the chicken GI tract, for instance exposure to the mycotoxin deoxynivalenol or selenium deficiency (Osselaere *et al.,* 2013, Yu *et al.,* 2015).

4.8 Future perspectives

The aims of this chapter were to identify actual topics related to gut health in pigs and poultry, and summarise the influence of dietary factors. Even if the goal to achieve a complete elimination of intestinal diseases appears over-ambitious and a complete ban of AMCs and (or) specific minerals (e.g. Cu and Zn) will not be possible under practical conditions, it has been shown by numerous studies in various animal species that an appropriate diet, a spectrum of zootechnical feed additives and other technological factors can have lasting positive effects on GI tract health, with benefits systemically. Future research efforts should focus on the rapid and comprehensive diagnosis of gut health and on the mechanistic description of the impact of specific dietary factors.

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5

Animal nutrition and immunity in pigs and poultry

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Summary points

- Mucosal tolerance is important for elimination of pathogens in the absence of an epithelium-damaging inflammatory response.
- Regulatory T-cells in the mucosa dampen or suppress antigen-specific T-cells and, thereby, damaging or protective immune responses.
- Dendritic cells regulate tolerance or immune activation by the induction and expansion of regulatory T cells.
- Homing of gut-activated immune cells can occur within the common mucosal immune system towards the upper airways, thereby, providing protection against infections.
- Nutritional compounds can exert immunomodulatory activity by influencing mucosal macrophages and dendritic cells.
- Nutritional compounds can exert innate immune training and thereby induce enhanced innate immune responses and cross-reactive activity.
- Vitamin A and its metabolite retinoic acid have potent immunoregulatory activities in the mucosal immune system by steering the production of immunoglobuline A antibodies and regulatory T cells.
- Priming of the immune system in the mother to enhanced immune activity can be (epigenetically) transferred to the offspring providing these with better immune protection.
- Immunomodulation by dietary components is a feasible option to steer immune competence and improved resistance.

Keywords: mucosal immunology, transgenerational, immune competence, gut and airways, animal feeds, nutrition, vaccination

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5.1 Introduction

In recent years, it has become well established that the microbial communities in the gut of animals and birds interact with host physiology through a variety of mechanisms. Interactions with immune and nutritional signalling are amongst these. Managing 'gut health' is, therefore, an important part of managing overall health and productivity of the whole animal. Optimising gut health is also of paramount importance to reduce therapeutic use of antibiotics and thus prevent antibiotic resistance. By using an interdisciplinary approach, it should be possible to further identify mechanisms underlying health and welfare. Associations between gut microbiota, nutrients, mechanics of immunity, and increased resistance of animals to pathogens merit exploration (Satyaraj *et al.,* 2011). With the identification of specific and sensitive biomarkers, we can objectively evaluate the impact of external factors, related to specific gut diseases, environment and climate, on animal health, productivity and welfare.

The gastro-intestinal tract (GIT) harbours in general the majority of immune cells in the body, with about 75% in humans whereas these figures are unknown in pigs and poultry. Gastro-intestinal epithelial cells are constantly monitoring the composition of the digesta in the gut and communicate with the underlying immune cells with intestinal microbes having a strong impact on this crosstalk. There is convincing evidence that intestinal microbes influence host immune development, immune responses, and susceptibility to intestinal diseases. Conversely, host factors affect the composition and metabolic activity of microbes, which in turn modulate disease susceptibility. Consequently, there is an intimate interaction in the GIT of animals between host epithelial cells (= host genotype), the residing microbes, and the animal feed. The innate immune system is responsible for early recognition of pathogens and pathobionts and for driving the innate and adaptive immune system in the required direction. The most dominant organ where immune development and immune recognition occurs is the GIT.

The pre- and perinatal environment that animals are exposed to, including the nutritional state of the mother, has large effects on performance, health and welfare of animals in later life (Palmer, 2011). Correspondingly, early nutrition seems to have long-term effects on the development of the immune system. The microbiota that colonise the GIT during the neonatal period play a crucial role in shaping the immune system and determining the immune competence of the animal, which, in turn, determines immune responses and immune tolerance later in life. The neonatal period is, therefore, crucial for both local and systemic innate and adaptive immune responses and consequently immune competence, later in life (Hansen *et al.,* 2012).

5.2 Immunity

5.2.1 Basic aspects of the immune system

The immune system provides a multi-layered defence with increasing specificity against pathogens ensuring health in humans and productive animals alike. Physical barriers including skin and membranes in the respiratory and GIT constitute the first barriers, and effectively prevent most pathogens from entering the organism (Isabelle and Oswald, 2006). However, if a pathogen manages to breach these barriers, which most readily occurs at the gut and respiratory surfaces, the innate immune system provides an immediate response. When pathogens are successfully able to evade this protection, vertebrates possess a third layer of protection, the adaptive immune system. The latter is activated by the innate response, has a profound amplification mechanism, but requires some time to evolve. Upon infection, the immune system develops long lasting and specific immunological memory of the invading organism. This allows for a more rapid and stronger recognition if an identical invasion occurs and results in a more efficient immune protection each time the specific pathogen is encountered (Fukuyama *et al.,* 2012), thereby reducing the productive consequences of such a future infection.

Also in all livestock species, such immune barriers are present and generally protect against pathological attacks effectively. This is especially required in the GIT, which is a dominant entry point for pathogens, toxins and other (dietary) antigens (Blecha *et al.,* 2001). A continuously produced mucus layer and a rapidly regenerating epithelial layer (once every 2-3 days in pigs and 3 days in chicken) provide further protection against such foreign materials (Uni *et al.,* 2001; Williams *et al.,* 2015). Underneath this mucus-covered epithelial cell layer is the mucosal immune system, which protects against infection, prevents the uptake of antigens, microorganisms, and other foreign materials, and coordinates the organism's immune response to this material (Kelly and Mulder, 2012). This mucosal immune system is highly specialised and is composed of innate and adaptive cells that are accumulated in, or in transit between, various mucosa-associated lymphoid tissues (MALT).

5.2.2 Mucosal immune system and tolerance induction

The mucosal immune system consists of all mucous membranes covering the aero-digestive tract (nasal cavity, oral cavity, airways and GIT) as well the urogenital tract, eye conjunctiva, inner ears, and ducts of all exocrine glands. The MALT is the largest mammalian lymphoid organ system and encompasses different lymphoid compartments where immune responses are initiated, such as the Peyers patches, mesenteric lymph node, solitary follicles in the intestine and the tonsils (Bailey, 2009). Each mucosal tissue has its own associated lymphoid tissue resulting in NALT

(nasal cavity), BALT (bronchus/lower airways), GENALT (urogenital tract) and GALT (GIT). The main function of the mucosal immune system is to protect against colonisation and invasion of air- or foodborne pathogens by immune exclusion. Consequently, while confronted with a large amounts of digesta, the healthy GALT must economically select appropriate effector mechanisms and regulate their intensity to prevent uptake of foreign antigens and avoid induction of tissue damage and immunological exhaustion (Holmgren *et al.*, 2005).

Similar to humans, also pigs have a Waldeyer's tonsillar ring, a ringed array of lymphoid tissue in the oropharynx (throat), that is the prime place were food components and pathogens can interact, before they are (partially) digested in the stomach and intestines. The palatine tonsils in the oropharynx are part of Waldeyer's ring, which includes the nasopharyngeal tonsil (adenoid), paired tubal tonsils and lingual tonsil as well. In the crypts, epithelium lymphocytes can be found, as well as dendritic cells (DCs) and macrophages (Perry and Whyte, 1998; Brandtzaeg, 2011).

The intestine is the largest immune organ in the body and actively responds to potentially harmful pathogens and antigens, while creating and maintaining tolerance (unresponsiveness) to harmless antigens, beneficial commensals and symbiotic microorganisms. The gastrointestinal mucosal barrier combines a physical barrier with the production of transmembrane Toll-like receptors (TLRs, recognising structurally conserved microbial molecules) and cytoplasmic nucleotide binding oligomerisation domains receptors as members of the innate immune system, in addition to the production of anti-microbial factors including defensins and cathelicidins (Kelly and Mulder, 2012). TLRs act as pattern recognition receptors binding microbial ligands present in the gut lumen and determine the interaction with host-immune defence, immune cell recruitment, and induction of mucosal inflammation, in order to maintain intestinal homeostasis (Aderem and Ulevitch, 2000).

A typical feature of the mucosal immune system is its capacity to distinguish between inducing an immune effector response when needed, while at the same time developing a state of oral tolerance to harmless (commensal and dietary) antigens. Typically for the gut immune system, there is an intense interaction between host cells, commensal, and pathogenic bacteria, all of which can also have an impact on systemic immune responses. The local presence of defined DC subsets, together with the vitamin A metabolite retinoic acid, and the presence of regulatory T cells (Tregs), are crucial in regulating gut tolerance and homeostasis. Gut microbiota generate signals that direct intestinal responses with effector T-cells against pathogens or, in the case of commensals, induce a state of tolerance via modulation of Tregs and release of immunosuppressive cytokines like interleukin (IL)-10 and transforming growth factor-β.

It has become clear that soluble antigens such as proteins are able to induce tolerance when given orally, whereas antigens in the form of particles or as part of a live organism are more likely to provoke active immunity. Oral application of soluble proteins generally induces tolerance, whereas particulate antigens or live organisms mostly induce active immune responses and often results in damage to the host. Tolerance thus reflects the inability of antigen presentation and activation of the immune response, thereby preventing proper priming of lymphocytes. Whereas single high doses of protein antigen cause anergy and/or deletion of antigen-specific T cells, multiple feeds of lower doses were predicted to generate regulatory FOXP3+ T-cells. Indeed, it seems that most 'inducible' T cells in the steady-state small intestine may be specific for food antigens in mammals and also chicken (Lillehoj *et al.,* 1996; Mowat, 2003).

The GALT functions independently of the systemic immune system and includes the Peyer's patches, the mesenteric lymph nodes and solitary follicles, which serve as the principal mucosal inductive sites for the initiation of immune responses. T cells, B cells, and accessory cell subpopulations populate the GALT. The gut-associated immune system is extremely dynamic and evolves with the various physiological stages of the animal. At birth (or hatching), the neonate's mucosal immune system is relatively undeveloped. A rapid development of the GALT occurs concomitantly with the development of digestive structures and functions. Nutrient supply and colonisation of intestinal microbiota accelerates development, resulting in a functional immune system shortly after birth or hatching. The GIT associated immune system activity and requirements vary therefore with a number of conditions of the host animal. These conditions are primarily: (1) stress associated with high levels of production; (2) weaning or hatching; (3) parturition; (4) reduced feed intake; and (5) sub-clinical disease status. Under these conditions or crucial stages in the animals life, it is essential to assure a nutrient supply that is in line with maximising GALT function and stimulating overall immunity if productivity and well-being of animals is to be optimised.

5.2.3 Developing immune system in young livestock animals

Using livestock animals, including chickens and pigs, under healthy, sustainable and responsible welfare conditions requires an integrated understanding of the complex interactions between nutrition, commensal microbiota of the GIT and mucosal immunity, and the consequences for production efficiency and animal health. This is especially important in young animals, as perturbations in these complex regulatory functions of the developing immune system during early life can have consequences for the development of chronic inflammatory conditions and overall health throughout life. Although the immune system is qualitatively complete at birth, exposures at a young age are essential for optimal priming and expansion of adaptive cell populations. This implies that during development animals go through several

critical windows for the immune system to attain proper functioning. These critical periods of development are highly vulnerable to insults, which may permanently alter the immune defence capacity. Due to the significant delays in the maturation of specific parts of these defences, a time window occurs for the development of tolerance, particularly to feed antigens, and to develop protection of immature tissues and organs from potentially harmful inflammatory actions. Maternal immune competence as reflected in the transfer of factors via the placenta and in colostrum and breast milk is relevant for continued protection of the young animal after birth.

During the gestation, the immune system of the foetus is generated and gradually acquires its function. The placenta does not only provide nutrients for the foetus, but also hosts a placental microbiome, which is hypothesised to have a function in the induction of tolerance to commensal bacteria by the developing pig foetus (Lecours *et al.,* 2011). This latter function is supported by an intra-uterine immune-suppressive environment, in which the placenta is preventing exposure of the foetus to pro-inflammatory immune responses. At birth, many immune cells are already present, but their responsiveness to pathogens is less profound compared to adults (Aura *et al.,* 2013). For example, TLR-induced anti-viral responses of plasmacytoid DCs are reduced at birth in pigs, but develop to a full response within weeks after birth (Jamin *et al.,* 2006). In addition, it takes several weeks after birth before B and T cell areas are formed in the BALT. Therefore, young animals, including piglets, are more vulnerable to infections and need additional protection (e.g. by maternal antibodies), which is provided by colostrum and milk upon suckling. In addition, young animals have an increased anti-inflammatory status (e.g. increased levels of IL-10), which suppresses immune responses (Johansson *et al.,* 2003). Likewise, similarities in the development of the immune system in young chickens has also been described, although there is still more to be discovered.

5.3 Immunomodulation by feed components

5.3.1 Nutrition and immune competence

In livestock production, the focus has shifted towards prevention, as it was realised that improved management and nutrition could reduce disease mortality and morbidity. At the same time, these husbandry measures affect the activity of the immune system and, thereby, can provide alternatives to the therapeutic use of antibiotics. Infectious diseases greatly impair animal welfare and efficiency of nutrient use and thus the environmental footprint of animal production. Nutrition may aid in minimising the incidence of the diseases by enhancing immune competence. Appropriate nutrition becomes even more critical as antibacterial, anti-parasite, and other additives that promote animal health, are eliminated due to consumer demands. Immune

competence is defined as the ability of the immune system to respond adequately to an antigenic stimulus by an appropriate immune response with a balance between tolerance and inflammation. It is, therefore, crucial to understand the underlying mechanisms of relevance in dietary immunomodulation.

The amount and composition of feeds provided to production animals throughout their production cycle vary, which impacts on both production and immune competence in different ways (Dawson *et al.,* 2017). In the GIT, a highly activated immune system ensures protective functions, but at the same time reduces production potential, as it requires nutrients and energy. Therefore, infections will affect the nutritional status and dietary requirements of the individual animal (Ulfman *et al.,* 2018). This energy requirement may compromise growth; the reduced feed intake will preserve homeostasis by decreasing endogenous losses, and reduces nutrient supply to potentially harmful bacteria.

During infections in the GIT, the activated immune response produces pro-inflammatory cytokines affecting hypothalamic neural structures, which regulate appetite and satiety. This hypothalamic activation reduces feed intake and the resulting reduction in energy intake reduces stress and associates with a reduction of the systemic immune responses, despite enhancing immune competence. Thus, diets need to be optimised at the level of nutrient density to regulate convincingly both immune competence and performance in a highly dynamic manner.

5.3.2 Macrophages as targets for dietary immunomodulation

Protein feeding differentially affects maturation of the innate and adaptive arms of the immune system. Macrophages are critically important in the immunomodulatory activity of feed components (Rowlands *et al.,* 2011). Also, downregulation of macrophage-mediated immunity, activation of aryl hydrocarbon receptor (AHR), and upregulation of heat-shock protein (HSP)70 chaperone gene expression show that protein ingestion modulates aspects of the gut-associated immune response, protection of cell proteins from stress (Kregel, 2002) and maintenance of integrity of the gut associated epithelial barrier (Kim *et al.,* 2014; Gross *et al.,* 2015). In addition, the composition and activity of the gut microbiota should be evaluated, since these influence feed effects and resulting gut immune responses. During microbial fermentation of proteins and other dietary constituents, critical metabolites are formed that have the capacity to modulate the immune competence of the animal (Viladomiua *et al.,* 2013; Liu *et al.,* 2015).

Homeostasis and a healthy porcine intestinal tract largely depend on gut-associated macrophage subpopulations, the gut microbiota and expression of AHR and HSP (Van Eden *et al.,* 2005; Jin *et al.,* 2014). These are critical in the induction and maintenance

of gut mucosal tolerance, while the microbiota respond to feed effects and the resulting gut immune response by metabolites produced during fermentation of proteins and other dietary components. Healthy gut barrier function is secured by the chaperoning activities of HSP in relation to cytoskeleton and tight junction proteins, cellular stress resistance and cellular life span. Anti-inflammatory effects of HSP are mediated through nuclear factor kappa B inhibition at the level of macrophages and the induction of Treg (Wieten *et al.,* 2010). Moreover, negative effects of indigestible proteins as well as positive effects of (*in vitro*) selected constituents on this co-induction can be investigated to the benefit of health and production characteristics.

The AHR has a critical modulatory role in various innate and adaptive immune responses, and determines the influence of microbiota and/or diet on the immune system (Zhang *et al.,* 2015). The cascade of AHR-driven innate immune signalling is reflected in IL-1α and IL-23 stimulated T cell subsets that produce IL-22, which is another target of AHR transactivation. The AHR receptor binds structurally diverse compounds that include pharmaceuticals, phytochemicals such as flavonoids and endogenous biochemical host cell products, and bacterial metabolites (Nguyen *et al.,* 2013). Macrophage transcriptome profiling revealed that activation induced expression of several enzymes controlling tryptophan catabolism, including IDO1 and tryptophan 2,3-dioxygenase, which catalyse the rate-limiting step in the kynurenine pathway and produce ligands for the AHR (Memari *et al.,* 2015). AHR ligands exhibit both agonist and antagonist activities, and there is evidence that some compounds exhibit tissue and cell-specific agonist or antagonist activities by modulating inflammatory response genes in colon epithelial cells (Pocar *et al.,* 2006; Jablonska *et al.,* 2011). In macrophages and DCs, interaction with the AHR results in anti-inflammatory activity. Various proteins, such as several heat shock proteins (Tsuji *et al.,* 2014) may be ligands for AHR in the cytoplasm.

5.4 Dietary immunomodulation in pigs

5.4.1 Immunomodulation and mucosal infections

Young pigs are known to be vulnerable to the combination of respiratory diseases called the 'porcine respiratory disease complex' which includes porcine reproductive and respiratory syndrome virus, Influenza virus, *Mycoplasma hyopneumoniae*, and several opportunistic bacteria (e.g. *Pasteurella multocida*) (Wang *et al.,* 2007). It is, therefore, crucial to enhance the immunological protection at the mucosal surfaces in airways and GIT (Murtaugh, 2014). The upper airways are protected by a mucosal immune system that is partly connected to other mucosa of the body, which is referred to as the 'common mucosal immune system'. The specific immune defence at this mucosa is partly formed by the occurrence of immunoglobuline A antibodies as the

result of antigen stimulation at inductive sites in the mucosal immune system (Shikina *et al.,* 2004; Holmgren *et al.,* 2005).

Nutritional interventions can, therefore, affect the immune system in the gut and the upper airways in response to viral (e.g. porcine reproductive and respiratory syndrome virus) and bacterial (e.g. *Bordetella bronchispetica*) polymicrobial infections and, thereby, form a major health threat to the pig industry (Opriessnig *et al.,* 2011). Until recently, antibiotics were extensively used in pig industry to treat infectious respiratory diseases and to enhance the immune responses after vaccination. The increasing risk for antibiotic resistance has stimulated the interest in the use of immunomodulators like cytokines, pharmaceuticals, microbial products, traditional medicinal plants and nutraceuticals, the latter being a component of the food that provides medical or health benefits (Hardy *et al.,* 2000).

The gut and airways of pigs contain an elaborate and fiercely active mucosal immune system (Došen *et al.,* 2007; Holt *et al.,* 2008). The intestine actively responds to potentially harmful pathogens and antigens, while creating and maintaining tolerance (unresponsiveness) to other antigens and beneficial commensal and symbiotic microorganisms. The gastrointestinal mucosal barrier thus combines a physical barrier with the production of transmembrane TLRs that act as microbial pattern recognition receptors binding ligands from the gut lumen (Rakoff-Nakoum *et al.,* 2004). These TLRs determine the interaction of pathogens with host-immune defence, immune cell recruitment and mucosal inflammation. The intestinal immune barrier consists of a mucus layer, epithelium and mucosal lymphoid follicles and collectively, regulates the microbial ecosystem and provides intestinal homeostasis. Immune trafficking mainly occurs in the small intestine, peaks in the ileum, and occurs to a lesser extent in the colon. Abnormalities and incompleteness in the regulatory capacity of this local immune system hamper the development of sustainable health status and compromise production performance. Improved understanding of basic mechanisms underlying mucosal immune tolerance induction, microbiota interaction and dietary immunomodulation enhances immune competence and disease resistance in next generation animals.

5.4.2 Common mucosal immune system and homing

Antigen-presenting cells comprise both macrophages and DCs and these cells can be found in different types of MALTs of the mucosal immune system, as they are major sites of microbial exposure. These MALTs are anatomically and functionally distinct, but share traits such as organised inductive sites where antigen is presented to T cells via antigen-presenting cells. In mice and humans, immune cells were found to migrate to the organ of their origin or to distant mucosal sites, a phenomenon called 'homing' (Murtaugh, 2014). There is also evidence for the existence of a common mucosal immune system in the pig (McDermott and Bienenstock, 1979; Zuercher

et al., 2002) as it was for example demonstrated that oral administration of Cholera toxin could potentially induce protective immunity in the reproductive tract of pigs (Hyland *et al.,* 2004).

Macrophages are critical regulators of processes aimed at maintaining homeostasis, and prominently contribute to inflammatory and immune responses, but also help maintain metabolic stability (Ezquerra *et al.,* 2009). These cells are extremely versatile to respond to environmental triggers, adapt their phenotype, and function accordingly. Different stages of activation of macrophages have been identified, and so-called classically activated, or M1-polarised macrophages, and alternatively activated M2-macrophages represent the ends of a full spectrum (Italiani and Boraschi, 2014). In general, M1 macrophages are catabolic, pro-inflammatory cells involved in anti-microbial host defence, while M2 macrophages are considered to be anabolic cells counteracting inflammation and stimulating tissue repair. Different polarisation states of macrophages are reflected in and regulated by the macrophages' metabolism. Typically, M1-polarised macrophages supply their energy need from aerobic glycolysis, while M2 macrophages have higher levels of mitochondrial respiration, serving oxidative phosphorylation (Murray *et al.,* 2014). Recent findings show that macrophage energy metabolism and inflammatory function are tightly linked. M1 activation enhances glycolysis and, thereby, fuels the macrophages with fast energy and biosynthetic precursors for the rapid killing of microbes. Simultaneously, glycolysis drives inflammatory responses in macrophages. Conversely, M2-polarised cells primarily utilise mitochondrial oxidative phosphorylation (oxphos) as ATP source, and oxidative mitochondrial metabolism attenuates macrophage-mediated inflammation. Because the epigenetic landscape is sensitive to environmental factors, its analysis should be included in future studies regarding nutritional interventions (Lin *et al.,* 2016).

Dendritic cells are well known for their regulatory capacities regarding the induced expression of homing molecules on the surface of activated lymphocytes, called imprinting (Johansson-Lindbom *et al.,* 2005; Kyrova *et al.,* 2014). First, lymphocytes will enter the blood circulation before homing to the effector sites, followed by differentiation into effector lymphocytes able to induce an immune response upon expression of CCR7 (Förster *et al.,* 2008). T-cells migrate to the intestine requiring homing receptors like α4β7-integrin and C-C Chemokine receptor 9 (CCR9), with their corresponding ligands on endothelial cells (e.g. mucosal vascular addressin-cell adhesion molecule 1, MAdCAM1) and intestinal epithelium (e.g. CCL25 or TECK, the only known ligand for CCR9). On the other hand, homing of immunoglobuline A producing B-cells to distant mucosal tissues is considered to be mediated by CCR10 and its chemokine ligand CCL28 (MEC). Also in pigs, chemokines, a family of peptides, are involved in homing of lymphocytes (Locati *et al.,* 2001; Johansen *et al.,* 2005; Johansson-Lindbom *et al.,* 2005).

GALTs play a critical role in homing of immune cells, and intestinal DCs produce, and can use, the dietary vitamin A metabolite retinoic acid to program gut-homing lymphocytes by upregulating expression of CCR9 and the major integrin α4β7, on activated T- and B-lymphocytes (Iwata *et al.,* 2004; Hammerschmidt *et al.,* 2011). From blood, these DC migrate towards lymphoid and non-lymphoid tissues where they reside under steady state conditions. As immunological sentinels, DCs are strategically located at sites of pathogen entry and react rapidly against selective pathogen-associated molecular patterns (Raymond and Wilkie, 2005). However, the number of DCs present differs between lymphoid tissues (MALTs) such as the Peyer's patch, isolated lymphoid follicles and the mesenteric lymph nodes.

It is well established that DC populations show a high level of heterogeneity, as their tissue localisation, maturation stage and local immunological environment guides their function (Guilloteau *et al.,* 2010). In other words, DC subpopulations may differ phenotypically and functionally, depending on multiple factors, including the location of the DC. This process is also called tissue-specific functional and phenotypic imprinting as mucosal DCs play a major role in imprinting of mucosal homing receptors on lymphocytes, and regulate mucosal tolerance (Johansen *et al.,* 2005; Summerfield *et al.,* 2015). It has been demonstrated that the number of intra-epithelial DCs is different between intestinal and respiratory compartments as a consequence of the fact that the number of antigens in the intestine greatly exceeds that of the respiratory tract (Bimczok *et al.,* 2006; Heo *et al.,* 2012).

5.5 Dietary immunomodulation in poultry

5.5.1 Innate immune training

The concept of trained immunity was developed from epidemiological observations in humans that vaccination did not only provided protection against the target disease but also extended to cross-protection against other (even unrelated) pathogens (Benn *et al.,* 2013). Cross-protection was already known from the adaptive immune response in which it occurs to some extent (e.g. after Salmonella vaccination), but this now also appeared to occur in the innate arm of the immune system (Netea *et al.,* 2011; Netea and Van der Meer, 2017). Trained innate immunity with cross-protection was shown in monocytes and natural killer cells after Bacille Camette-Guérin vaccination against *Mycobacterium tuberculosis*, which protects against all-cause mortality by reducing neonatal sepsis, respiratory infection and fever (Aaby *et al.,* 2011). This vaccine-based innate protection was still detectable after three months and appeared to be mediated by increased H3K4 trimethylation in monocytes (Kleinnijenhuis *et al.,* 2012; 2014).

This shows that, besides the adaptive immune response, also the innate immune system can adapt to previous infections and develop memory (Netea and Van der Meer, 2017) to ensure that after a primary infection an enhanced innate immune response is induced, not only in response to the same secondary exposure, but also to unrelated (heterologous) infections. Thereby, trained or innate immune memory is the opposite of immune tolerance, induced by exposure to endotoxin (by lipopolysaccharides) and that can result in sepsis, whereby, previous contact with lipopolysaccharides induces immunological unresponsiveness to subsequent lipopolysaccharides exposure (Kurtz, 2005). The decisive mechanisms by which factors or pathways determine to induce tolerance or immune training upon infection remain largely unknown.

Innate immune cells cannot distinguish single bacterial or viral species but can distinguish different pathogens via a group of pathogen recognition receptors, such as TLRs, nod-like receptors, and C-type lectins (e.g. dectin-1) (Ifrim *et al.,* 2014; Tartey and Takeuchi, 2017). These receptors can recognise pathogen-associated molecular patterns or endogenous danger signals from apoptotic or dead cells (danger associated molecular patterns) and can subsequently lead to trained immunity or tolerance in some cases, but not all. Adaptive immune memory is very specific for one type of antigen or pathogen, whereas innate immune memory is not species-specific. In trained immunity histone methylation and acetylation of H3K4me1 or H3K27Aac of latent enhancers of pro-inflammatory cytokine genes takes place during initial activation, resulting in the enhance epigenetic status of immune cells leading to long-term memory (Quintin *et al.,* 2012; Saeed *et al.,* 2014).

5.5.2 Nutrition-based innate immune training

Interestingly, recent findings indicate that food components can mimic pathogen-associated molecular pattern effects and are associated with long-term epigenetic, metabolic and functional reprogramming and, thereby, induce trained immunity. Transgenerational transfer of this information depends on genetic background and molecular epigenetic mechanisms that affect, in particular, macrophage differentiation during ontogeny and tissue development. It has long been assumed that in contrast to specific immunity, innate immune cells, and innate immune responses in general, have no memory function. Recent findings, however, showed that the innate immune system exhibits immunological memory ('training'). Memory for innate host defence, first reported in plants and invertebrates, indicates that stimulation of monocytes and macrophages results in nonspecific protection from reinfection via epigenetic reprogramming (trained immunity). Adaptive immune functions are not fully matured in young animals, thus innate immune mechanisms, including activity of monocytes and macrophages are most important for disease resistance in early life of pigs and poultry. If the functionality of the innate immune system decreases before the adaptive immune system becomes fully activated, animals can be susceptible for infections and are prone to diseases (Figure 5.1). The 'immunity gap', which occurs

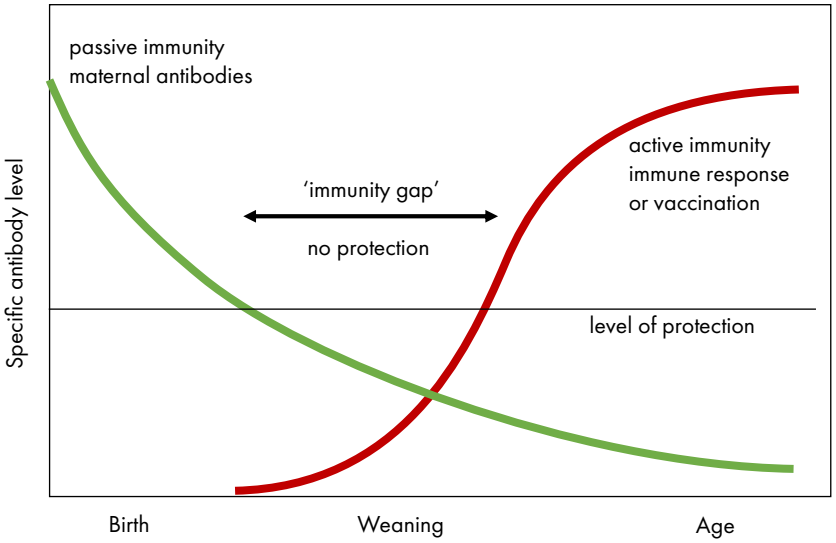

Figure 5.1. The 'immunity gap'. This, which occurs around the time of weaning refers to this decreased exposure to milk containing protective antibodies from their mother and the transition to ingestion of solid feed. During this transition, the immune system in piglets is still developing and attaining full functioning, which puts the animal in an immune-compromised position. The health risks and performance consequences posed by the immunity gap are not insignificant and reduction of this gap is indicated.

around the time of weaning refers to this decreased exposure to milk containing protective antibodies from their mother and the transition to ingestion of solid feed in piglets. During this transition, the immune system is still developing and attaining full functioning, which puts the animal in an immune-compromised position. Therefore, it is important to minimise the gap in immune protection as much as possible. In this respect, training of innate immune cells with a limited number of (infectious and dietary) innate antigens may bridge this immunity gap and subsequently have long-term effects on disease resistance to a wide variety of pathogenic microorganisms.

The mechanism of trained immunity was revealed by the enhanced responsiveness of isolated human monocytes challenged (after priming) with 1,3-(D)-β-glucan derived from *Candida albicans* (Quintin *et al.,* 2012). β-glucans are widely present in the diets for pigs and poultry (Thompson *et al.,* 2010; Meena *et al.,* 2013). It was found that β-glucan in trained macrophages induced epigenetic changes in trimethylation of H3K4, via binding to Dectin-1 that included methylation and acetylation of H3K4m1, H3K4me3 and H3K27ac when compared to non-trained macrophages (Saeed *et al.,* 2014). Cell metabolism is important in monocyte to macrophage differentiation. Resting and tolerant macrophages rely on oxidative phosphorylation, while activated (trained) macrophages shift to aerobic glycolysis (Warburg effect) via the dectin-1-akt-mTOR and HIF-1α pathway (Cheng *et al.,* 2014; Saeed *et al.,* 2014). Resting

macrophages have a functional tricarboxylic acid cycle that, together with glycolysis, enhances membrane synthesis and induces TLR-mediated activation in DCs (Saeed *et al.,* 2014). Two other metabolic pathways that are important *in vitro* and *in vivo* for trained immunity induction are glutaminolysis and cholesterol synthesis pathway (Arts *et al.,* 2016; Bekkering *et al.,* 2018). β-glucan induced trained immunity via the Dectin-1 pathway is connected to cell metabolism, metabolites and epigenetic changes by selected feed components to trained immunity.

5.5.3 Feed-based transgenerational priming of the mucosal immune system

Transgenerational epigenetic priming of immunity with consequences for feeding and management strategies of the maternal line can be investigated using the chicken as a model. Matching of the environment of the mother (including environmental conditions, diet and microbiota) with that of the young, safeguards that the offspring is well prepared through a memory for that specific environment. In modern poultry husbandry, this connection is, however, largely lost. This mismatch in priming of offspring affects immune competence and may significantly contribute to increased susceptibility and prevalence of infectious and digestive/metabolic diseases (weight loss, change in feed conversion). In practise, offspring may perform according to the standard (match) or perform unexpectedly poor (mismatch). The healthy intestinal and respiratory tract of chickens, similar to mammals, harbour an efficient and highly active mucosal immune system. Aberrations in maintaining full capacity of this local immune system can decrease production performance and impede development of a sustainable health status throughout the animals productive lifespan. Leakage of the gut or airway system due to mucosa degradation can consequently result in serious enterococcus problems, locomotor issues and the need for antibiotics. More knowledge of the mechanisms underlying mucosal immune tolerance induction, microbiota interaction, and dietary immunomodulation enhances the ability to improve immune competence and disease resistance in the animals of the future (Berghof, 2013).

This may be of interest for traditional broilers that depend more on the innate defence due to their relatively short lifespan, but even more for slower growing breeds and parent stocks of layers who will rely more on their developing adaptive immune system in later stages of their lives. In addition, altered innate immune activity may also contribute to establishment of a stable intestinal microbiota. The phenotypes of activated innate immune cells are maintained and transferred to next generations of cells by epigenetic mechanisms, such as DNA modifications that regulate activity of genes involved in the innate immune response. Transgenerational induced epigenesis or priming is an adaptive effect whereby females respond to environmental challenges such as hygienic conditions (infections, vaccinations) or diet related factors (levels, ingredients, energy or intestinal microbiota), and prepare memory in their offspring.

This shapes the phenotype and determines the immune competence of the next generation through the maternal line.

The ban on antibiotics, changing housing conditions, and current breeding conditions of poultry, urges more knowledge on whether, which, and how transgenerational priming of mother birds affect the immune competence of their offspring, and whether (mis) matching of maternal and neonatal environments (including diet in early life) account for variation in immune phenotypes, microbiota composition, disease resistance, metabolic disorders and misbehaviour of poultry.

5.6 Future perspectives

The concepts of immunomodulation in pigs and poultry as outlined in this chapter are summarised in Figure 5.2. This figure outlines the interactions between feed compounds and infectious exposure in relation to husbandry conditions, feeding strategies, hygiene conditions and vaccination status on immune competence. Therefore, the interactions with macrophages, T-cells and B-cells determine the balance between immune activation and tolerance induction that are crucial in mucosal immune system in the GIT. As a consequence, this balance determines the final outcome of the disease or health status of the individual animal. Novel insights, including those presented here, will set the stage for further research in these fields (e.g. homing of activated specific immune cells in the common mucosal immune system and cross-protective trained innate immunity) with consequences for the health and performance of pigs and poultry.

5.6.1 Nutrition-based research in pigs

Infectious diseases greatly impair animal welfare and efficiency of nutrient use and thus the environmental footprint of animal production. Nutrition may aid in minimising the incidence of diseases by enhancing immune competence, which is critical as antibacterial, anti-parasitic, and other additives that promote animal health, are increasingly eliminated due to consumer demands (Sibilaa *et al.,* 2007; Schokker *et al.,* 2014). Immune competence is defined as the ability of the immune system to respond adequately on an antigenic stimulus by an appropriate immune response with a balance between tolerance and inflammation. In order to better understand dietary immunomodulation, more extensive studies in DC biology are implicated (Brown and Gordon, 2003).

Only a limited number of studies in pigs focussed on the common mucosal system and homing of immune cells, including chemokines and integrins at the effector sites in mucosal tissues (Stock *et al.,* 2013; Wilson and Obradovic, 2015). As feed interventions have high potential value as preventive measures for infections, by enhancing immune

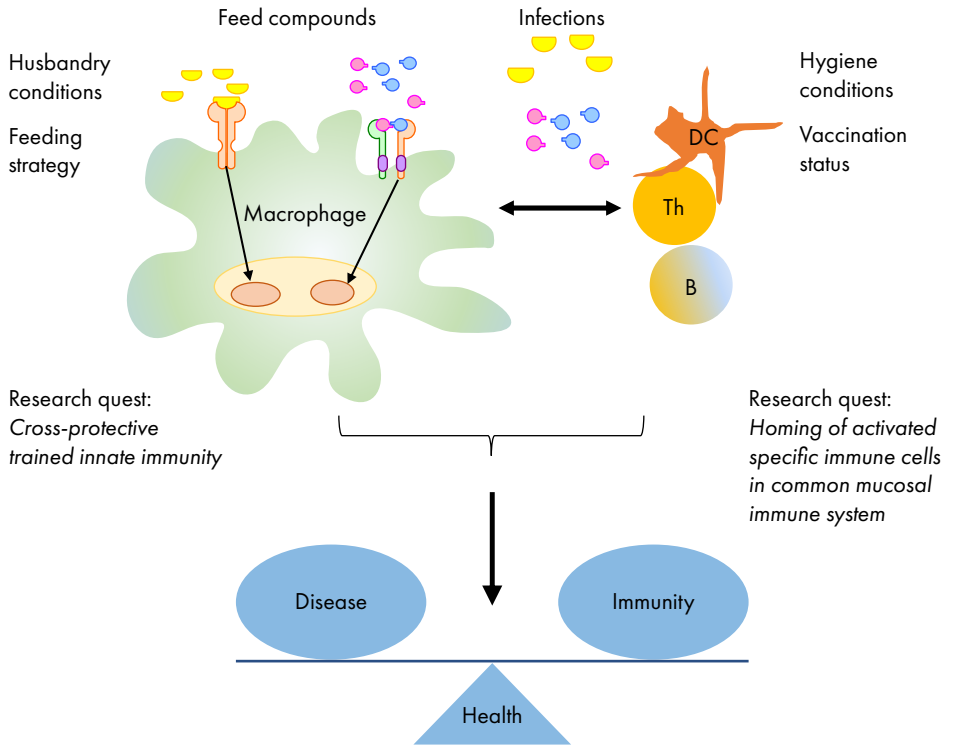

Figure 5.2. Current perspectives in research on nutrition and protective immunity in pigs and poultry. Research quests as outlined here are presented together with the main immunomodulators (feed components and infections). In addition several confounding but relevant factors are presented that together influence the balance between diseased states and development of protective immunity. This balance reflects the level of immune competence of the individual and finally determines the health status of the animal.

competence and maintaining proper homeostasis, the mechanisms by which dietary factors and its metabolites effect homing in the mucosal immune system and how homing in general exerts its function are essential to understand, especially regarding homing to tissues that encounter crucial pathogens in early life, known to highly affect health and production (e.g. the respiratory system).

During development of gut associated chronic inflammation, a decreased nutrient intake and enhanced epithelial leakage occurs. Inappropriate feed composition reduces animal welfare and health, thereby, affecting production performance negatively and poses the need to use therapeutic antibiotics. A better understanding of basic immunological mechanisms involved in dietary-mediated gut immune competence contributing to improved gut health and prevention of energy consuming inflammation provides the possibility to rationally select dietary proteins and their metabolites in pig feed (Teodorowicz *et al.*, 2018). A properly functioning GIT in

pigs, with an improved resistance to pathogens and other stressors will improve the health and welfare of pigs and leads to a further reduction in the use of antibiotics in pig farming and savings on animal health related costs.

5.6.2 Nutrition-based research in poultry

The development of immune competence and vaccination efficacy in broiler chickens, slower growing breeds and parent stock of layers, largely determines health and performance. As the worldwide trend in the industry is to come, via a gradual reduction, to ultimately a ban on the use of antibiotics as a growth promotor, a better understanding of the influence of transgenerational priming and early life conditions on the development of immune competence is pivotal.

Research on immunomodulatory function of dietary components in poultry has usually not included regulatory mechanisms. It is, therefore, necessary to acquire better insights in how feed (components) modulate the immune competence in mother hens and, even more importantly, how this immune competence is transferred to the offspring, which is challenged with ever more complex environmental and dietary cues. Here, the practical implications of understanding and applying trained innate immunity by feeds can have a large impact. Outstanding questions in this respect are: What are the causal feed factors that result in trained immunity induction by a dietary component in poultry? Does the epigenetic status of an individual chicken, based on previous infections play a role, or not? Alternatively, did the diet influence the responsiveness of the monocytes isolated from their blood? In order to answer these questions whole-genome transcriptome and epigenome studies need to be performed in poultry. Ultimately, insight in the feed associated triggers for innate immune cell training may, thereby, help to design not only better feed interventions, but also combined strategies with novel vaccine formulations, to protect production animals, including poultry, against infection and disease.

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6

Nutritional modulation to improve health and welfare

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Summary points

- Diverse composition and physicochemical properties of the carbohydrates in feed.
- Types and levels of dietary carbohydrates' influence on the microbial ecology, end-products of fermentation and the gut health.
- Types and levels of dietary carbohydrates' influence on site of digestion and types and levels of products absorbed and assimilated.
- Types and levels of dietary fibre's influence on satiety and behaviour of sows and influence on the farrowing process.

Keywords: pigs, carbohydrates, gastrointestinal tract, microbiota, digestion, absorption, glucose, short-chain fatty acids

6.1 Introduction

The production of pork has been increasing tremendously and is now in the range of more than 109 million tons annually (FAO, 2013). This level has been reached through intensification of the swine production systems, improved knowledge of nutrition and selective breeding programmes. For many years it was common practise to use in-feed antimicrobial growth promoters to improve growth rate and to control gut health. However, public concern over the heavy use of in-feed antibiotics have caused the European Union to ban the use of in-feed antibiotics in Europe because it became generally accepted that heavy use could result in antibiotic residues in pig meat and select for the survival of resistant bacteria and strains that could be transferred to

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other bacteria, thus making them resistant (Gebreyes *et al.*, 2006; Aarestrup *et al.*, 2008). A further concern in modern pig production is that sows needs to be fed restricted. During pregnancy, the domestic sow's energy requirement for maintenance and foetal growth is much lower than their desired *ad libitum* intake and they are, therefore, fed approximately 50% of their *ad libitum* intake to avoid obesity and metabolic related health problems (Meunier-Salaün *et al.*, 2001). As a consequence, pregnant sows feel hungry during a large part of their pregnancy (D'Eath *et al.*, 2009). Hunger in pregnant sows has been raised as an animal welfare issue and according to current European Union legislation (Council-Directive-2001/88/EC, 2001), pregnant sows must have access to fibrous food, or straw, to satiate them and to satisfy the need to chew. Feed components, fibre in particular, however, has been identified as a means to improve the health and welfare of the animals by modulating the substrate available for the microbiota, which can influence the microbial community, the products formed during microbial fermentation and the type and rate of, absorbed nutrients (Verstegen and Williams, 2002; Bach Knudsen *et al.*, 2012).

The main purpose of the current chapter is to discuss the potential of nutritional modulation with emphasise on dietary carbohydrates for improved health and welfare of pigs.

6.2 Feed composition with special emphasis on carbohydrates

After weaning, the pigs obtain almost all their nutrients from plant-based feeds (Bach Knudsen and Jørgensen, 2001). Plant based feeds have a far more heterogeneous composition within the individual feed but in particular when feeds of different botanical types are compared. For instance, cereals which form the basis for most of the pig's energy supply and up to 50% of the protein supply, are an assembly of complex structure with distinct differences in tissue composition between the endosperm part and the outer cell tissues (Surget and Barron, 2005; Bach Knudsen, 2014). When comparing different types of feedstuffs, e.g. cereals against legumes (Bach Knudsen, 1997; Bach Knudsen and Lærke, 2018), the picture become even more complex not only because of different plant families but also because some of the feedstuffs, e.g. distillers dried grains with solubles, soyabean meal, rapeseed meal and cake have been processed in one or another way before being used as a feed ingredient for pigs (Serena and Bach Knudsen, 2007; Pedersen *et al.*, 2014). The heterogeneity of the feedstuffs, however, makes it possible to produce mixed diets with distinct chemical compositions (Theander *et al.*, 1989; Bach Knudsen *et al.*, 2013) and physicochemical properties (Zhou *et al.*, 2018).

6.2.1 Carbohydrates

Carbohydrates are diverse molecules that chemically can be classified according to their molecular size (degree of polymerisation; DP), as sugars (DP=1-2), oligosaccharides (DP=3-9) and polysaccharides (DP≥10), with the latter consisting of starches and non-starch polysaccharides (NSPs), and glycosidic bonds (Cummings and Stephen, 2007; Englyst *et al.*, 2007; Bach Knudsen *et al.*, 2013; Bach Knudsen and Lærke, 2018) (Table 6.1). Based on the chemical classification it is possible to group the carbohydrates according to how they potentially will be digested. Digestible carbohydrates represent the carbohydrates that can be digested by the hosts' enzymes and absorbed in the small intestine. In this group we find sugars (monosaccharides and disaccharides) present in plant materials or milk products and most of the starches. For the non-digestible carbohydrates (NDC), there are no endogenous enzymes present in the gastrointestinal tract that can cleave these bonds but the microbiota, which have a much larger hydrolytic capability then the host (Flint *et al.*, 2012), can potentially degrade NDC. In this latter group of carbohydrates, we find the majority of oligosaccharides that are present naturally in plants (raffinose oligosaccharides, fructooligosaccharides) or added to compound feeds as additives (e.g. galactooligosaccharides), resistant starch (RS) and NSP. The molecular linkages in RS are the same as for digestible starch but RS is unavailable for enzymatic digestion in the small intestine because the starch is physically trapped in intact cell wall structures or when coarsely ground materials are fed (called RS type 1, RS_1); because of the structure of the raw starch granules (RS type 2, RS_2); because the starch has retrograded and re-crystallined during cooling (RS type 3, RS_3) or has been chemically modified (RS type 4, RS_4) (Englyst *et al.*, 1992). The largest NDC is the NSPs, which consist of a series of soluble and insoluble polysaccharides predominantly present in primary and secondary plant cell walls (Selvendran, 1984; Carpita and Gibeaut, 1993; McDougall *et al.*, 1996; Bach Knudsen, 2014). It is by far the most complex part of the carbohydrate fraction because of the large number of different building blocks and the diversity in linkages to different hydroxyl groups and orientations. The building blocks of NSPs are the pentoses arabinose and xylose, the hexoses glucose, galactose and mannose, the 6-deoxyhexoses rhamnose and fucose, and the uronic acids glucuronic and galacturonic acids (or their 4-O-methyl ethers). So, compared to sugars, oligosaccharides and starch, there are more building blocks (10 common monosaccharides), which can exist in two ring forms (pyranose and furanose), and these residues can be linked through glycosidic bonds at any one of their three, four or five available hydroxyl groups and in two (α or β) orientations. As a result, NSP can adopt a large number of three-dimensional shapes and, thereby, offer a vast range of functional surfaces. NSP make up the major part of the cell walls where it typically represents 90-95% of the dry matter (DM).

Table 6.1. The major dietary carbohydrates and lignin.[1]

Category	DP	Type of component	Endogenous enzymes	Prebiotic CHO
Monosaccharides	1	glucose		-
		fructose		-
Disaccharides	2	sucrose	+	-
		lactose	+	-
Oligosaccharides	3	raffinose	-	+/-
	4	stachyose	-	+/-
	5	verbascose	-	+/-
	3-9	fructo-oligosaccharides	-	+++
		xylo-oligosaccharides	-	+
		trans-galactooligosaccharides	-	+
Polysaccharides				
A. Starch	≥10	rapidly digestible	+	-
		slowly digestible	+	-
		resistant	+	++
B. Non-starch	≥10			
Cell wall NSP		cellulose	-	-
		β-glucan	-	+/-
		arabinoxylans	-	+/-
		xyloglucans	-	-
		rhamnogalacturans	-	-
		galactans	-	-
Non-cell wall NSP		fructans/inulin	-	+++
		mannans	-	-
		guar gum	-	-
		pectins	-	-
Lignin			-	-

[1] DP = degree of polymerisation; CHO = carbohydrates; NSP = non-starch polysaccharides.

6.2.2 Lignin

Lignin is not a carbohydrate but formed by the polymerisation of coniferyl, *p*-coumaryl and sinapyl alcohols (Davin *et al.*, 2008). Lignin may be covalently linked to polysaccharides both directly through sugar residues and indirectly via ferulic acid esterfied to polysaccharides. Lignin stabilises the polymers and consequently cements and anchors the cellulose microfibrils and other matrix polysaccharides. In this way it stiffens the cell walls making them very rigid and difficult to degrade by the microorganisms in the large intestine.

6.2.3 Physicochemical properties of fibre

The diverse composition and structure of the fibre fraction influences the physicochemical properties – hydration and viscosity – of the feed and the behaviour of the fibres in the digestive system (McDougall *et al.*, 1996; McDonald *et al.*, 2001; Zhou *et al.*, 2018). Knowledge concerning the physicochemical properties of a feed is important as they can interfere with the digestion and absorption processes in all segments of the gastrointestinal tract. The hydration properties are characterised by the solubility, swelling, water holding, and water binding capacity. The latter two have been used interchangeably in the literature since both reflect the ability of a fibre source to immobilise water within its matrix.

6.3 Modulation of the digestion and absorption processes by dietary means

6.3.1 The gastrointestinal tract

The gastrointestinal tract of pigs can be considered as a tube with regions that have different structure and functional elements, which provide optimal conditions for the digestion and absorption processes (Figure 6.1). Gastric emptying regulates the flow of digesta from the stomach to the small intestine (Low, 1990) and the digesta moves at a higher velocity in the proximal small intestine compared to the more distal segments (Wilfart *et al.*, 2007). The digesta in the stomach and the upper part of the small intestine is composed primarily of dietary components, and the endogenous secretions from the stomach, intestine, pancreas and gallbladder (Johansen and Bach Knudsen, 1994; Johansen *et al.*, 1996). In these regions, the main contributor to the hydrolytic capacity comes from the endogenous enzymes secreted in the stomach and small intestine and the enzymes located on the brush border of the small intestine (Kidder and Manners, 1980). The mucosa of the small intestine 'traps' the nutrients released by the hydrolytic processes (glucose from starch; amino acids and peptide from proteins and fatty acids and monoglycerol from lipids) and from here they are absorbed into the body (Gray, 1992). The epithelial layer is a semipermeable membrane that efficiently regulates the exchange of materials between the body and the luminal contents and is metabolically highly active. Furthermore, the secretions and the glycoproteins of the brush border membrane influence the adherence and the metabolic activity of bacteria (Kelly *et al.*, 1994). There are also regional differences in the mucosal architecture. The height of the villi is higher in the proximal (e.g. the regions with high nutrient influx) than the distal small intestine (320-350 µm vs 220-260 µm; Jin *et al.*, 1994). In the colon, there is barely any villi present (Jin *et al.*, 1994; Brunsgaard, 1997).

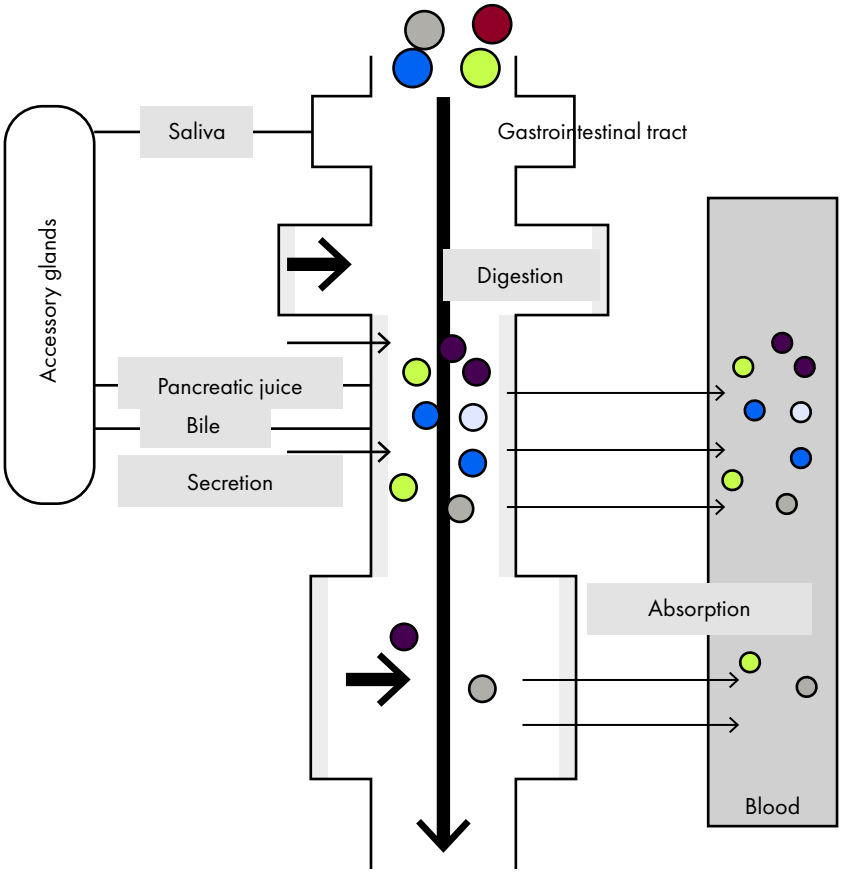

Figure 6.1. The gastrointestinal tract of pigs consisting of the mouth, the stomach, the small intestine and the large intestine (caecum and colon).

The large intestine is characterised as an anaerobic fermentation chamber with a low oxygen concentration, low flow rate, and high moisture content – all of which are conditions that favour bacterial growth, which may reach 10^{11}-10^{12} viable counts per gram fresh material (Jensen, 2001; Louis *et al.*, 2007). The microbial ecosystem contains hundreds of species of anaerobic bacteria, with each species occupying a particular niche and with numerous interrelationships between them (Flint *et al.*, 2012). The products of fermentation of undigested dietary components in the large intestine are short-chain fatty acids (SCFA) and an aray of other small organic metabolites, which are absorbed and enter the portal vein system (Bach Knudsen, 2005), used as substrate for intestinal cell growth and renewal, or excreted in the faeces (Bergman, 1990). Gases formed are excreted through flatus and expiration (Jensen and Jørgensen, 1994), and microbial biomass as well as undigested and non-fermented components excreted by defeacation.

6.3.2 Digestion and absorption processes

Immediately after weaning at 3 weeks of age, there is an insufficient production of pancreatic enzymes required for starch digestion (Efird *et al.*, 1982). However, of the brush-border enzymes it is only the activity of maltase and glycoamylase which is directly affected by feed intake in the post-weaning period (Kelly *et al.*, 1991). These conditions are the most likely cause for the compromised digestive capacity of the small intestine up to 10 days post weaning as compared to piglets at 14-28 days post weaning, growing pigs and sows (Table 6.2). Gelatinising the starch, which increase the surface area and, thereby, the interactions between the starch and the digestive enzymes may, however, increase the digestibility of starch in piglets 10 days post-weaning (Hopwood *et al.*, 2004) to a level only slightly lower than in growing pigs where gelatinised starch is almost completely digested (Bach Knudsen *et al.*, 2006).

The compromised digestibility of starch in the immediate post-weaning period has a profound influence on the possibility to modulate the flow of nutrients from the small to the large intestine by dietary means. This is illustrated in Table 6.3, which shows the calculated amounts of polysaccharides flowing from the small to the large intestine when feeding diets varying in dietary fibre between 7-120 g/kg DM. As it appears, starch is the dominating polysaccharide that pass from the small to the large intestine irrespective of the level of NSP in the first two weeks after weaning, whereas NSP become increasingly important depending on the dietary concentration of NSP, onwards from 14 days post weaning. These conditions could potentially overload the large intestine with readily fermentable nutrients, thereby, reducing the digestibility

Table 6.2. The digestibility (% of intake) of starch and non-starch polysaccharides in the small intestine and total tract of piglets, growing pigs and sows.[1]

Life stage	n	Small intestine		Total tract	
		Starch	NSP	Starch	NSP
Piglets					
0-10 days post-weaning[2]	9	75	3	99	57
14-28 days post-weaning[3]	8	95	14	100	67
Growing-finishing pigs[4]	78	96	21	100	70
Sows[5]	3	93	30	99	64

[1] n = number of diets; NSP = non-starch polysaccharides.
[2] From Lærke *et al.*, 2003 and Hopwood *et al.*, 2004.
[3] From Gdala *et al.*, 1997, Jensen *et al.*, 1998 and Pluske *et al.*, 2007.
[4] From Bach Knudsen *et al.*, 2008; average body weight 63 kg, range 37-120 kg.
[5] From Serena *et al.*, 2008b.

of NSP as has been seen in growing pigs when high loads of fermentable starch reaches the large intestine (Nielsen *et al.*, 2014). Under experimental conditions, the digestibility of NSP in the immediate post-weaning period is also slightly lower than in older pigs but it cannot be excluded that the high load of readily fermentable carbohydrates present in the large intestine contributes to digestive disturbances typically seen just after weaning. A further phenomena to be noted is that when a low fibre diet is supplemented by either soluble or insoluble fibre, the increase in faecal dry weight is much higher with piglets than with growing pigs and sows (Bach Knudsen *et al.*, 2012). The likely cause is the lower digestibility in the small intestine up to 2-weeks post-weaning not only of starch but of all nutrients and that the intestinal plasticity in piglets is lower compared to older pigs.

From 2 weeks post weaning and onward, the dietary fibre level is by far the most important factor controlling the flow of nutrients from the small to the large intestine. This is illustrated in Figure 6.2 and Table 6.4 which was compiled from data based of 21 studies involving 78 diets varying widely in dietary composition (Bach Knudsen *et al.*, 2013). The small intestine is the compartment where by far, the most of the nutrients are digested and absorped; on average close to 100% of the sugars, ~97% of the starch, ~75% of the protein and ~72% of the fat disappears during the passage of the small intestine (Table 6.4). In response to an increased intake of fibre, the digestion in the gastrointestinal tract occurs more aborally and with a concomitant increase in the flow of organic and inorganic materials from the small to the large intestine

Table 6.3. Calculated amounts of carbohydrates available for fermentation in the large intestine at 0-14 and after 14 days post weaning in pigs when fed diets varying in non-starch polysaccharide (NSP) content.

Period post weaning	Dietary non-starch polysaccharides (g/kg DM)		
	7	80	120
0-14 days[1], feed intake of 300 g/d			
Starch (g)	52	46	42
NSP (g)	2	24	36
Total carbohydrates (g)	54	70	78
>14 days[2], feed intake of 600 g/d			
Starch (g)	15	13	12
NSP (g)	4	43	65
Total carbohydrates (g)	19	56	77

[1] The digestibility coefficients used to calculate table values are based on data from Lærke *et al.*, 2003; Hopwood *et al.*, 2004.

[2] The digestibility coefficients used to calculated table values are based on data from Gdala *et al.*, 1997; Jensen *et al.*, 1998; Pluske *et al.*, 2007.

(Figure 6.2, Table 6.4). Approximately 50% of the residues passing from the small to the large intestine are made up of carbohydrates primarily NSP and to a lower extent starch. Not only does the intake of fibre influence the flow of NSP but it has also a significant influence on the flow of crude protein (calculated as N×6.25), lignin and the residue, whereas there is hardly any effect on the flow of starch and fat. The positive relationship between fibre and protein is due to the encapsulation of protein in intact cell structures (Johansen *et al.*, 1997), the presence of protein as a structural part of cell walls and an effect of viscosity and water holding properties caused by fibre (Jha and Berrocoso, 2016). The latter makes the digesta more bulky, thereby, stimulating the endogenous secretion of digestive enzymes (Larsen *et al.*, 1993; Bartelt *et al.*, 2002). The analysis of the relationship between the intake of dietary fibre and the flow of organic matter (OM) also revealed a positive intercept; when feeding 2000 g of DM to pigs, the intercept is 88 g probably representing mucopolysaccharides and other organic materials not accounted for in the analysis. Since residual sugars were not accounted for in all studies, small amounts of sugars will also be present in the residue.

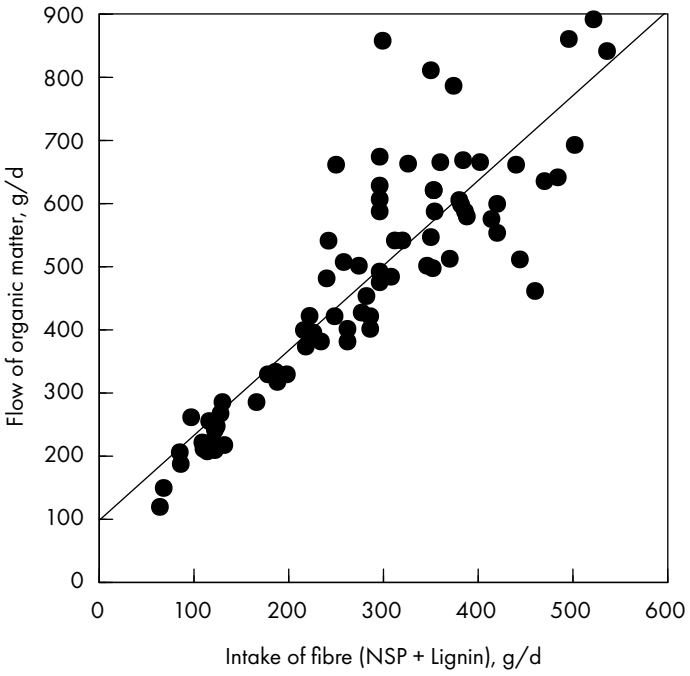

Figure 6.2. The influence of the intake of fibre (non-starch polysaccharides (NSP) + lignin) on the flow of organic matter at the ileum of growing-finishing pigs (Average BW, 63 kg; range 37-120 kg). For further details see Table 6.3.

Table 6.4. Intake, and recovery of nutrients (g per day) at the ileum and in the faeces, and the effects of fibre on the ileal and faecal recovery of nutrients in growing pigs.[1,2]

Component	Intake g/d	Ileal recovery g/d	Effect of fibre			Faecal recovery g/d	Effect of fibre		
			Intercept	Slope	R^2		Intercept	Slope	R^2
Dry matter	2,000	536	121	1.5	0.75	273	-6	1.0	0.70
Organic matter	1,903	475	98	1.3	0.77	231	-20	0.9	0.70
Crude protein (N×6.25)	351	88	43	0.2	0.27	56	12	0.16	0.63
Crude fat	130	36	29	0.03	0.03	35	24	0.05	0.09
Carbohydrates:									
Sugars	99	ND[3]				ND[1]			
Starch	984	31	19	0.04	0.03	3	-0.5	0.01	0.09
NSP	244	191	-2	0.7	0.77	79	-35	0.4	0.58
Lignin[4]	36	36[2]	1	0.1	0.40	36[2]	0.9	0.13	0.40
Residue[5]	59	100	2	0.3	0.30	29	-16	0.2	0.21

[1] The data in this table was compiled from 21 published and one unpublished report representing 78 diets. The intake was calculated based on 2.0 kg of dry matter and converted to macronutrients from the reported chemical compositions. The recovery at the ileum and in the faeces was calculated based on the digestibility coefficients reported by Bach Knudsen *et al.*, 2013.

[2] ND = not determined; NSP = non-starch polysaccharides.

[3] Sugars not determined in most studies and sugars in the ileum and the faeces consequently part of the residue fraction.

[4] It is assumed that lignin is not broken down during passage in the gut of pigs.

[5] Residue calculated as: 1000 – (ash + crude protein (N×6.25) + fat + sugars + starch + NSP + lignin).

In growing-finishing pigs (average BW 63 kg; range 37-120 kg) approximately half of the OM entering the large intestine were fermented during passage through the large intestine but with large difference between the nutrients; 37% of the crude protein, 59% of the NSP, 71% of the non-identified residues and 90% of the starch disappears (Table 6.4). The structural features of the NSP and its lignification, however, will have a substantial influence on how much of the NSP that can be degraded. For instance, the digestibility of cellulose and arabinoxylan is much higher from non-lignified materials than from lignified materials (Graham *et al.*, 1986; Bach Knudsen and Hansen, 1991; Bach Knudsen *et al.*, 1993; Longland *et al.*, 1993; Glitsø *et al.*, 1998). Moreover, due to the close association of polysaccharides and lignin in lignified cell walls, the whole complex becomes very insoluble and the main cell wall polysaccharides are virtually degraded to the same degree (Bach Knudsen and Hansen, 1991; Glitsø *et al.*, 1998). This is in contrast to non-lignified materials where cellulose is less well digested compared to hemicellulose polysaccharides (Bach Knudsen and Hansen, 1991; Glitsø *et al.*, 1998). Nevertheless, the amount of OM degraded in the large intestine increases in response to the fibre concentration; i.e. the degradation is 170 g OM per day with a fibre concentration of 150 g/kg DM and 286 g OM per day with a dietary fibre

concentration of 200 g/kg DM (Table 6.4). For sows fed diets with 429-455 g/kg DM of fibre, the degradation of OM can reach as high as 355-503 g/d (Serena *et al.*, 2008b).

Like it is the case with the flow of OM from the small to the large intestine, the fibre level has a profound effect on the faecal output of OM and its composition (Figure 6.3, Table 6.4). The majority of the OM in faeces is made up of NSP, lignin and protein; the latter deriving from undigested feed components and microbial matter. Jha and Berrocoso (2016) concluded after reviewing the literature that inclusion of dietary fibre and reduction of crude protein in pig diets seems to be an efficient nutritional strategy that may counteract the negative effect of protein fermentation in the pig gut by reducing ammonia concentration, shifting nitrogen excretion pathways in the gut and minimising the negative impact of intensive pig production on the environment.

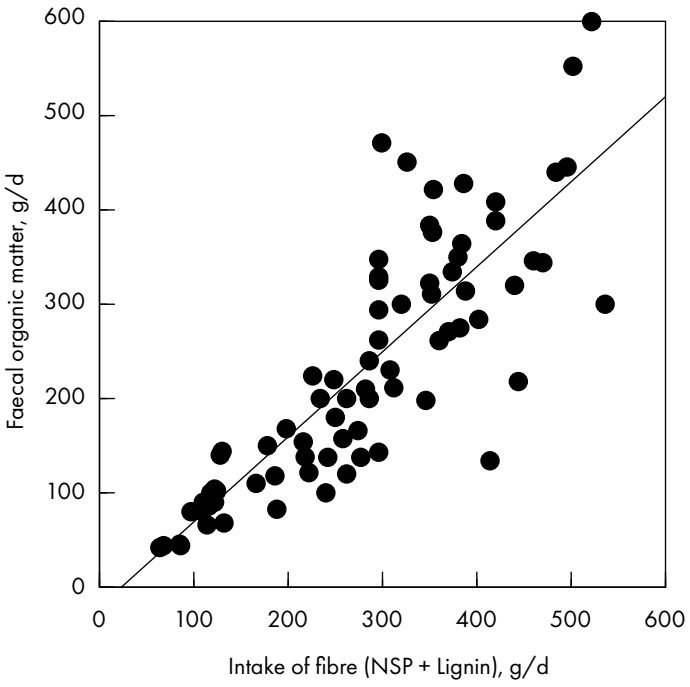

Figure 6.3. The influence of the intake of fibre (non-starch polysaccharides (NSP) + lignin) on faecal output in growing-finishing pigs (Average BW, 63 kg; range 37-120 kg). For further details see Table 6.3.

6.4 Modulation of microbial community and microbial end-products

6.4.1 Microbial community

The bacteria in the large intestine have, beside the endogenous substrates from endogenous enzymes, mucus, and exfoliated cells, access to the dietary residues that escape digestion in the small intestine. There is a nutrient gradient along the length of the large intestine with the highest concentration in caecum and the proximal colon and the lowest in distal colon and rectum (Bach Knudsen *et al.*, 2013). The borderline between the region with plenty and a scarce supply of carbohydrates, however, will be influenced by the level and composition of the carbohydrate residues passing from the small to the large intestine. Some types of NSP (Glitsø *et al.*, 1999; Le Gall *et al.*, 2009) and RS (Nielsen *et al.*, 2014) may be slowly degradable. The high nutrient concentration in the caecum and proximal colon compared to the distal colon leads to high microbial growth and SCFA generation and consequently high SCFA concentration and low pH in the proximal compared to the distal part of the large intestine (Bach Knudsen *et al.*, 1993; Jensen and Jørgensen, 1994; Glitsø *et al.*, 1998). In some situations, the profile of SCFA may also change, usually in the way that acetate increases at the expense of propionate from the caecum/proximal colon to the distal colon (Bach Knudsen *et al.*, 1991).

The microbiota of piglets and older pigs mainly belongs to the *Firmicutes* and *Bacteroidetes* phyla but with a higher ratio between the two phyla as the pigs get older. The ratio was 10 times higher in 2, 3 and 6 month pigs compared to 1 month piglets (Zhao *et al.*, 2015). As the pigs matured, it seemed that the composition of the microbiota became more stable in faeces. In adult pigs, there was a significant difference in the microbial profile between the contents of the small intestine and the large intestine. The dominant genera in the small intestine belonged to anaerobe or facultative anaerobe categories whereas the main genera in the large intestine were all anaerobes (Zhao *et al.*, 2015). In adults, the main bacterial groups comprise the following bacteria: *Streptococcus* spp., *Lactobacillus* spp., *Eubacterium* spp., *Fusobacterium* spp., *Bacteroides* spp., *Peptostreptococcus* spp., *Bifidobacterium* spp., *Selenomonas* spp., *Clostridium* spp., *Butyrivibrio* spp., *Escherichia* spp., *Prevotella* spp. and *Ruminococcus* spp. (Leser *et al.*, 2002; Kim and Isaacson, 2015).

Already at weaning, the gastrointestinal tract of pigs is densely populated with bacteria (Zhao *et al.*, 2015), but the community is very unstable (Janczyk *et al.*, 2007; Pieper *et al.*, 2008). It takes at least 5-10 days for the intestinal bacterial community to be re-established and to adapt its activity to the new feeding situation with complex plant materials at the expense of liquid nutrients from milk (Janczyk *et al.*, 2007; Pieper *et al.*, 2008). As long as the microbial community is not disturbed by enterobacteria,

members of the lactobacilli family are the dominating bacterial groups and lactic acid (LA) by far the most important metabolic end-product. The concentration of LA increases several-fold within the first 11 days after weaning and with a concomitant drop in pH (Janczyk *et al.*, 2007; Pieper *et al.*, 2008). While it is difficult to change the flora composition in the immediate post-weaning period, prebiotic carbohydrates in the form of fructose containing oligo- and polysaccharides can at latter stages be used specifically to stimulate lactic acid producing bacteria (*Lactobacillus* spp. together with *Bifidobacterium* spp.) (Mikkelsen *et al.*, 2004; Mølbak *et al.*, 2007; Wellock *et al.*, 2008; Barszcz *et al.*, 2016) resulting in a higher ratio between lactobacilli and coliform and reduced luminal pH in digesta (Wellock *et al.*, 2008). However, no difference in the composition of the microbiota in the ileum and faeces could be detected using the terminal restriction fragment length polymorphism method when feeding diets contained dried chicory roots with fructan concentrations ranging between 30-160 g/kg DM (Hedemann and Bach Knudsen, 2010). Stimulation of LA producing bacteria is generally considered beneficial as attachment of these harmless bacteria to the mucosa may protect the animal from gut infection. Since fructose containing oligo- and polysaccharides are readily fermentable, they further reduce luminal pH (Houdijk *et al.*, 2002; Bach Knudsen *et al.*, 2003) and potentially reduce the proliferation and establishment of pH sensitive enteropathogenic strains of *Escherichia coli, Salmonella, Shigella* or some clostridia (Gibson and Wang, 1994; Macfarlane *et al.*, 2006). In a study where inulin and alginate were used as prebiotic carbohydrates and fed to pigs at a commercial farm and an experimental farm, respectively, there was a more pronounced decrease in the age-related enterococci numbers when inulin was added to the diet and this effect was stronger at the commercial farm (Janczyk *et al.*, 2010). Moreover, when alginate was included in the diet, the enterococci in the gastrointestinal tract of piglets from the experimental farm remained at constant and high numbers throughout the study. These observations further confirm the hypothesis that it is not all NDC that have prebiotic properties and that the NDC may act differently depending on the hygienic conditions at a farm (Janczyk *et al.*, 2010).

Fructooligosaccharides, inulin, RS and some forms of NSP have been found to influence the microbiota composition in older pigs. Feeding a diet containing dried chicory roots and sweet lupins rich in fructans and galactans to growing pigs were found to stimulate *Bifidobacterium thermoacidophilum* and *Megasphaera elsdenii* (Mølbak *et al.*, 2007), which inhibited *Brachyspira hyodysenteriae* to be established (Thomsen *et al.*, 2007). A shift in the composition of the bacterial populations was found when feeding inulin of variable chain lengths to young pigs (Patterson *et al.*, 2010), when inulin was compared to cellulose (Yan *et al.*, 2013) as was the case when RS_2 (Nielsen *et al.*, 2014) and RS_3 (Haenen *et al.*, 2013a,b) were fed to pigs. High amylose RS_2 maize has further been used as a versatile prebiotic for use with probiotic bacteria and in combination with fructooligosaccharides used to raise faeces bifidobacteria numbers (Brown *et al.*, 1997, 1998). There are also indications that NSP with a more diverse sugar residue composition than inulin and RS can influence the

microbial composition as indicated by the stimulation of the proliferation of butyrate producing microorganims (i.e. *Faecalibacterium pausnitzii*, *Roseburia intestinalis*) by arabinoxylan provided as part of a whole grain cereal diet (Nielsen *et al.*, 2014) and when provided as a concentrate (Belobrajdic *et al.*, 2012).

In addition to the horizontal variation in bacterial composition, there is also a vertical gradient of species distribution (Kelly *et al.*, 1994; Macfarlane *et al.*, 2006). The mucosa provides an environment that differs physically and chemically from those of the digesta. It is also recognised that the bacteria associated with the mucosa are likely to have a greater potential to influence the host than those present in the digesta (Kelly *et al.*, 1994; Macfarlane *et al.*, 2006). However, much more is known about the bacteria of the digesta and how to influence them by dietary mean than those attached to the mucosa.

6.4.2 End-product formation

The concentration of SCFA in the colon digesta increases post-weaning when piglets are transferred from milk to solid feed (Van Beers-Schreurs *et al.*, 1998; Bruininx *et al.*, 2004). Although the concentration of SCFA in the digesta of the large intestine in piglets (Wellock *et al.*, 2008) is comparable to what is found with older animals (growing pigs and sows) (Bach Knudsen and Hansen, 1991; Serena *et al.*, 2008a), the type of substrate may have a profound influence on the composition of fermentation end-products. The range of carbohydrates that arrive in the large intestine from the diet is enormous. While starch will dominate in the immediate post-weaning period (Table 6.3), fibres in various forms will later on be the major contributor (Bach Knudsen *et al.*, 2013) (Table 6.4). The high load of starch in the immediate post-weaning period is responsible for the relatively high butyrate concentration at that stage (Pluske *et al.*, 2007), which further can be stimulated by the inclusion of RS_2 from raw potato in the diets of piglets (Hedemann and Bach Knudsen, 2007) but also for growing pigs (Van der Meulen *et al.*, 1997a; Sun *et al.*, 2006). In older pigs and sows where NSP are the main substrate for microbial fermentation, rate and overall degree of degradation of these polymers is influenced by the chemical nature of the plant fibre, the solubility, and the degree of lignification. In the caecum and proximal colon, the carbohydrate supply is usually sufficient to support a high activity of the microbial community, while carbohydrates become a limiting factor for high SCFA generation in the more distal locations of the colon (Bach Knudsen *et al.*, 1993; Glitsø *et al.*, 1998). In these regions, other substances like protein and endogenous materials are of greater importance (Jha and Berrocoso, 2016). These conditions influence the concentrations and molar proportions of organic acids, the concentrations of SCFA and the ratio between saccharolytic derived SCFA (acetate, propionate, and butyrate) and proteolytic derived acids (iso-butyrate, and isovalerate) decline from the proximal to the distal large intestine (Bach Knudsen *et al.*, 1993). The concentration of potential harmfull components like NH_3, indoles, phenols, secondary bile acids are also higher in the distal compared to the proximal colon (Glitsø *et al.*, 1998; Belobrajdic *et al.*, 2012; Jha and Leterme, 2012).

6.5 Modulation of carbohydrate derived absorption products

Several studies have shown that the supply of carbohydrate deriving nutrients to the body takes place in two phases; a phase with rapid and high influx of nutrients (absorptive phase) lasting 4-5 h after a meal, and a phase with low influx of nutrients (post absorptive phase) lasting until the next feeding (Giusi-Perier *et al.*, 1989; Rérat, 1996; Bach Knudsen *et al.*, 2000; Bach Knudsen *et al.*, 2005; Theil *et al.*, 2010; Ingerslev *et al.*, 2014) (Figure 6.4). Reducing sugars are the dominating products deriving from carbohydrate assimilation in the absorptive phase with levels that are 4-8 times higher than of SCFA. In the post-absorptive phase, however, SCFA become increasingly important and the amount of SCFA may equal the amount of reducing sugars the last hours before the next meal.

The dietary carbohydrate composition influences rate and type of products derived from carbohydrate assimilation. Thus, glucose and sucrose appear to be absorbed more rapidly than starch and, particularly, lactose (Rérat *et al.*, 1984a,b). From another series of experiments it also appears that maltose was absorbed to the portal vein more rapidly than starch (Rérat *et al.*, 1993). The type of starch has also been found to influence the rate of glucose absorption (Van der Meulen *et al.*, 1997a,b; Regmi *et al.*, 2011; Ingerslev *et al.*, 2014), while the fibre level only seems to have an influence on the rate of glucose absorption when added to the diet as a fibre isolate. Viscous guar-gum, concentrated β-glucan and concentrated arabinoxylan added to either a semi-synthetic diet or a low-fibre diet reduces the postprandial appearance of glucose in the portal vein (Ellis *et al.*, 1995; Hooda *et al.*, 2010; Christensen *et al.*, 2013). In contrast, neither insoluble (wheat bran) nor soluble fibre (sugar-beet fibre, oat bran and rye) sources seem to modify nutrient absorption (Michel and Rerat, 1998; Bach Knudsen *et al.*, 2000; Theil *et al.*, 2010; Christensen *et al.*, 2013; Ingerslev *et al.*, 2014).

LA appears in the portal vein in the early phase of absorption and is better synchronised with the absorption profile of starch than SCFA (Bach Knudsen *et al.*, 2000; Serena *et al.*, 2007; Ingerslev *et al.*, 2014). In some studies, the estimated absorption of LA is close to the absorption of SCFA but it cannot be excluded that the absorption of LA in experiments with catheterised pigs is overestimated as a significant proportion of LA can be derived from glucose oxidation in the gut (Vaugelade *et al.*, 1994).

The absorption of SCFA occurs at a slower rate than glucose (Figure 6.4). Diurnal variations of SCFA concentrations in portal blood have only been reported when high levels of readily fermentable carbohydrates (lactose or sugar alcohols) were fed (Giusi-Perier *et al.*, 1989; Rérat *et al.*, 1993) or when refeeding after prolonged fasting (Rérat *et al.*, 1987; Bach Knudsen *et al.*, 2005). The relative proportion of energy absorbed as reducing sugars or SCFA, however, is strongly influenced by the dietary carbohydrate

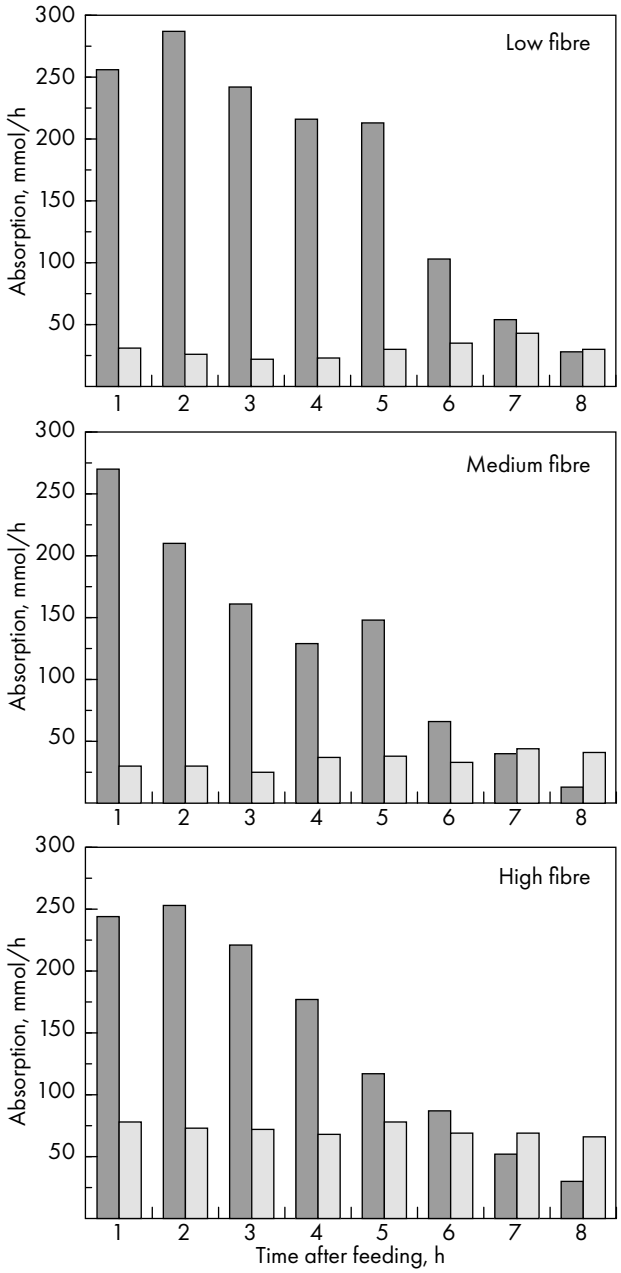

Figure 6.4. Absorption (mmol/h) of glucose and short-chain fatty acids after consumption of a diet low, medium and high in fibre. The pigs were fed three times daily at 8 h interval. Data adapted from Bach Knudsen *et al.*, 2000; Bach Knudsen *et al.*, 2005.

composition. Thus, a significant rise in absolute and relative contribution to the energy supply of the animals is seen when the level of carbohydrates fermented in the large intestine is raised either by fibres, RS, poorly absorbed sugars or sugar alcohols (Giusi-Perier *et al.*, 1989; Rérat *et al.*, 1993; Bach Knudsen *et al.*, 2000; Bach Knudsen *et al.*, 2005; Ingerslev *et al.*, 2014). For instance, the proportion of energy absorbed as SCFA was only ~4% for the low NDC maize starch diets while it was 44% when a high NDC potato diet was fed (Table 6.5). An even higher energy contribution from SCFA was seen in sows that were fed a high-fibre diet (429 g/kg DM) where 52% of the energy derived from SCFA compared with 12% in a low-fibre diet containing 177 g/kg DM fibre (Serena *et al.*, 2009).

Adult animals can tolerate and handle higher levels of fibre than is the case with young growing animals. In an experiment with sows fed either a low fibre diet (170 g/kg DM)

Table 6.5. Effect of meal size and intake of digestible starch and non-digestible carbohydrates on portal concentrations and fluxes of glucose and short-chain fatty acids, and the proportion of energy absorbed as glucose and short-chain fatty acids.[1,2]

Diet	Intake, g				Glucose		SCFA		Absorbed energy (%)	
	Meal size	Dig. starch	NDC		mmol/l	mmol/h	µmol/l	mmol/h	Glu	SCFA
			RS	NSP						
LF wheat bread	1,300	746	4	77	8.10	175	775	30	93.0	7.0
HF wheat bran	1,300	663	3	140	7.69	127	854	30.8	90.5	9.5
HF oat bran	1,300	605	3	140	7.66	132	908	37.1	89.1	10.9
HF rye bread	1,250	676	13	254	6.60	157	1,140	76.9	82.4	17.6
HF wheat bread	1,250	610	7	275	6.43	117	1,001	66.5	80.2	19.8
Maize starch	860	536	9	39	8.85	146	459	13.9	96.0	4.0
Pea starch	860	535	15	36	6.90	105	454	17.8	93.1	6.9
Maize starch	1,250	762	20	66	8.14	185	480	19.1	95.7	4.3
Maize:potato (1:1) starch	1,250	609	189	66	6.94	109	1,240	60.3	80.6	19.4
Potato starch	1,250	361	458	66	5.97	49	1,620	88.9	55.9	44.1
LF diet	1,248	611	7	66	8.8	257	533	37	94.1	5.9
HF RS	1,380	586	140	135	8.5	203	945	66	87.6	12.4
HF AX	1,539	576	11	197	8.7	247	1,287	102	84.8	15.3

[1] AX = arabinoxylan; Glu = glucose; HF = high fibre; LF = low fibre; NDC = non-digestible carbohydrate; NSP = non-starch polysaccharides; RS = resistant starch; SCFA = short-chain fatty acids.

[2] Data from Van der Meulen *et al.*, 1997a,b; Bach Knudsen *et al.*, 2000, 2005; Ingerslev *et al.*, 2014.

or diets high in insoluble or soluble fibre (430-450 g/kg DM) it was found that the high fibre diets increased the amount of undigested residues in the gastrointestinal tract and influenced the absorption profile of nutrients (Table 6.6) (Serena *et al.*, 2008b, 2009) towards more being provided as SCFA relative to glucose. The shift in the provision of energy from glucogenic towards ketogenic (Figure 6.5) result in higher systemic levels of non-esterified acetic acid (Yde *et al.*, 2011) and reduced diurnal variations in the uptake of energy and fluctuation in insulin (Serena *et al.*, 2009). These conditions will keep the sows satiated for a longer period of time and can potentially reduce the incidence of aggressiveness, stress and/or stereotype behavior in sows incurred by hunger (De Leeuw *et al.*, 2004, 2005a,b).

Several studies have investigated the effects of feeds high in fibre in pregnant sows and gilts fed restrictively. In most studies, feeding motivation has been measured in relation to feeding time. A lower feeding motivation both before and after feeding has been reported in pregnant gilts fed fibrous feeds (Robert *et al.*, 1997, 2002). Fibrous feeds can potentially influence feeding motivation in a number of ways. Firstly, substitution of starch for fibre results in a shift in the nature of absorbed energy from readily absorbed glucogenic energy to more slowly released ketogenic energy

Table 6.6. The influence of fibres on the weight of gut content, concentration of insulin in plasma, and absorption of carbohydrate derived nutrients.[1,2]

Item	Low fibre	High fibre	
		insoluble	soluble
Dietary composition (% of DM)			
Starch	51.8	23.9	21.7
Dietary fibre	17.5	45.3	43.0
Gastrointestinal content (kg)			
Stomach	4.3^{b}	5.6^{a}	6.1^{a}
Small intestine	1.5^{b}	2.1^{a}	2.4^{a}
Large intestine	5.1^{b}	8.6^{a}	7.5^{a}
Absorption (mmol/h)			
Glucose[3]	419	189	124
Short-chain fatty acids	133^{c}	218^{b}	321^{a}
Concentration (pmol/l)			
Insulin	138^{a}	98^{b}	87^{b}

[1] Data from Serena *et al.*, 2008a, 2009.
[2] $^{a-c}$means within a row without a common superscript are different (P<0.05).
[3] Diet (D), time (T) and D×T are all statistically significant.

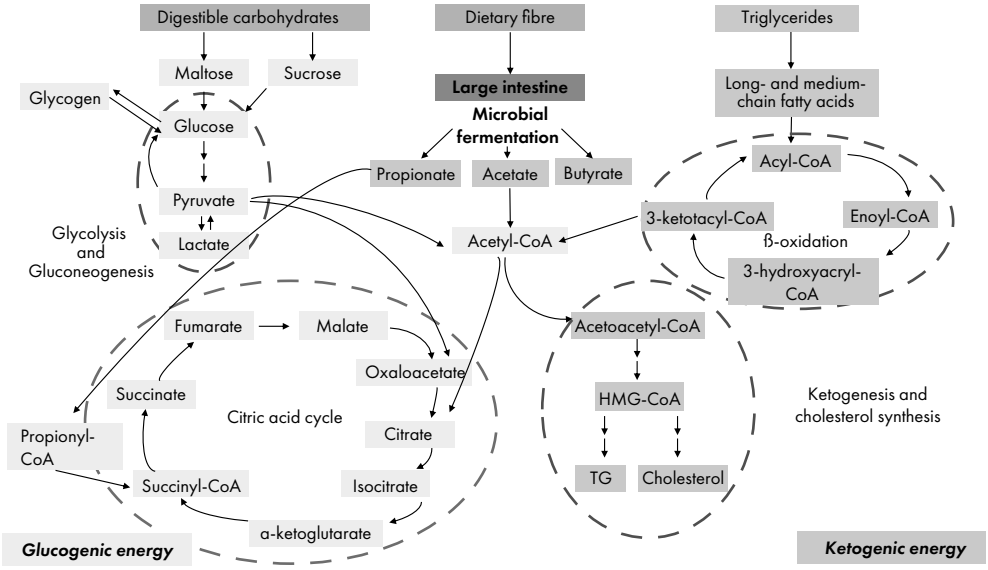

Figure 6.5. Basic catabolic pathways of carbohydrate and lipid energy metabolism (from Blad *et al.*, 2012).

(Table 6.6, Figure 6.5) (Serena *et al.*, 2009). Secondly, a direct consequence of the reduced diurnal variation in absorbed energy is a lower fluctuation in insulin (De Leeuw *et al.*, 2004, 2005a; Serena *et al.*, 2009). Thirdly, fibre-rich diets resulted in more materials in the stomach and the remaining gut system (Serena *et al.*, 2008a). Therefore, sows consuming high-fibre diets can be expected to be satiated for a longer period of time due to the physical presence of digesta in the gut, which influences the stretch and chemoreceptors in the stomach and duodenum. In the long run, the lower diurnal variation in energy uptake will also keep the sows satiated for a longer period of time (Read *et al.*, 1994). However, although feeding sows with fibrous diets has been shown to alter the plasma concentrations of many of the observed metabolites, and most of the changes indicate that consumption of the fibrous diet increased satiety, it is a great challenge to satiate pregnant sows for a longer period. Most studies show that the satiating effect of high fibre diets only lasted the first hours after feeding (Bergeron *et al.*, 2000; Ramonet *et al.*, 2000; Meunier-Salaün *et al.*, 2001; Jensen *et al.*, 2012, 2015).

In addition to the beneficial effects of dietary fibre on behavioral and welfare in gestating sows, recent studies have further pointed to beneficial effects of dietary fibre in relation to reproductive performance. It has been suggested by Oliviero *et al.* (2009) that inclusion of dietary fibre in sow diets may improve the farrowing process. A recent study concluded that high dietary fibre supplementation to late gestating sows

in the last two weeks before expected farrowing reduced the proportion of stillborn piglets and consequently reduced total piglets mortality as fewer live-born piglets died due to poor viability at birth and due to piglets diarrhea (Feyera *et al.*, 2017). These aspects warrant further investigations, particularly concerning the mode of action of dietary fibre and the optimal fibre source to be used.

6.6 Conclusions

The composition of the carbohydrate fraction has a profound influence on the site of nutrient digestion, the microbiota, the end-products derived from carbohydrate assimilation as well as types and rates of nutrients absorbed. These aspects are all important for the health and welfare of piglets, growing-finishing pigs and sows and can proactively be used to optimise the feeding of pigs from birth to maturity.

6.7 Future perspectives

Although we have a substantial knowledge on how carbohydrate components are digested and fermented in the gastrointestinal tract, there are still areas that warrant further investigations. These include the use of specific carbohydrate components in the immediate post-weaning period to promote a healthy microbiota and how dietary fibre supplementation in diets to late gestation sows can be used to reduce piglet mortality.

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7

Nutrigenomics and its perspective in nutrition

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Summary points

- 'Omics' techniques can be utilised to study the role of nutrients on poultry and swine physiology and growth, helping to fine-tune nutritional requirements and animal production.
- Three 'omics' techniques utilised to study the interaction of nutrition and molecular changes are transcriptomics, proteomics and metabolomics.
- In swine, nutrigenomics is helping to further the understanding of how nutrition modulates gut health and immunity during weaning.
- In poultry, 'omics' techniques are enhancing knowledge of the mechanisms behind nutritional strategies as alternatives to antibiotics.
- In both species, nutrigenomics techniques are being utilised to better understand the relationships between diet and liver, muscle and adipose metabolism.
- Nutrigenomics techniques are also being applied to studies on how nutrition alters gut microbiota and offspring epigenetics in both pigs and poultry.
- In the future, use of nutrigenomics will enhance nutritional management, especially during periods of immunosuppression.

Keywords: bioinformatics, gut health, metabolism, microbiome, poultry, swine

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7.1 Introduction

The biological complexity of agricultural animals unavoidably requires a systems biology approach, i.e. a way to systematically study the complex interactions in the animal using a method of integration instead of reduction (Loor *et al.*, 2015). Important goals of systems biology are to uncover the underlying links (pathways, regulatory networks, and structural organisation) within and between tissues (e.g. adipose and liver; skeletal muscle and adipose; gut microbiota and epithelia), and also to discover new emergent properties that may arise from examining the interactions between all components of a system. This integrative approach provides the means to arrive at a holistic view of how the organism functions (Deusch *et al.*, 2015; Vailati-Riboni *et al.*, 2017). *In silico* work with human genome and experimental work with model organisms have underscored the applicability of high-throughput technologies (e.g. microarrays, next generation sequencing) to discern functional biological networks (Bordbar and Palsson, 2012).

'Omics' tools are helping scientists elucidate the roles of diet and specific food components on human health, as a way to increase our knowledge about common diseases such as obesity (Chadwick, 2004), coronary heart disease (Talmud, 2004), or cancer prevention (Davis and Hord, 2005). Cattle, pigs, and poultry represent economically-important livestock species. Application of omics tools and bioinformatics in livestock science are already allowing a better understanding of animal physiology and its association with feedstuffs, hence, enabling nutritionists to design functional diets that enhance animal performance based on exploiting an animal's full genetic potential. In the long-term, nutrigenomics research is poised to fine-tune nutrient requirements and increase the quality of animal products (i.e. technological proprieties, health, safety).

Ghormade *et al.* (2011) summarised the post-genomic era opportunities that the novel field of nutrigenomics has created, or will be able to create, for livestock science and nutrition. In the pig industry, nutrigenomics tools have been leveraged for their economic benefits and to improve human nutrition and health. However, the use of advanced and more holistic type of analysis is still not widespread. This chapter focuses on the use of transcriptomics and bioinformatics as tools to study the role of nutrients on pig and poultry physiology and growth. Furthermore, we discuss available computational tools that can generate useful functional information from 'omics' datasets. The goal is to provide specific examples of how these combined approaches could advance our understanding of tissue function beyond the classical biochemical pathways.

7.2 Methodology overview

Modern, high throughput 'omics' techniques along with proper statistical analysis methods should be considered as complimentary research tools for analysing nutrigenomics data to generate accurate, comprehensive, and unbiased information. Application of 'omics' provides a holistic view of the overall physiology and molecular adaptations of an organism in terms of genes and genome (transcriptomics), proteins and proteome (proteomics) and metabolites and biological pathways (metabolomics). To date, most published nutrigenomics studies have focused on one of the technologies to infer about the role of nutrients or the environment on animal physiology, and few published studies have integrated two or more technologies (Table 7.1). For example, a recent study with poultry utilised transcriptomics and metabolomics to evaluate the molecular basis of the liver response to heat stress in chickens (Jastrebski *et al.*, 2017). In the following sections we describe briefly the general aspects of transcriptomics, proteomics and metabolomics.

7.2.1 Transcriptomics

A number of organism-specific and in general transcriptome (mRNA) databases have been created with well-defined annotation methods to increase the in-depth knowledge of differentially expressed genes and overall genome research. Between 1995 and 2008, DNA microarray was the tool of choice for differential gene expression analysis. However, this technique is rapidly being replaced with next generation sequencing technologies that allow us generating information about all RNA species in a given sample, including splice variants (Oshlack *et al.*, 2010; Anders *et al.*, 2013). In-depth biological questions regarding mutations, splice variants, evolution, and genome structure and function have been addressed with RNA-sequencing (RNA-seq) (Costa-Silva *et al.*, 2017). This technique is becoming cheaper and more affordable. It is likely that livestock nutrigenomics work in the near future will be solely performed with this approach.

Application of next generation sequencing via different sequencing platforms such as Illumina HiSeq, Roche 454, SOLiD generates tens of millions of sequencing 'reads' (Koboldt *et al.*, 2012; Anders *et al.*, 2013; Ratan *et al.*, 2013). The selection of a sequencing platform depends on the experimental design, desired end results and specific requirements (Hrdlickova *et al.*, 2017). The raw reads are filtered through a quality control and quality assurance method using software packages such as FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). There are several pipelines that allow users to obtain the desired results from RNA-seq data (Yalamanchili *et al.*, 2017). For example, Trapnell *et al.* (2012) developed a tool to process reads by mapping to a reference genome using one of several mapping aligner tools such as bowtie 2 (Langmead and Salzberg, 2012) and tophat2 (Kim *et al.*, 2013a). The mapped reads are then assembled into transcripts using 'Cufflinks' software (Trapnell *et al.*,

Table 7.1. Applications of omics technologies for use in nutrigenomics studies with swine and poultry.

Animal	Type of analysis	Tissue	Dietary treatment	Effect	Reference
Swine	proteomic	muscle	linoleic acid	Influenced the abundance of proteins related to energy metabolism, fatty acid oxidation and synthesis, amino acid metabolism, defence, transport and other miscellaneous processes.	Zhong *et al.*, 2011
Swine	transcriptomic	muscle	low protein (14.5% DM)	Can modulate intra-muscular fat content by altering gene pathways involved in lipid biosynthesis and degradation; however negatively impacts protein synthesis pathways.	Hamil *et al.*, 2013
Swine	transcriptomic	muscle	L-carnitine	Enhance expression of genes involved in cytoskeletal protein binding, insulin-like growth factor-1 binding, transcription factor activity, and insulin receptor binding, while downregulating pro-apoptotic transcription factors.	Keller *et al.*, 2011
Swine	transcriptomic and proteomic	liver	soy or casein proteins	Expression of genes involved in the metabolism of stress response exhibited significant changes in the transcription level and indicated an increased oxidative stress response, also at the protein level.	Schwerin *et al.*, 2002; Junghans *et al.*, 2004
Swine	transcriptomic	intestine	glutamine	Supplementation increased intestinal expression of genes that are necessary for cell growth and removal of oxidants, while reducing expression of genes that promote oxidative stress and immune activation.	Wang *et al.*, 2008
Swine	transcriptomic	intestine	threonine deficiency	Immune and defence responses, energy metabolism and protein synthesis genes were downregulated. Changes in the expression of the gene encoding for glucose cotransporter and regulators of paracellular permeability were also reported.	Hamard *et al.*, 2010
Swine	metabolomic	intestine	highly fermentable proteins	Mass spectrometry revealed increased abundance of metabolites associated with arachidonic acid metabolism in colon, but no changes in urine.	Pieper *et al.*, 2012
Swine	transcriptomic	leukocytes	selenium	Gene activities indicate a modulation of multiple physiological pathways, such as immune responses, inflammatory response, oxidative stress status and cholesterol metabolism, providing benefits to pigs' health and performances.	Song *et al.*, 2013
Swine	transcriptomic	leukocytes	fermented soybean meal	Diet regulates genes potentially related to the reduction of inflammatory response and anti-oxidative activity in pigs after a LPS challenge.	Roh *et al.*, 2014
Poultry	transcriptomic	intestinal lymphocytes	carvacrol, cinnamaldehyde or *Capsicum* oleoresin	Can modulate genes expressing functions of antigen presentation, humoral immune response, and inflammation, and lipid metabolism and small molecule biochemistry.	Kim *et al.*, 2010; Lillehoj *et al.*, 2011

Table 7.1. Continued.

Animal	Type of analysis	Tissue	Dietary treatment	Effect	Reference
Poultry	transcriptomic	intestinal lymphocytes	anethole, turmeric or garlic metabolites	Enhance genes relevant for host of the anti-inflammatory response and protective immunity, during a challenge with avian coccidiosis.	Kim *et al.*, 2013a,b,c
Poultry	transcriptomic	liver	inulin	Supplementation affects genes of basal cell metabolism, immune system processes and fatty acid metabolism, improving animal performance, health and meat quality.	Sevane *et al.*, 2014
Poultry	transcriptomic	muscle	chromium picolinate	Modulates expression of microRNA involved in the regulation of protein synthesis.	Pan *et al.*, 2013
Epigenetic effect on offspring					
Swine	transcriptomic	offspring liver and muscle	high protein sow diet (30% w/w)	Liver of offspring experience alteration in energy sensing pathways, nitrogen, lipid and energy metabolism, while muscle tissue undergo similar alteration, including alteration in cell-cycle regulation and nucleic acid metabolism.	Oster *et al.*, 2011-2012a
Swine	transcriptomic	offspring liver and muscle	low protein sow diet (50% of requirements)	Sow diet cause a change in liver lipid metabolism of the offspring, while disturbing muscle tissue gene expression of growth-related pathways into adulthood.	Oster *et al.*, 2012b; Doring *et al.*, 2013
Swine	transcriptomic and proteomic	offspring liver and muscle	folic acid in sow diet	Sow folate deficiency extremely downregulate lipid metabolism-related genes and associated metabolic. Supplementation instead increases the content of liver proteins that regulate immune response, energy metabolism, and intermediary metabolism, while it downregulates proteins associated with cellular signal transduction, proteolysis, and cell migration regulation	Li *et al.*, 2013; Liu *et al.*, 2013
Poultry	transcriptomic	embryo	selenium (*in ovo* injection)	Genes involved in adipocyte determination and differentiation, fatty acid uptake, triacylglycerol synthesis and lipolysis inhibitors are up-regulated. Osteogenic and myogenic genes are down-regulated, and genes related to oxidative stress response during adipogenesis are up-regulated.	Hassan *et al.*, 2014
Poultry	transcriptomic	chick intestine	minerals and vitamins in hens diet	Affect genes related to intestinal turnover, proliferation and development, metabolism and feed absorption.	(Rebel *et al.*, 2006)

2010). These transcripts are further processed into differentially expressed genes using the 'Cuffdiff' tool from the Cufflinks software package. Alternatively, accordingly to another pipeline that was published by Anders *et al.* (2013), the mapped reads along with annotation or assembled transcripts are counted and then normalised before statistical analysis. In this case, the statistical analyses are performed using 'R Bioconductor' software including, but not limited to, 'DESeq' (Anders and Huber, 2010), 'edgeR' (Robinson *et al.*, 2010) and limma (Ritchie *et al.*, 2015). After statistical analysis, the differentially expressed genes are counted and can be further analysed to allow for functional interpretation in the context of the experimental designs. The RNA-seq technique has been applied in both poultry and swine nutrition as a means to improve health and meat quality (Cardoso *et al.*, 2017; Van Goor *et al.*, 2017).

7.2.2 Proteomics

Proteome analysis relies on a variety of analytical methodologies. We briefly highlight the two most commonly used technologies, i.e. western blot and mass spectrometry. Both provide information of protein identification and characterisation within cells, tissues or organisms. However, each technique has its own merits and demerits in terms of sample handling, experimentation and analytics. Proteomics studies have been conducted in swine and poultry to understand various pathophysiological conditions (Frohlich *et al.*, 2016, Peng *et al.*, 2018).

The western blot technique is an analytical technique that is used to detect specific proteins based on their molecular weights or peptide size. Protein samples are first quantified and then denatured before running on gel electrophoresis. The denatured proteins are separated on a gel based on their polypeptide lengths. Peptides are then transferred to a membrane either composed of nitrocellulose or polyvinylidene fluoride for staining purposes with antibodies of interest (Renart *et al.*, 1979; Towbin *et al.*, 1979). The results are analysed by means of software packages e.g. ChemiDoc (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

In mass spectrometry, a neutral protein sample is ionised usually through an 'electron bombarding' procedure. The ionised molecules are then separated based on a mass to charge ratio (m/z). The corresponding results are displayed on a mass spectra which represent the characteristics of either the molecular mass of molecules and/or structure of proteins or peptides (Fenn *et al.*, 1989; Vidova and Spacil, 2017).

7.2.3 Metabolomics

As in proteomics, metabolomics analyses also rely on mass spectrometry. This type of analysis along with transcriptome profiling is helpful in understating the biological pathways related to metabolism (Wu *et al.*, 2014; Goldansaz *et al.*, 2017). There are chromatographic techniques that are combined with mass spectrometry such as gas

chromatography coupled to mass spectrometry and liquid chromatography coupled to mass spectrometry. Both techniques work on a similar principle (Haggarty and Burgess, 2017). The gas chromatography coupled to mass spectrometry technique involves the separation of metabolites or biochemical compounds using a gas chromatograph. The liquid chromatography coupled to mass spectrometry technique involves the separation of metabolites or biochemical compounds in a liquid phase (usually water) by chromatography before they are introduced to an ion source (e.g. electron).

7.3 Nutrigenomics in practice

7.3.1 Swine nutrition

The triangle of metabolism: liver, adipose and muscle

These tissues have received the most emphasis by scientists and nutritionists in the context of understanding how the animal behaves under particular nutritional management or how it reacts to specific environmental stressors or management practices (Table 7.1). Regarding the latter, omics analysis have been helpful in understanding how the liver responds to stress (Ren *et al.*, 2018) or fasting, together with adipose tissue (Tao *et al.*, 2017). Also, how muscle metabolism, important for pork production, responds to specific nutritional restrictions (Liu *et al.*, 2017), or to management practices designed to exploit compensatory growth as a nutritional strategy (Lametsch *et al.*, 2006). The understanding of differences or similarities among different types of tissue reserves is an important aspect of the basic knowledge that animal scientists could accomplish with this holistic type of analyses. For example, by simultaneously comparing the transcriptome of subcutaneous and visceral adipose depots, Wang *et al.* (2013) were able to determine the differences underlying the immune function in swine.

Regarding the interaction with specific diets and nutrients, there is ample space for generating knowledge with these technologies and published data are scarce. To date, most of the nutrigenomics work in swine has been as a model for human nutrition, with little interest on generating data useful for the pork industry. A good portion of the existing applicable work concerns sources of fat for pig diets, where the response of muscle and adipose tissue has been analysed to determine dietary effects. Use of sunflower oil in growing pigs as a source of mono unsaturated fatty acids was reported to modulate the fatty acid metabolism of the back fat, and induce a greater turnover. The upregulation of genes related to cell viability indicated a protective role of mono unsaturated fatty acids against oxidative stress and DNA damage. Park *et al.* (2012) studied how type of fat (from beef tallow, olive, soybean or coconut oil) affects fatty

acid composition and insulin signalling-related gene expression in longissimus dorsi muscle. Dietary supplementation with linoleic acid was correlated with proteomic changes that contributed to greater intramuscular lipid content, a trait related to marbling and pork quality (Zhong *et al.*, 2011).

Concerning marbling, Hamill *et al.* (2013) observed that a low protein diet in finishing pigs increased it by altering gene pathways involved in lipid biosynthesis and degradation. However, this nutritional approach negatively impacted protein synthesis pathways, with potential adverse consequences for lean tissue growth. Therefore, its application was suggested by the authors only in the finishing period, when pigs are managed mainly to increase adiposity rather than muscle mass. Muscle tissue also has been found to be responsive to carnitine supplementation (Keller *et al.*, 2011). Carnitine may have beneficial effects on skeletal muscle through stimulating the anabolic insulin-like growth factor-1 pathway and suppressing pro-apoptotic and atrophy-related genes, which are involved in apoptosis of muscle fibres and proteolysis of muscle proteins (Keller *et al.*, 2011). These results underscored how carnitine supplementation can be effective for muscle mass, rather than merely controlling the tissue fatty acid oxidation capacity.

Despite its central role in coordinating metabolism, few have utilised omics tools to study liver-diet relationships. This is opposite to other species (e.g. bovine), where these novel technologies are gaining importance in the analysis of hepatocyte responses. Junghans *et al.* (2004) studied the effect of protein source on the porcine liver, specifically the role of high-value sources such as casein and soybeans in feeding young animals. Data revealed that feeding soybean proteins increased the hepatic proteome related to oxidative stress. This analysis also was important because it correlated the proteome with the transcriptome on the same animals (Schwerin *et al.*, 2002). This is an example of how omics approaches can be integrated in an attempt to obtain a more holistic understanding of the animal. More recently, a proteomic approach was implemented to assess the effect of supplemental zinc oxide in weaned pigs (Bondzio *et al.*, 2013). The authors detected increases in expression of proteins involved in transport, stress response, metabolism, apoptosis and cellular signalling, all of which suggested a healthier and more active liver.

Swine immunity and the gastro-intestinal tract

Swine performance in the face of a disease challenge is becoming progressively more important. Hence, scientists are applying nutrigenomics to better understand the link between nutrients and the immune response to improve animal performance. It is well-established that bioactive food compounds can interact with genes affecting transcription factors, protein expression, and metabolite production in various tissues of an organism. However, it is often forgotten that rations first must 'pass' through the gastro-intestinal tract (GIT) where food compounds (macro- and micronutrients)

are digested, absorbed or fermented before they can reach other body tissues. In the GIT, feedstuffs interact with, and are partly metabolised by enterocytes, where they manifest some of their possible effects, from growth and morphology of the intestinal epithelium, to improving its functionality (i.e. absorption, activity, metabolism) and health.

Weaned piglets are often the target of nutrigenomic immuno-focused studies, as weaning magnifies the stress load of the animal increasing the disease incidence during this period. To better understand the impact on the GIT, a microarray analysis of small intestine enterocytes from (Zhu *et al.*, 2014) revealed how weaning can induce cell cycle arrest, enhancing apoptosis and inhibiting cell proliferation, and increase inflammatory signals and the associated transcription factors. The lower feed intake upon weaning also impacts intestinal gene expression profile (Bauer *et al.*, 2011). Nutritional strategies have been developed to address these issues. For example, amino acid supply and balance in the diet was reported to be critical for GIT functionality and health. For different amino acids and nitrogen-containing compounds (e.g. arginine and N-carbamylglutamate), leucine, and valine data for the interaction of nutrients with enterocyte morphology and its transcriptome have been largely generated with basic transcriptomic tools such as real time-polymerase chain reaction (Wu *et al.*, 2010; Morales *et al.*, 2012; Wu *et al.*, 2012; Yang *et al.*, 2013).

There are reports of high-throughput technologies to better understand the molecular basis underlying the mechanisms of action. Dietary supplementation of glutamine can prevent intestinal dysfunction and atrophy in weanling piglets, but the underlying mechanism(s) are largely unknown. Data from a microarray analysis revealed how supplementation of glutamine in early-weaned piglets (Wang *et al.*, 2008) increased the intestinal expression of genes necessary for cell growth and removal of oxidants, while decreasing the expression of genes that promote oxidative stress and immune activation. At a functional level, the glutamine treatment enhanced intestinal oxidative-defence capacity, prevented jejunal atrophy, and promoted small intestinal growth and body weight gain.

Ren *et al.* (2014) analysed the jejunal proteome in weanling piglets, and data revealed the importance of an amino acid balanced diet. The results indicated that amino acid supplementation to a protein restricted diet improved gut health and mucosal immunity, intestinal nutrient absorption and transport, and enhancing weight gain and feed efficiency. As an example of balancing amino acid intake, Hamard *et al.* (2010) focused on threonine, as the GIT seems to readily absorb and retain it, suggesting its contribution to maintaining a regular gut physiology. Transcriptome analysis in threonine-deficient piglets revealed upregulation of genes involved in immune and defence responses coupled with downregulation of energy metabolism and protein synthesis. Furthermore, microarray analysis highlighted changes in the expression of genes encoding glucose transporters and genes regulating paracellular permeability.

These results support the idea that adequate threonine is important for proper gut functionality.

The increased consumer awareness of drug use has enticed researchers to find feasible alternatives to alleviate GIT problems during weaning. For example, in an attempt to mitigate the immunological stress of weaning challenges with enterotoxigenic *Escherichia coli* (ETEC) have been coupled with different dietary supplements such as zinc. Zinc is used widely as an immunonutrient and modern 'omics' tools are helping understand its efficacy. Transcriptomic analyses revealed how zinc oxide supplementation (Sargeant *et al.*, 2010) decreases markedly the expression of immune response genes concerned with inflammation, and possibly related to the stage of infection, suggesting a mechanism that might influence ETEC infection. Furthermore, proteomic data indicated that zinc oxide supplementation improved the redox state and prevented apoptosis in the jejunum of weaning piglets, thereby alleviating weaning-associated intestinal dysfunction and nutrient malabsorption (including amino acids) (Wang *et al.*, 2009).

Other novel compounds such as phytonutrients are being tested for their ability to be incorporated in diets. Plant extracts such as capsaicin, turmeric oleoresin and garlic extract (Liu *et al.*, 2014), can increase the expression of genes related to integrity of membranes in ETEC-infected pigs, indicating enhanced gut mucosa health. In addition, they can decrease the expression of genes associated with antigen presentation or other biological processes of the immune response, indicating that they attenuate overstimulation of the immune response caused by *E. coli*. These changes were associated with a decrease in diarrhoea incidence in ETEC-challenged piglets fed the diet supplemented with various extracts.

Other than dietary composition, the form in which the diet is supplied also can affect the gut environment. Fermentability, for example, in post-gastric fermenters such as pigs, needs to be accounted for when formulating diets. A metabolomics analysis was conducted on colonic contents of growing pigs fed either high- or low-fermentable carbohydrates or crude protein (Pieper *et al.*, 2012). Irrespective of dietary fermentable carbohydrate, metabolite identification with mass spectrometry and annotation using the Kyoto Encyclopedia of Genes and Genomes metabolic pathways revealed increased abundance of metabolites associated with arachidonic acid metabolism in the colon of pigs fed a high concentration of fermentable crude protein. In the same experiment, urinary metabolites did not reveal distinguishing patterns. Arachidonic acid is normally oxygenated and further transformed into a variety of products, which mediate or modulate inflammatory reactions (Samuelsson, 1991). Thus, a nutritionist should control diet fermentability to avoid developing detrimental conditions in the GIT.

On the same topic, research on resistant starch highlighted its possible use in swine diets to improve colon health and functionality. Haenen *et al.* (2013) performed a genome-wide transcript profiling of colon tissue in growing pigs fed a diet with high levels of resistant starch. The diet induced major changes in colonic gene expression, e.g. induction of oxidative metabolic pathways and suppression of immune response and cell division pathways. The resistant starch diet favoured the growth of microbial populations producing organic acids and inhibited a range of potentially pathogenic microbial groups, thus, leading to the most desirable gut environment. Resistant starch also increased colonic short-chain fatty acid concentration. The short-chain fatty acids fermented from dietary fibre have been recognised as trophic agents that stimulate epithelial cell proliferation in the large intestine (Sangild, 2006). Pea fibre is available as a feedstuff in rural areas devoted to pea production and can be fed to pigs. Microarray data (Che *et al.*, 2014) revealed that growing pigs fed pea fibre had improved colonic barrier, bacterial profiles, and production of short-chain fatty acids, all of which may be associated with unique gene expression profiles encompassing signalling pathways related to better colonic immune response and function.

Besides effects on the GIT tract alone, similar effects have been reported at a systemic level. For instance, organic selenium improved the expression of genes in blood leukocytes that are related to enhanced immunity of pigs (Song *et al.*, 2013), while mannan oligosaccharide supplementation modulates the expression of non-immune and immune-related genes in the same cells. These data indicate that supplementation with 0.2% mannan oligosaccharide (Bio-Mos, Alltech Inc., Nicholasville, KY, USA) benefits the animal by enhancing the immune responses of the pig upon an infection, while preventing overstimulation of the immune system (Che *et al.*, 2011). In addition, supplementation of fermented soybean meal can modulate expression of genes related to inflammatory response and anti-oxidant activity in the whole blood of piglets after an immune challenge (LPS) (Roh *et al.*, 2014). As a consequence, animals fed fermented soybean meal compared with controls had lower serum cortisol, a beneficial effect for growth of nursery pigs during the postweaning period.

In summary, improving and maintaining gut health will optimise its functionality during the digestion process. Such an effect is particularly important during specific periods of the pig life cycle, e.g. the early days after birth. At weaning pigs are exposed to stress that can impair intestinal function and protection, thus, increasing the risk for developing health disorders. The use of nutrigenomics approaches along with measures of animal performance and health clearly has demonstrated that providing nutrients with an immunostimulant capacity will optimise the weaning transition and preserve the performance in pigs.

7.3.2 Poultry nutrition

The gut system and the antibiotic dilemma

The poultry GIT, even more than in swine, plays a central role in the nutritional state of the animal. During the first weeks after hatching, the intestine grows allometrically compared with the rest of the body, at such a high rate that supplementation in the first 96 h post-hatching can have a long-term effect on the animal (Brennan *et al.*, 2013b). Maintaining a healthy digestive system is important to exploit the full genetic potential of these animals; however, this often requires prophylactic use of antibiotics in diets to contain and prevent outbreak of intestinal pathogens that would decrease productivity. Scientists have taken advantage of modern nutrigenomic technologies to study the interaction of the diet with the gut immune system to enhance our understanding of mechanisms and efficacy of different nutritional management approaches.

Microarray analysis has been widely used in broiler nutrigenomics studies. Phytonutrients with immunomodulatory capacity have great potential in modern poultry diets to help maintain a healthy and robust digestive system. Transcriptomic analysis of the tissue itself or the intestinal mucosa leukocytes clearly revealed that products such as carvacrol, cinnamaldehyde, and oleoresin from *Capsicum* spp. (Kim *et al.*, 2010; Lillehoj *et al.*, 2011), anethole (Kim *et al.*, 2013c), garlic metabolites (Kim *et al.*, 2013d) or turmeric (Kim *et al.*, 2013b) are efficacious on the GIT immune response and protection. These compounds can modulate the expression of genes regulating immunity and physiology (e.g. energy and protein metabolism), supporting the idea that plant-derived phytochemicals possess immune-enhancing properties in chickens. Furthermore, anethole, turmeric and garlic metabolites have been tested for their efficacy against pathogens such as *Eimeria acervulina*, attenuating the induction of inflammation that causes gut damage in commercial poultry production (Kim *et al.*, 2013b,c,d).

Prebiotics such as yeast cell-wall products upregulated the expression of oxidative phosphorylation genes in the jejunum, and other genes important in cellular stress response (Xiao *et al.*, 2012). When tested against a common antibiotic (bacitractin), the gene expression profiles in yeast cell-wall-supplemented broilers revealed that biological functions and pathways related to improved health and metabolism were activated (Brennan *et al.*, 2013a). Results from this microarray study indicates that birds given yeast cell-walls underwent changes at a genomic level that corresponded to slower gut cell turnover and, therefore, increased energy utilisation for growth. Probiotics also have been studied using 'omics' tools. The study of the enterocyte proteome in broilers fed *Enterococcus faecium* revealed several differentially expressed proteins related to immune and antioxidant systems, indicating that these chickens employed less nutrients and energy to deal with immune and antioxidant stresses.

These recent findings from nutrigenomics studies clearly offer new avenues for developing effective drug-free alternative strategies for disease control for poultry infectious diseases.

The chicken liver and muscle metabolism

The liver transcriptome represents a photograph, a freeze-frame of metabolism at the sampling time, and different environmental and management conditions such as fasting (Richards *et al.*, 2010), food withdrawal, catching and transport stress (Sherlock *et al.*, 2012) can modify it. The recent increase of feedstuff mycotoxin content due to climatic conditions has sparked interest in understanding their effect on the liver genome. Aflatoxin B1 and deoxynivalenol can have effects on this organ at concentrations as low as 2 to 2.5 mg/kg of feed (Yarru *et al.*, 2009a; Dietrich *et al.*, 2012). These toxins can influence the nutritional and immune status of hepatocytes. Adsorbants (i.e. clays) are being used as a physical solution to diminish the amount of mycotoxins absorbed. However, Yarru *et al.* (2009b) demonstrated that diets enriched in turmeric could have a positive effect counteracting the effect of aflatoxins. Turmeric had a protective effect on the expression of antioxidant-, biotransformation-, and immune system-related genes in the liver of chicks fed aflatoxin B1.

Improvements in systemic immunity were reported with fibre supplementation of inulin to chickens, a fructan used as carbohydrate storage by plants (Sevane *et al.*, 2014). The authors detected an increase in expression of pathways involved in augmenting the immune response of animals. Furthermore, inulin increased the expression of other pathways involved in growth and performance, and production of long-chain fatty acids. Along with improving meat fatty acid profiles, when fed to chickens, inulin acted as a prebiotic and a useful alternative to antibiotics for improving performance and general immunity.

Regarding skeletal muscle metabolism and its relationship with meat yield and quality, thus far, few dietary components have been subjected to 'omics' analysis. For example, proteomics was used to assess the effect of methionine and levels of antioxidants in broiler diets (Stagsted *et al.*, 2004, Corzo *et al.*, 2006, Zhai *et al.*, 2012). Transcriptomics was used to investigate various mechanisms of action on muscle oxidant capacity and metabolism of different sources of dietary antioxidants (algae extracts against the synthetic vitamin E) (Xiao *et al.*, 2011).

Despite the lack of data, next generation sequencing is becoming the method of choice in studies of nutrigenomics of poultry. Pan *et al.* (2013) used RNAseq to study the effect of chromium picolinate (CrPic) on the skeletal muscle genome. The results provided a valuable clue regarding the role of microRNA target genes in the mechanism whereby dietary supplementation of CrPic alters protein synthesis in skeletal muscles of broilers. In fact, Solexa sequencing (Illumina, San Diego, CA,

USA) revealed 57 differentially expressed microRNAs in both control and treated chickens. Those affected microRNAs in birds supplemented with 10 mg/kg of CrPic may play an important role in the proliferation and differentiation of skeletal muscle. Furthermore, CrPic increased serum total protein concentration, while it decreased glucose and triglycerides, which further increased insulin sensitivity in tissues.

7.4 Offspring programming – the epigenetic role of diets

It is becoming increasingly apparent that the environment in utero in which a foetus develops may have long-term effects on subsequent health and performance. Thus, novel nutritional strategies are being studied to better exploit the full genetic value of modern livestock breeds. The goal is to 'program' the offspring in utero to fully express their potential after birth. This involves complex epigenetic mechanisms in which the whole genome, or part of it, are modulated by the environment.

Sow nutrition during gestation is mainly focused on how it may affect the subsequent lactation, but several effects of pre-farrowing diet have been detected on metabolism of litter, suggesting a prenatal effect on genetic predisposition. Nutritionists pay close attention to the energy supply during pregnancy to regulate sow weight and body reserves at farrowing. However, dietary protein supply during gestation can have a great impact on future performance of litters. Oster *et al.* (2014) demonstrated how the liver of pig offspring appeared to be more resilient to nutritional modulation during gestation compared with skeletal muscle. Despite the fewer variations detected, the offspring liver can experience changes in gene expression due to the protein supply during gestation.

A microarray study demonstrated that a high level of protein in the sow diet (~30% w/w) can affect hepatic (Oster *et al.*, 2011) and skeletal muscle gene expression (Oster *et al.*, 2012a), leading to what is commonly known as *in utero* growth retardation. The transcriptomic analysis revealed an altered hepatic responsiveness of energy-sensing pathways involving genes related to growth factor signalling pathways, stress/immune response and the metabolism of energy, nitrogen, lipids and nucleic acids. Similar to changes in the liver transcriptome, skeletal muscle experienced both short- and long-term effects for mRNA expression of genes related to cell cycle regulation, energy metabolism, growth factor signalling pathways, and nucleic acid metabolism (Oster *et al.*, 2012a).

The same analysis was conducted on litters from sows fed approximately 50% of the recommended protein requirements. The hepatic transcriptome revealed downregulation of key genes involved in *de novo* fatty acid synthesis, whereas

several genes associated with lipolysis and phospholipid biosynthesis were shown as upregulated (Doring *et al.*, 2013). No alterations were detected on the hepatic lipid profile, but the authors hypothesised a long-term effect on total body lipids. In skeletal muscle, the low-protein maternal diet altered the expression of genes related to cell cycle regulation, growth factor signalling, lipid metabolism, energy metabolism, and nucleic acid metabolism (Oster *et al.*, 2012b). Some of these changes were also reported into adulthood, around 6 months of age. Proteomic analysis confirmed the adipose tissue transcriptome data, i.e. the low-protein diet increased abundance of proteins related to glucose and fatty acid metabolisms, lipid transport, and regulation of apoptosis, while the high-protein diet induced changes in proteins putatively involved in amino acid metabolism or protein turnover (Sarr *et al.*, 2010).

Molecular 'omics' technologies have also been applied to study the effect of maternal folic acid supplementation during pregnancy on the offspring liver (Liu *et al.*, 2013) and muscle tissues (Li *et al.*, 2013). The findings suggested that maternal folic acid supplementation altered the expression of several hepatic genes that were involved in metabolic regulation and oxidative responses, while muscle data revealed a folate effect on genes associated with myogenesis and intramuscular fat deposition in piglets.

There are fewer published studies dealing with the role of epigenetics in poultry. An example in the nutrition of poultry comes from the work of Rebel *et al.* (2006). Hens were fed a commercial mix with or without vitamins and mineral supplementation, and intestinal samples from their offspring were collected for microarray analysis. Chicks from supplemented hens had an upregulation of genes affecting intestinal turnover, proliferation and development, metabolism and feed absorption. Thus, the maternal diet influenced the functionality of the offspring intestine already at day 3 and 14 of age.

'*In ovo*' technologies provide a clear opportunity for the supplementation of nutrients to embryos (Johnston *et al.*, 1997), as a way to alter the environment in which the animal develops. In practice, this approach turns out to be the poultry version of in utero nutrition. Selenium injections *in ovo* have been tested for their effect on the embryo and future chickens (Hassan *et al.*, 2014). Genes that protect cells against oxidative stress were upregulated during selenium treatment, possibly ameliorating oxidative stress during adipogenesis. *In ovo* injection of selenium also increased the expression of genes involved in adipocyte determination and differentiation, fatty acid uptake and triacylglycerol synthesis, leading to an increase in embryo adipose tissue mass and causing adipocyte hypertrophy.

The above findings will have implications for the regulation of adipose mass with micronutrient levels during early developmental stages. Another example is the injection of glutamine complexes with diamond nanoparticles that enhanced expression of genes correlated with muscle cell proliferation and differentiation,

suggesting an effect on accelerating growth and maturation of muscle cells (Grodzik *et al.*, 2013).

Other applications of *in ovo* technologies involve the use of silver nanoparticles as a protective carrier for molecules like ATP to (Schokker *et al.*, 2015)provide an extra-energy source to enhance molecular mechanisms involved in muscle cell proliferation (Sawosz *et al.*, 2013). *In ovo* injection of carbohydrates also has been evaluated. Glucose injection had a modulatory effect on humoral-related immunity, while fructose or ribose improved the cellular immunity in broiler chickens (Bhanja *et al.*, 2014).

7.5 Gut microbiota in pigs and poultry

In pigs and poultry, the GIT comes into contact with trillions of exogenous microorganisms early after birth or hatching (Han *et al.*, 2018; Kers *et al.*, 2018). Thereafter, a complex symbiotic community of microbes representing all kingdoms of life colonises the GIT and is termed the 'gut microbiota'. The gut microbiota exerts a substantial impact on various functions in the host animal. As the host develops, the microbiota becomes very diverse and develops a mutualistic relationship with the host (Schokker *et al.*, 2015; Cheng *et al.*, 2018). An increasing body of evidence has revealed enormous benefits of the gut microbiota to the host by providing nutrients from indigestible dietary substrates and modulating the development and function of the digestive and immune system that are essential for host development and physiology (Clavijo and Florez, 2018; Metzler-Zebeli *et al.*, 2018). Hence, at least in monogastrics, it is increasingly obvious that disrupting the microbial composition in the gut, coined dysbiosis, has been associated with many metabolic, inflammatory, and neurodevelopmental disorders (Oakley *et al.*, 2014; Arguello *et al.*, 2018). It is now firmly established that the diet plays a fundamental role in shaping the composition of gut microbiota and, thus, impacts host-microbe interactions to determine host growth and development (Frese *et al.*, 2015; Kers *et al.*, 2018). Together, this suggests that a comprehensive understanding of the diet-microbe-host interactions in the near future will help develop new dietary strategies that take into account the needs of the commensal gut microbiota with those of the host, leading to promote growth, maximise feed efficiency, and protect host against pathogenic bacteria. Examples of published work examining the effects of dietary substrates on gut microbiota in pigs and poultry are summarised in Table 7.2.

7.6 Future perspectives

'Omics' technologies have contributed to the understanding of how nutrition may alter biological pathways in pigs and poultry, allowing for a more holistic evaluation of the relationship between nutrition and physiology. The research to date illustrates

Table 7.2 Summary of published studies examining the influence of nutrition on gut microbiota in swine and poultry.

Model	Dietary treatment	Effect	Reference
Swine	Protein restriction	Stimulated beneficial microbial colonisation and metabolite production of the hindgut; promoted gut barrier function. • ileum: ↑ *Peptostreptococcaceae*, ↑ *Escherichia-Shigella*, ↓ *Clostridium* • colon: ↑ *Peptostreptococcaceae*, ↑ *Clostridium*, ↑ *Turicibacter*, *RC9*	Fan *et al.*, 2017
Swine	SUCRAM (artificial sweetener)	Reduced *Campylobacter* populations suggesting that SUCRAM may reduce contamination of meat and boost food safety. • small intestinal mucosa,: ↑ Helicobacteraceae, ↓ Campylobacteraceae • duodenal mucosa: ↑ *Lactobacillus*, ↓ *Campylobacteraceae*	Kelly *et al.*, 2017
Swine	Protein restriction	Caused a shift in microbial composition and metabolite production in the hindgut; maintained growth performance. • caecum: ↑ *Prevotella*, ↑ *Coprococcus*, ↑ *Peptostreptococcaceae*, ↑ *Lachnospiraceae*, ↑ *Erysipelotrichaceae*, ↓ *Lactobacillus* • colon: ↑ *Sarcina*, ↑ *Mogibacterium*, ↑ *Subdoligranulum*, ↑ *Coprococcus*, ↑ *Peptostreptococcaceae*, ↑ *Clostridiaceae*, ↑ *Erysipelotrichaceae*, ↓ *Streptococcus*	Zhou *et al.*, 2016
Swine	Highly-resistant starch	Induced beneficial microbial composition and metabolite production of the hindgut which may impact gut health. • colon: ↑ *Turicibacter*, ↑ *Ruminococcus*, ↑ *Coprococcus*, ↓ *Sarcina*, ↓ *Dorea* • caecum: ↑ *Coprococcus*, ↓ *Sarcina*, ↓ *Clostridium*	Sun *et al.*, 2016
Swine	Low-fat/high-fibre or high-fat/low-fibre	Stimulated beneficial microbial colonisation and metabolite production of the hindgut which may induce better gut health. • low-fat/high-fiber faeces: ↑ *Lactobacilli*, ↑ *Bifidobacteria*, ↑ *Faecalibacterium*	Heinritz *et al.*, 2016
Swine	Grape seed proanthocyanidins	Shifted microbiome composition and phenolic compound production in faeces • faeces: ↑ *Lachnospiraceae*, ↑ *Clostridales*, ↑ *Lactobacillus*, ↑ *Ruminococcaceae.*	Choy *et al.*, 2014
Poultry	Sorghum versus corn grain	Sorghum did not alter growth performance but stimulated the colonisation of beneficial bacteria and reduced the population of unfavourable populations. • small intestine: ↓ *Clostridium*, ↓ *Weissella* • caecum: ↑ *Lactobacillus*, ↓ *Desulfotomaculum*	Fagundes *et al.*, 2017
Poultry	Xylo-oligosaccharides	Improved growth performance and gut health through the stimulation of butyrate-producing bacteria. • colon and caecum: ↑ *Clostridium*, ↑ *Lactobacillaceae*, ↑ *Anaerostipes butyraticus*	De Maesschalck *et al.*, 2015

the potential for molecular 'omics' techniques to provide knowledge needed for fine-tuning nutritional requirements to increase production and health of poultry and pigs, especially during periods of immunosuppression. However, many studies have utilised only one of the 'omics' technologies and tend to only consider one organ. Therefore, an important undertaking in future work will be the integration of techniques and tissue types. Focusing on greater integration of more than one component of the system will generate novel biological data to further our knowledge of animal biology and support the use of nutrigenomics in nutritional management of pigs and poultry.

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8

The adverse effects of heat stress on the antioxidant status and performance of pigs and poultry and reducing these effects with nutritional tools

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Summary points

- Climate change, especially high ambient temperature, will have further harmful impact on agricultural production, including animal husbandry.
- In addition to nutritional knowledge, physiological and biochemical knowledge is paramount to solve the problem of increasing temperature on animal performance.
- Heat stress adversely affects the antioxidant system and energy metabolism of animals and, therefore, the amount and quality of the animal origin food product (e.g. meat) is reduced.
- Different nutritional methods are available to compensate the harmful effects of heat stress in order to produce high quality meat for human consumption.

Keywords: poultry, pig, nutrition, heat stress, antioxidant status, performance

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8.1 Introduction

Interest in climate change and its implications for animal production has drastically increased in recent years. The findings of various international studies show that animal production should be affected more in the near future. IPCC-2014 (Intergovernmental Panel on Climate Change), which in its reports provides the most comprehensive global review of climate changes, issued their latest, Nobel Prize winning fourth report (AR4: IPCC's Fourth Assessment Report) in 2007 (Babinszky *et al.*, 2011a).

In addition to research on nutritional and climatic effects on animal production, there is an increasing need for making climate change and its effects a subject of regular education and a focus of agricultural extension services. As neither the present status of climate change nor its expected development are an unequivocal fact – particularly in consequence to the expected impact of the international treaties for climate protection (Kyoto Protocol, 1998) – the continuous monitoring of the process, of changes and their influence are necessary both from the meteorological and from the user sides (Babinszky *et al.*, 2011a).

The question most frequently raised in connection with climate change is its impact on agriculture (crop and livestock production), and from a broader perspective on our food supply.

It is also known that climate change, in addition to crop production, also has a negative impact on livestock production (Collin *et al.,* 2001; Gonzalez-Esquerra and Leeson, 2005; Huynh *et al.*, 2005; Babinszky *et al.*, 2011c). For animals with high genetic potential, heat stress is a bigger risk factor than cold stress (Niaber and Hahn, 2007). This is the reason why more research focus is required on the possibility of reducing the harmful effects of heat stress. In order to more fully understand how heat stress affects the performance of pigs and poultry, it is necessary to understand the processes pertaining to the utilisation of dietary energy and the impact of heat stress on the antioxidant system of animals.

This chapter focusses on the impact of adverse effects of heat stress on the antioxidant status and energy metabolism, and performance of pigs and poultry. It also aims to demonstrate how to repair the damaged antioxidant system and to improve the performance of animals by means of nutritional tools.

8.2 Impact of heat stress on the antioxidant system of animals

8.2.1 The three level antioxidant system

It is well known that heat stress induces inflammatory processes which results in free radical formation. Stress leads to increased production of reducing equivalents: nicotinamide-adenine-dinucleotide (NADH), nicotinamide-adenine-dinucleotide-phosphate (NADPH), glutathione (GSH) and increases the activity of enzymatic defences (Berry and Kohen, 1999). Reactive oxygen species (ROS) production takes place mainly in the mitochondria, where they are produced by different enzymes of the electron transport chain (xanthine-oxidase, NADPH-oxidase or the cytochrome P450). Furthermore, the lipoxygenase and monooxygenase catalysed reactions also generate ROS. However, living organisms cannot eliminate the entire amount of ROS (Halliwel, 1991). Lipid peroxidation is a process which is generated naturally in small amounts in the organism through the effect of free radicals (Mylonas and Kouretas, 1999).

As can be seen in Figure 8.1, electrons are transferred along the complexes of the electron transport chain. Leaked electrons are able to react with molecular oxygen (O_2)

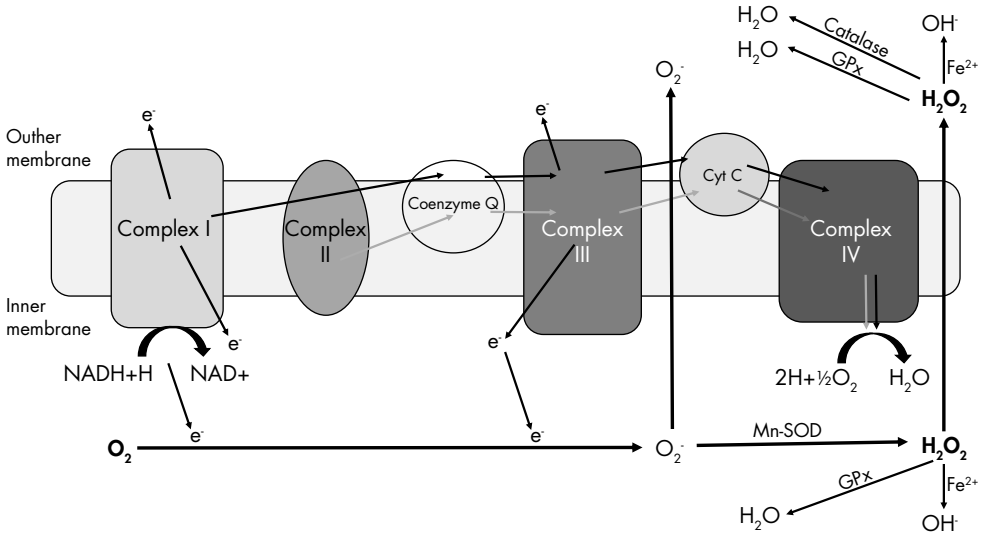

Figure 8.1. Electron transfer along the electron transport chain (Frisard and Ravussin, 2006). Cyt C = cytochrome c oxidase; e^- = electron; Fe^{2+} = iron; GPx = glutathione peroxidase; H = hydrogen; H_2O = water; H_2O_2 = hydrogen peroxide; Mn-SOD = manganese superoxide dismutase; NAD^+ = oxidised nicotinamide-adenine-dinucleotide; NADH = nicotinamide-adenine-dinucleotide; O_2 = oxygen; O_2^- = superoxide anion radical; OH^- = hydroxyl radical.

and form superoxide anions (O_2^-) which are formed under normal conditions within the electron transport chain. With inhibition of electron transport, O_2^- production increases due to the reduction of NADH dehydrogenase.

Free radicals are produced during normal cellular metabolism and can damage constituents of the cell, therefore leading to abnormal cell function and cell death. There is a positive relationship between free radical production and metabolic rate (Frisard and Ravussin, 2006).

The increased oxidative damage may be caused by dysfunction of damaged mitochondria, producing more free radicals than total oxygen consumption, as well as a decline in enzyme activity in Complex I, II, and IV in mice (Desai *et al.*, 1996). Alterations in the complexes result in reduced binding activity and increased production of free radicals. The reduced oxidative capacity can result in accumulation of fatty acids and fat metabolites in the mitochondria. Increased oxidative processes lead to lipid peroxidation and result in dysfunction of mitochondria and reduced oxidative capacity (Schrauwen and Hesselink, 2004). Increased oxidative damage and decreased oxidative capacity can result in increased ROS production, reduced natural defences and mutations in mitochondrial DNA. Heat stress can implicate mitochondrial impairment in the accumulation of oxidative damage. This mechanism provides a direct connection between the electron transport chain and the accumulation of oxidative stress (Frisard and Ravussin, 2006).

The antioxidant defence system plays a very important role in the reduction of the heat stress generated lipid peroxidation process. In heat stress, the antioxidant-pro-oxidant balance is upset and the balance is shifted to the pro-oxidant phase and lipid peroxidation can cause necrosis in the organism. To re-establish the balance, the three level antioxidant defence system will be activated (Figure 8.2). The elimination is done by the first level of the antioxidant system which is working at the same time as the detoxification and regeneration of the second level pathway. The third level is activated after the damage is done, to repair and eliminate damaged cells. Heat stress increases the production of reducing equivalents (NADH, NADPH, and GSH) and increases enzyme activity (Berry and Kohen, 1999).

First level: direct enzymatic pathway

This first level includes the neutralisation of free radicals (oxygen and nitrogen centred) by enzymes (Figure 8.2). An increased amount of free radicals is formed under heat stress which can be partially eliminated by the direct enzymatic pathway. Superoxide anions are formed in the largest concentration in the reaction of molecular oxygen and electrons, which are generated by the electron transport chain. A superoxide anion has to be scavenged or converted into less harmful molecules. It is present in the cytosol and mitochondria. In this reaction, oxidised glutathione (GSSG) is formed

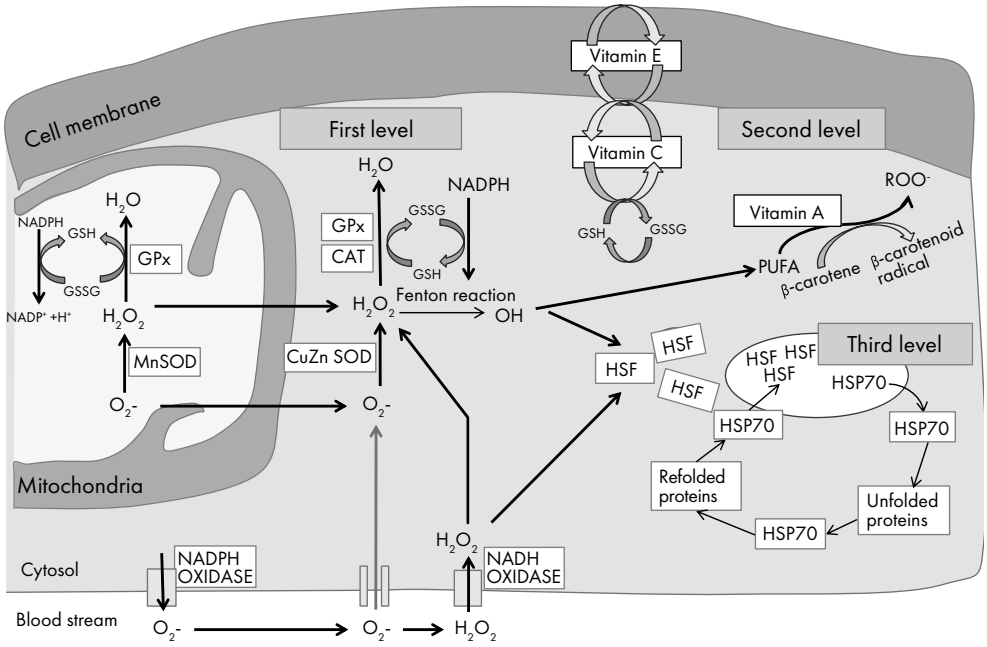

Figure 8.2. Three level antioxidant system (Horváth *et al.*, 2016). CAT = catalase; CuZn SOD = copper/zinc superoxide dismutase; GPx = glutathione peroxidase; GSH = glutathione; GSSG = glutathione disulphide; H_2O = water; H_2O_2 = hydrogen peroxide; HSF = heat shock factors; HSP70 = heat shock protein 70; Mn SOD = manganese superoxide dismutase; NADH = nicotinamide-adenine-dinucleotide; $NADP^+$ = oxidised nicotinamide-adenine-dinucleotide-phosphate; NADPH = nicotinamide-adenine-dinucleotide-phosphate, O_2^- = superoxide anion radical; OH = hydroxyl radical; PUFA = polyunsaturated fatty acids; ROO^- = peroxyl radical.

through the SH-bridge, meanwhile GSSG is transformed back to GSH in the NADPH dependent reaction catalysed by glutathione-reductase (GR). This reaction ensures the oxidative balance in healthy organisms (range of reduced and oxidised form 500:1). GR and the glutathione peroxidase (GPx) are selenium dependent enzymes. They have selenocysteine and selenomethionine in their active centre instead of sulphur-containing amino acids. In the absence of selenium, glutathione-S-transferase is activated and reduces the organic peroxides (Baker *et al.*, 1988). Activity of GPx is dependent on the constant availability of GSH and NADPH which are supplied by the pentose phosphate pathway.

Catalase removes H_2O_2 and converts it into water and molecular oxygen, using iron or manganese cofactor. It is located in the peroxisomes and erythrocytes (Conner and Grisham, 1996; Jeeva *et al.*, 2015).

Second level: small antioxidants molecules

The second level of the antioxidant system includes the detoxification and regeneration reactions of the small molecule antioxidants (Figure 8.2). Vitamin A can combine with peroxyl radicals before they cause peroxidation to lipids (Birben *et al.*, 2012). Glutathione is able to donate a hydrogen or an electron and also to regenerate ascorbate (Meister, 1994; Noctor and Foyer, 1998). Vitamin C is one of the most important small antioxidant molecules with a strong reducing action. As can be seen in Figure 8.3, the reaction with a free radical results in a stable oxidised form with one electron, mono-(de) hydro-ascorbate-radical (MDHA). It is capable of binding a subsequent free radical and form a dehydroascorbate (DHA) radical. In the detoxification process, where the superoxide anion is eliminated, hydrogen peroxide is formed, which is transformed into water by the ascorbic acid peroxidase. The regeneration of ascorbic acid can occur in two different ways. In the first, dehydroascorbate-reductase (DHAR) transforms DHA into ascorbic acid during the oxidation of GSH. The work of the DHA/DHAR system results in GSSG, which is transformed into GSH by GR using NADPH. The second way is a reaction catalysed by the dehydroascorbate-reductase

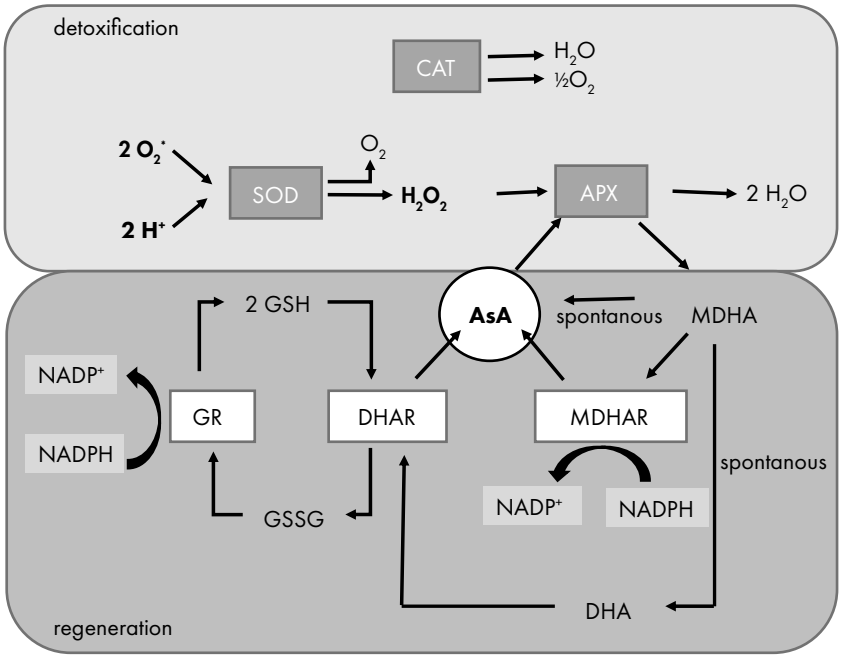

Figure 8.3. The detoxification and regeneration method of ascorbate (Gross *et al.*, 2013). APX = ascorbic peroxidase; ASA = ascorbic acid; CAT = catalase; DHA = dehydroascorbic acid; DHAR = dehydroascorbic acid reductase; GR = glutathione reductase; GSH = glutathione; GSSG = glutathione disulphide.; MDHA = monodehydroascorbic acid; MDHAR = monodehydroascorbic acid reductase; SOD = superoxide dismutase.

(MDHAR), in which MDHA is reduced to ascorbic acid. This reduction can also be spontaneous. In the course of the other regeneration pathway, which works simultaneously, GSH reduces dehydroascorbic acid to ascorbic acid at the same time with the oxidation of NADPH. The DHA is also formed spontaneously from MDHA (Rose and Bode, 1993; Braun *et al.*, 1997; Pignocchi and Foyer, 2003).

It is well known that ascorbate is not essential for poultry because the kidney, the liver or both organs are capable of synthesis (Chatterjee, 1973; Bánhegyi *et al.*, 1997; Maurice and Lightsey, 2007). Liver necrosis begins because of heat stress and this is indicated by the increased concentration of lipofuscin. Since heat shock reduces the synthesised amount of ascorbic acid, the elimination pathway of the H_2O_2 becomes insufficient and the process of lipid peroxidation accelerates.

Vitamin E has high antioxidant property and is of high importance as a small antioxidant molecule in animal nutrition because only plants can synthesise it (Chan and Decker, 1994). α-Tocopherol is a lipid soluble antioxidant, which can protect the unsaturated fatty acids from peroxidative damage. It also plays a significant role in the protection of the endoplasmic reticulum and other membrane systems due to its chain breaking property. The evidence of its antioxidant property is that one vitamin E molecule can protect 2000 phospholipid molecules against oxidative damage (Packer, 1992).

Vitamin E transforms peroxide radicals (e.g. H_2O_2) in cell membranes. During this process, vitamin E loses one proton and a less active and resonance stable, oxidised vitamin E is formed. This is called an α-tocopheril radical (Duthie, 1996; Kregel, 2002; Blokhina *et al.*, 2003). Regarding the cell's antioxidant status, the reduction of tocopherols from oxidised form to biological active form has high importance (Porter, 1992). In this process, the presence of vitamin C (Chan, 1993, Tanaka *et al.*, 1997) and GSH (Wu *et al.*, 2004) are very significant. In this reaction, L-ascorbic acid is a proton donor; it can reduce the α-tocopherol radical and, thereby, regenerate the biologically active vitamin E.

Third level: chaperon proteins

Heat shock proteins (HSP) are one of the most outstanding research areas today (Figure 8.2 and 8.4). They are small proteins and are grouped by their molar weight: HSP110/90/70/60. Chaperones are present in all living cells, where they coordinate transport and apoptotic processes. Endogenous and exogenous stress factors can induce the production of chaperones by enhancing their expression. The synthesis of chaperones is promoted by the heat shock factors (HSF) (Morimoto, 1998; Santoro, 2000; Åkerfelt *et al.*, 2010).

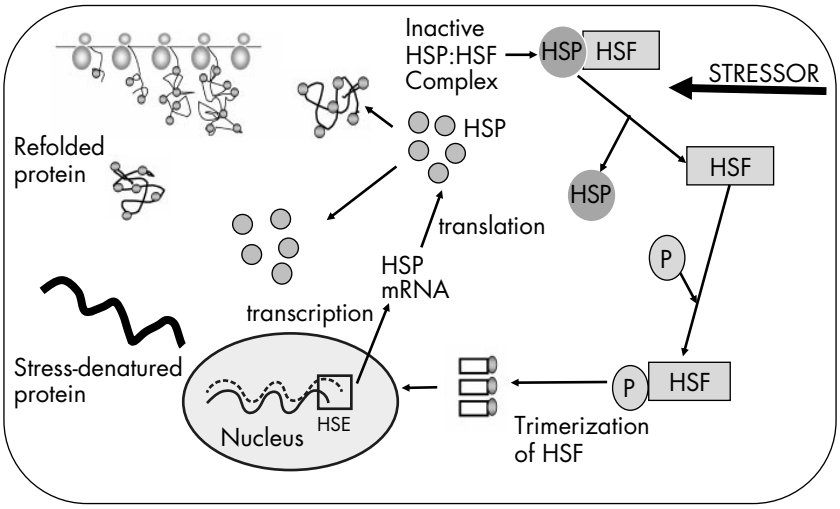

Figure 8.4. The activator of HSP70 expression and mechanisms of action (based on Kregel, 2002). HSF = heat shock factor; HSP = heat shock protein; ROS = reactive oxygen species.

HSF are located in the cytosol; they are bonded by HSP in an inactive state. As can be seen in Figure 8.4, in the case of a stressor (e.g. heat stress), the chaperones dissociate from the HSF. Then, HSF are phosphorylated, form trimers and migrate into the nucleus where they bind to the heat shock elements. This linkage initiates the transcription of HSP mRNA and it leaves the nucleus to the cytosol, where new HSP are synthesised. (McMillan *et al.,* 1998; Kregel, 2002).

The best-known representative of chaperones is HSP70, which is present in cytoplasm. The most important functions of chaperones are: repair of protein structures, degradation of permanently damaged proteins and control of apoptotic pathways. They inhibit c-jun-kinase with the inhibition of the Cascade-8 receptor. Inhibiting the depolarisation of mitochondrial membrane, they can also regulate the outflow of cytochrome-c (Asea, 2005). In addition, chaperones also control the development of the immune response, because they stimulate the cytokine synthesis and the release of ROS, which are necessary for phagocytosis (Lehner *et al.*, 2000; Panjwani *et al.*, 2002).

8.2.2 The effect of heat stress on the antioxidant system in poultry and pigs

It is known that heat stress causes and increased ROS formation, which can lead to disruption of mitochondrial function, decreased vitamin concentrations, dysfunction in the antioxidant enzymes, increased lipid peroxidation and oxidative stress and also cause DNA damage. Short term heat stress (35 °C, 3 h/d) decreased the activity of mitochondrial respiratory chain according to Yang *et al.* (2010). Lin *et al.* (2006)

examined the effect of heat stress (32 °C for 6 h/d) on the liver and heart of broilers. They determined thiobarbituric acid reacting substances (TBARS) which are formed during lipid peroxidation and the amount shows the rate of lipid peroxidation. They found that the level of TBARS increased in liver but there was no difference in the TBARS concentration in heart indicating that the rate of lipid peroxidation was larger in the liver. Cui *et al.* (2016) found that heat stress caused reduced liver weight and also increased apoptosis in pig liver. The activity of GPx, superoxide dismutase and catalase in blood samples increased under heat stress in poultry (Altan *et al.*, 2003; Ramnath *et al.*, 2008; Akbarian *et al.*, 2015) and also in pigs (Yang *et al.*, 2014; Liu *et al.*, 2016). It is known that the intake of vitamins and micro minerals decreases during heat stress (Patience *et al.*, 2005; Lin *et al.*, 2006). Based on the so-called 'antioxidant theory', if the amount of antioxidants and vitamins decrease in the blood, lipid peroxidation will increase, which can lead to cell and tissue damage. The increased amount of oxygen free radicals and/or heat stress induces the expression of HSP (Salo *et al.*, 1991; Zulkifli *et al.*, 2009; Lara and Rostagno, 2013; Liu *et al.*, 2016).

8.2.3 Reducing the negative effects of heat stress with different nutritional tools

Feed additives e.g. vitamins and micro minerals which have direct or indirect antioxidant effects can be used to reduce the effects of heat stress (Renaudeau *et al.*, 2012). Vitamin A (15 000 IU/kg feed) supplementation decreases lipid peroxidation in poultry (Kucuk *et al.*, 2003). Vitamin C (500 mg/kg feed) increased plasma ascorbic acid concentration and stress response, while also decreasing HSP 70 expression in poultry (Mahmoud *et al.*, 2004) and decreasing rectal temperature in pigs (250 mg/kg feed; Adenkola *et al.*, 2009). Vitamin E in poultry diets (200, 250 mg/kg feed) decreased lipid peroxidation, as well as the activity of superoxide dismutase, catalase and GSH, GR concentrations in their blood (Sahin *et al.*, 2002a; Maini *et al.,* 2007) and improved resistance against heat stress in pigs (Zhao and Guo, 2005; Cottrell *et al.*, 2015; Liu *et al.*, 2016). Zinc supplementation (30, 60 mg/kg feed) also decreased lipid peroxidation in poultry (Kucuk *et al.*, 2003; Sahin *et al.*, 2006; Kucuk, 2008) and improved gut health in pigs (Fernandez *et al.*, 2014; Pearce *et al.*, 2015). Selenium supplementation (0.2-1 mg/kg feed) decreased lipid peroxidation (Sahin *et al.*, 2002a) and increased GPx and GSH concentration in poultry blood (Mahmoud and Edens, 2003; Harsini *et al.*, 2012) and in pigs (Liu *et al.*, 2016).

8.3 Impact of heat stress on energy metabolism in pig and poultry

8.3.1 Thermoneutral zone and thermoregulation of farm animals

In order to better understand how climate change affects livestock performance, it is necessary to become acquainted with the basis of livestock production, and particularly with the processes pertaining to the utilisation of dietary energy, since the ambient temperature has a major impact on the energy metabolism of food producing farm animals. The concept and importance of the thermoneutral zone and the thermoregulation of animals are briefly reviewed below for this purpose.

Physiological processes are associated with heat production, which is the sum total of non-productive energy utilised by the animal and of the energy 'lost' in the course of converting dietary nutrients. Non-productive energy is used for maintenance, i.e. it satisfies the energy requirement of such essential physiological processes as the maintenance of body temperature, the nervous system, organ functions, ion pumping and the energy requirement for minimal activity. The extra heat produced in the course of digestion, excretion and metabolism of nutrients is called the heat increment. Within a certain range of ambient temperatures and with constant feed and nutrient intake, the total heat production of the animal remains constant (Figure 8.5). Therefore, when energy intake changes, the thermoneutral zone changes. At

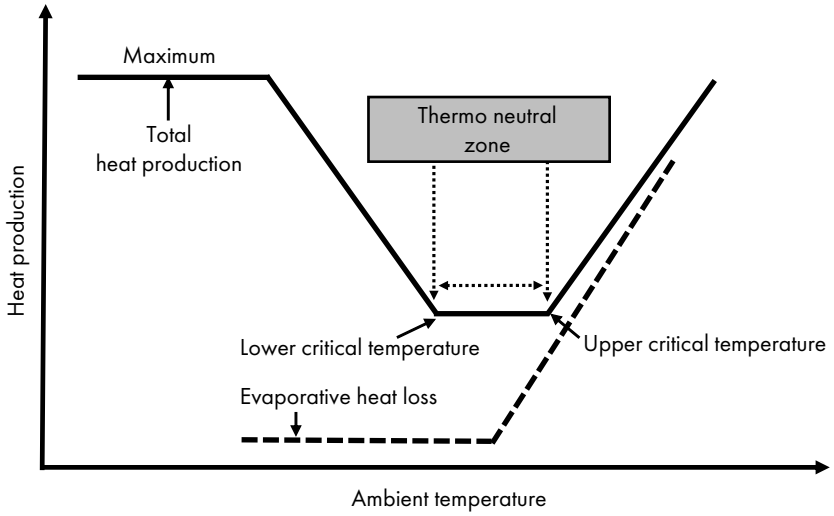

Figure 8.5. Relationship between ambient temperature and heat production of farm animals.

Table 8.1. Recommended thermal conditions for swine (FASS, 1999).

Type and weight	Preferred range (°C)	Critical temperature (°C)	
		Lower	Upper
Lactating sow with piglets	15-26 (sow)	15 (sow)	32 (sow)
	25-32 (piglets)	25 (piglets)	35 (piglets)
Prenursery, 3-15 kg	26-32	15	35
Nursery, 15-35 kg	18-26	5	35
Sow, boar>100 kg	10-25	-20	32

higher intake, the lower critical temperature is lowered and also the upper critical temperature is lowered.

This temperature range is called the thermoneutral zone. In a thermoneutral environment, the heat production of the animal is at its lowest level, and thus the dietary energy can be used for production (growth, egg and milk production) efficiently. Unfavourable temperatures (too cold or too hot environments) lead to increased heat production by the animal, i.e. there is more loss of energy, and in consequence, less energy remains for production at the same level of energy intake, and the efficiency of energy utilisation deteriorates. The upper and lower critical temperatures for different types of pigs are shown in Table 8.1.

8.3.2 Impact of heat stress on energy metabolism in pigs and poultry

Studies on the relationship between climate change and the performance of farm animals primarily focus on the interactions between meteorological factors (e.g. air temperature, relative humidity, air movement, radiation, photo periods, precipitation, weather fronts, air pressure) and voluntary feed intake. Since a close relationship exists between feed intake and the heat production of animals, any alteration of the voluntary feed intake and/or the digestible energy level of the ingested feed will alter the heat production of the animal, as well. In addition to body weight, condition and production level, it is the ambient temperature and humidity that most influence voluntary feed intake. The influence on voluntary feed intake is so significant also because the most common limiting factor in livestock production is sufficient intake of digestible nutrients to meet the requirements of production. This is because animals generally prioritise available nutrients to support maintenance needs first, followed by growth or milk production, and then reproduction. Breeds of cattle, pig and poultry with high production levels are highly sensitive to changes in the environment and

can only achieve their genetic potential in a thermoneutral environment where their requirements are met. Otherwise, adaptation to the adverse effects of the environment is associated with energy losses and in consequence with a loss of production. If the harsh environmental factors are lasting and intense, they can lead to a decline of immune resistance and finally to disease.

As can be seen in Figure 8.6, increased thermal dissipation during heat stress in a pig reduces physical activity that produces metabolic heat, and increase radiant and evaporative heat loss. The resultant shift in the physiological and metabolic status of the pig places a premium on heat dissipation at the expense of processes beneficial for efficient pig production.

8.4 Impact of heat stress on pigs and poultry production and elimination of adverse effects by nutrition tools

8.4.1 Pigs

The warming of the climate can mean more days per year of heat stress load for the pig, especially for the adult animal, affecting several production traits. In the breeding animal, the constrained heat dissipation causes appetite and feed intake

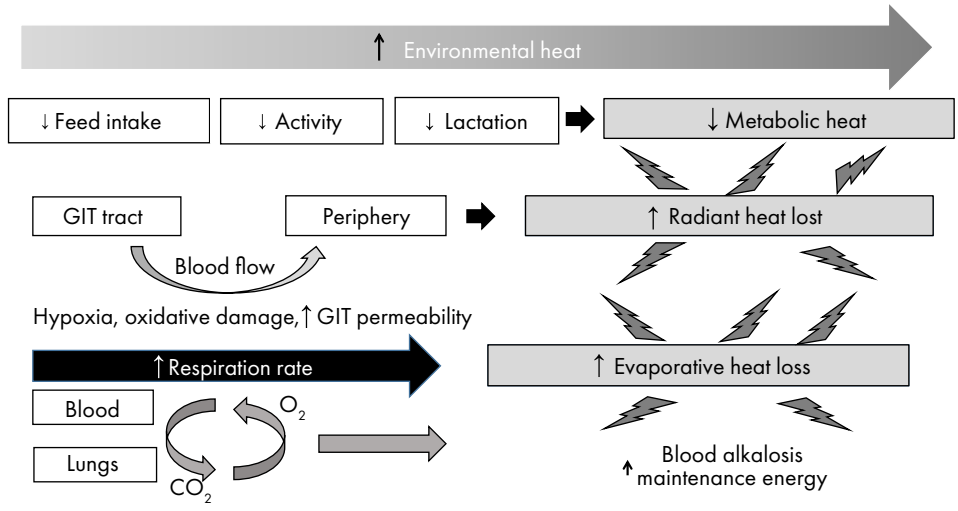

Figure 8.6. Overview of heat management in the pig (Cottrell *et al.*, 2015).

problems. In the case of extreme high temperatures, the associated heat dissipation difficulties may lead to passive hyperthermia, which has an adverse impact on feed intake. In consequence, lactating sows do not consume their daily ration and, as a result their milk production is suboptimal, piglets receive less milk, grow more slowly, and starvation and mortality may become more prevalent. According to the studies, however, feeding high fat diets (125 g fat per kg of dry matter) to sows during lactation during the hot season leads to decreased heat production, which may influence the energy and feed intake of the sows.

Feeding high fat diets also improves the energetic efficiency of feed energy into milk production when compared to sows fed high starch diets (with low dietary fat levels), because synthesising milk fat from dietary fat is more efficient than synthesising dietary fat from e.g. dietary carbohydrates (Babinszky, 1998). Heat may also have negative consequences on fertility parameters. The quality of eggs and sperm deteriorates, embryo mortality between day 1-15 increases, maturity is delayed, and the number of sows returning to oestrus is higher. The heat dissipation, evaporation capacity of pigs is limited, which is the reason why they are sensitive to heat stress. Heat becomes an adverse factor when the ambient temperature exceeds the thermoneutral zone of the pig. In nursery pigs, the unfavourable influence of warming is less expressed because their heat requirement in the early phase of growth is high and their heat tolerance is better towards the end of the nursery phase. However, warming may lead to a considerable loss of both appetite and weight gain (Babinszky *et al.*, 2011a).

For fattening pigs, a hot environment leads to loss of appetite, decreased feed intake and poorer feed conversion, which suggest that pigs are less able to utilise the dietary energy efficiently, and thus more than 35% of the metabolisable energy in the diet is used for maintenance (Baker, 2004). As a result of heat stress, fattening parameters decline, weight gain decreases even more than feed intake, and meat quality parameters (pH_2) suffer, as well (Table 8.2).

The reduction in feed intake is 20% at 25 °C, 40% at 30 °C, and 60% at 35 °C. After hot periods of 30-33 °C, pigs display compensatory growth, overcome their heat stress and grow further, but they cannot overcome temperatures of 36 °C. Temperatures higher than their thermoneutral zone by 8 °C can also affect the digestibility of nutrients (Quiniou *et al.*, 2000). The decreasing quantity of ileal digestible lysine has a negative impact on the daily weight gain, daily protein deposition and feed conversion rate as well (Babinszky and Halas, 2009). Due to the deviation from the specific ileal digestible lysine / digestible energy ratio, the fat content of the body increases, i.e. the quality of meat may deteriorate.

The most important action for alleviating the impact of heat stress is to open up enclosed buildings, in order to increase their cubic capacity. Outdoor pens become more important for breeding animals, and further solutions can be to establish using

Table 8.2. Fattening performance of pigs fed restricted or *ad libitum* kept in heat stressed vs a normal environment (Wittmann *et al.*, 1997).

Parameter	Heat stress		Thermoneutral	
	Rationed	*Ad libitum*	Rationed	*Ad libitum*
Initial weight (kg)	33	33	27	27
Finishing weight (kg)	103	104	100	106
Weight gain (g/d)	609	619	632	685
Feed intake (g/d)	1.98	2.08	1.96	2.20
Feed conversion rate (kg/kg)	3.25	3.36	3.10	3.21
pH_2	5.70	5.71	5.57	5.59

sprinkler systems, wallows, and to cool the buildings with adiabatic systems or heat exchangers.

8.4.2 Poultry

The thermoregulation system of poultry differs considerably from that of mammals. They have no sweat glands, and due to their feathers, they can dissipate less heat through their skin; evaporative cooling is achieved by panting. The efficiency of evaporative cooling improves as humidity declines. The efficiency of heat dissipation is indicated by the rate of increase in body temperature, the changes in blood pH and the appearance of heat stress proteins. The feed intake of poultry species will drop rapidly with extreme high temperatures. In layer hens, this reduction can be as high as 30-35% at 30 °C, when compared to values measured at 20-22 °C. In excessive heat, the elevated body temperature associated with feeding means an additional load on the bird struggling with an already higher body temperature. This kind of passive hyperthermia can be prevented if birds are denied access to feed during the hottest times of the day. The temperature of 33 °C is determining for poultry, because up to this point, through a better feed conversion rate and lower basal metabolic rate, birds are able to compensate for the energy loss caused by the lower feed intake. Above 33 °C, the feed and energy intake declines to such an extent that birds are not able to compensate for it, and the energy balance of the body can become negative. This means that energy content of the body declines and thus production declines rapidly or can even be negative. In broiler chickens, body protein decreases, body fat content increases; skin damage and haemorrhages in muscle tissues are frequently encountered during processing.

Broilers were observed to respond to high ambient temperatures with decreased protein synthesis and increased protein breakdown (reviewed by Lin *et al.*, 2006). This appears to be supported by trial findings that report lower body protein and muscle tissue protein, in addition to higher fat levels in heat stress (Aksit *et al.*, 2006). The deterioration of meat quality is not limited to the altered protein/fat ratio, as the mobilisation of minerals and vitamins from tissues due to heat stress (Sahin *et al.*, 2009) further compromises the nutritive value of eggs and meat (Fouad *et al.*, 2016). The prevalence of other deficiencies of meat quality, such as high drip loss, too pale colour (Aksit *et al.*, 2006; Table 8.3), and PSE (pale, soft and exudative) meat also increase and these contribute to a significant decline in consumer confidence.

Another consequence of summer heat is that poultry species lay smaller eggs (the change in hen eggs is 5-6 g, turkey eggs 10-11 g and goose eggs 10 g). The heat stress can also affect the egg shape index, and may impair eggshell thickness and strength. Acute heat stress may alter the functioning of the reproductive tract and have an adverse effect on fertility. Heat stress also induces immune suppression, the concentration of antibodies decreases and the risk of viral and mycoplasma infection increases. Measures to control heat stress involve changes in the housing technology (over-pressure and exhaust ventilation systems, natural ventilation, cooling panels, humidifiers) and in the feeding technology (granulating, fat supplements, vitamin C and E supplements), as well as the additional solution of improving the heat tolerance of birds by applying heat treatments at a young age. Therefore, changing weather

Table 8.3. Effect of rearing temperature on carcass and breast meat percentage, meat composition and some meat quality traits (Aksit *et al.*, 2006).[1]

Rearing temperature[2]	Yield		Meat nutrient composition			Meat colour parameter[3]		
	Carcass (%)	Breast (%)	Moisture (%)	Protein (%)	pH	L*	a*	b*
Control	73.8^{a}	29.9^{a}	74.7^{a}	23.5^{a}	6.00^{a}	48.94^{a}	2.99^{a}	4.19^{a}
28-22 °C	72.9^{b}	29.5^{a}	74.4^{a}	22.1^{ab}	5.95^{ab}	53.04^{b}	4.49^{b}	5.71^{b}
34 °C	71.5^{c}	28.4^{b}	72.4^{b}	21.6^{b}	5.89^{b}	53.94^{c}	5.21^{b}	4.44^{a}
SEM	0.2	0.2	0.2	0.3	0.02	0.30	0.28	0.31
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003

[1] Means in the same column with different superscript differ significantly.

[2] Control = temperature was maintained at 22 °C; 28-22 °C = temperature was 28 °C from 10:00 h to 17:00 h and at 22 °C from 17:00 h to 10:00 h; 34 °C = temperature was kept at 34 °C from 3 to 7 weeks of age.

[3] L*= lightness; a* = redness, b* = yellowness.

conditions are likely to force many livestock producers to prepare for moving into buildings where the microclimate can be regulated.

Farm animals must gradually adapt to the changing conditions of the environment, as rapidly changing environmental factors or prolonged extreme conditions may radically decrease production due to the evolving heat stress. Summarising the relevant scientific findings on pig and poultry studies, it can be stated that, in general, six different nutritional interventions are recommended in the case of high ambient temperature (heat stress) (Babinszky, 1998; Noblet *et al.*, 2001; Schrama *et al.*, 2003; Babinszky *et al.*, 2011b,c; Horváth *et al.*, 2016):

- using more antioxidant vitamins (vitamin A, C, E, etc.) and micro minerals (e.g. zinc, selenium) in the diets to support the antioxidant system of the animals;
- supplementing diets with monovalent ions (Na- and K-bicarbonate, K-hydrocarbonate, K-sulphate) to alleviate the reduction of water retention in the animal's body;
- feed a more concentrated diet to (partly) counteract low feed intake;
- feeding higher fat content diets to reduce heat production of animals;
- feeding low protein diets with synthetic amino acids according to the ideal protein concept;
- adding dietary betaine.

Betaine (trimethylglycine) is an intermediate metabolite in the catabolism of choline, which can modify osmolarity, acts as a methyl donor, and has potential lipotropic effects (Metzler-Zebeli *et al.*, 2008). Schrama *et al.* (2003) showed that under thermoneutral conditions, dietary betaine supplementation (1.23 g/kg) reduced the total heat production of pigs.

8.5 Conclusions

It is clear that climatic conditions will directly influence animals and their productivity with the expectation that climatic conditions will likely involve higher temperatures. Associated with heat stress are changes in the antioxidant system. In recent years, much knowledge has been obtained from studies on this topic. A short survey is presented in this chapter. The climatic changes can also influence changes in feed intake. This, in turn, will affect energy metabolism and thus heat production in animals.

Based on the literature findings, it can be stated that different methods are to compensate the harmful effects of heat stress: using more antioxidant vitamins (e.g. vitamins C, E) and micro minerals (e.g. zinc, selenium) in the diets, using more energy and nutrient concentrated diet, feeding higher fat content diets, feeding low protein diets with synthetic amino acids according to the ideal protein concept and adding dietary betaine to diets.

8.6 Future perspectives

Although the technological advances in the everyday management (i.e. housing, ventilation and cooling systems) and different nutritional strategies have partially alleviated the negative consequences of heat stress, it continues to be a significant financial burden for farmers. As such more knowledge on the relationship between heat stress and nutrient supply and its pathophysiology impacts on e.g. carbohydrate and lipid and energy metabolism, anti- and prooxidant balance and on immune status and intestinal function in animals is needed for future animal production. The effect of different types of heat stress (permanent high, or cyclical: morning, evening relatively low and at midday and afternoon high ambient temperature) on the energy and nutrient metabolism and the defence systems of animals are also a key issues. It is very likely that further molecular biological knowledge and research will be needed in the future to answer these questions to produce high quality animal food products despite climate change (heat stress).

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9

Using non-invasive synchrotron-based analytical techniques in animal nutrition: a novel approach

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Summary points

- Advanced synchrotron-based bioanalytical techniques are able to reveal interactive association between feed intrinsic structure at both cellular and molecular levels and nutrient metabolism and availability in animals.
- These cutting-edge techniques can bring a new level of understanding of feed structure on a molecular basis in relation to biological functions in animals.

Keywords: synchrotron radiation, molecular nutrition, molecular structure, macromolecular sensitivity and response to processing/treatments, nutrient metabolism and availability

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9.1 Introduction

Cutting-edge advanced synchrotron radiation-based infrared microspectroscopy (SR-IMS) and synchrotron radiation-based X-ray spectroscopy have been developed as rapid, direct, non-destructive and bioanalytical techniques (Yu *et al.*, 2004; Yu, 2012a). These advanced techniques are making contributions to advances in animal nutrition, feed structure, feed chemistry, and feed science technology (Yu, 2012a; Rodríguez-Espinosa *et al.*, 2019). Conventional 'wet' chemical methods often result in destruction of the intrinsic structures of feeds during processing for analysis. In contrast, these spectroscopy techniques take advantage of the brightness of synchrotron radiation and small effective source size and are, thereby, capable of exploring the molecular chemistry within microstructures of a biological tissue without destruction of the inherent structures at ultra-high spatial resolutions within cellular dimension (Marinkovic *et al.,* 2002; Yu *et al.*, 2004). To date, little application of these advanced non-invasive techniques exists in feed science and animal nutrition.

Synchrotron radiation is produced as accelerated electrons in ultra-relativistic speed passing through a magnetic field, which is extremely high flux and bright light with a broad spectrum (CLS, 2013; Synchrotron Radiation, 2013). In other words, 'synchrotron radiation' is actually a 'photon', which is produced from a giant particle accelerator, called 'synchrotron facility or center'. This giant particle accelerator usually has several major components which include: Electron Gun, Linear Accelerator, Booster Ring, Storage Ring, Beamline and End Experiment Station. Scientists usually work at the experimental station (CLS, 2013; Synchrotron Radiation, 2013).

Synchrotron radiation covers the full electromagnetic spectrum, which includes infrared, soft X-ray and hard X-ray (Australian Synchrotron, 2013). Usually scientists select specific wavelengths or a broadband light of synchrotron radiation to investigate the structure of molecules. First, synchrotron radiation is collected and directed by a collimating mirror system to an experimental end-station, and a broadband white light or a required narrow bandwidth spectrum is selected by a monochromator or modulated by utilising a Michaelson interferometer directed at the sample. A detector system is employed to acquire very large amounts of data, control experiments, and measure the amount of light that is absorbed, reflected and/or scattered by the molecules. In this way, synchrotron radiation can be used to study matter on a molecular basis (CLS, 2013; SSRF, 2013; Synchrotron Radiation, 2013).

When bright infrared synchrotron radiation is coupled with a Fourier transform infrared microspectroscopy end-station, it is called synchrotron-radiation-based Fourier transform infrared microspectroscopy often abbreviated as 'SR-IMS' or 'SR-FTIRM'. Figure 9.1 shows a synchrotron-based analytical technique 'SR-IMS' with the Bruker System at SPring-8, Japan Synchrotron Radiation Research Institute (JASRI, beamline scientist Dr Yuka Ikemoto). Figure 9.2 shows a 'SR-IMS' with both

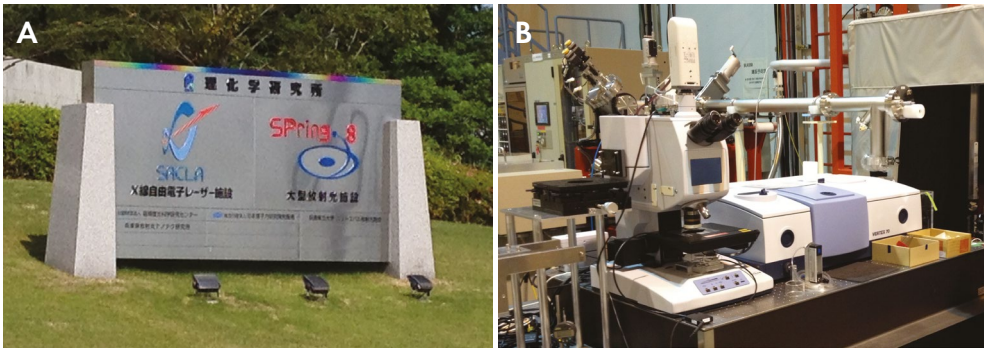

Figure 9.1. Advanced synchrotron radiation-based Fourier transform infrared microspectroscopy (SR-IMS) at SPring-8, Japan Synchrotron Radiation Research Institute (JASRI). (A) SPring-8 (synchrotron facility), Japan; (B) synchrotron radiation-based infrared microspectroscopy (SR-IMS at SPring-8).

Thermo-Nicolet and Bruker Systems at the National Synchrotron Radiation Research Center (NSRRC, beamline scientist Dr Yao-Chang Lee) in Taiwan. These analytical techniques can explore the chemical make-up at a cellular and molecular level within intact tissues without destruction of inherent structures, unlike conventional wet chemical analysis (Wetzel *et al.*, 1998; Yu *et al.*, 2004; Dokken, 2006; Miller and Dumas, 2006; Yu, 2010; Yu *et al.*, 2019).

The SR-IMS and SR-FTIRM have mainly been applied to research fields in medicine, physics, and chemistry (Miller *et al.*, 1998; Holman *et al.*, 2002; Marinkovic *et al.*, 2002; Miller and Dumas, 2006; Marinkovic and Chance, 2006). Since the development of this technique, it has made contributions to advances in science and technology (Marinkovic and Chance, 2006; Miller and Dumas, 2006). However, to our knowledge, there is little application to date of these advanced synchrotron-based analytical techniques in agricultural sciences research in fields such as feed science, feed processing, animal nutrition, food science, crop breeding, etc. One of the reasons is that researchers in agricultural areas are mostly unaware of these developed advanced non-invasive analytical techniques.

This chapter reviews applications of synchrotron-based analytical techniques as non-invasive techniques in animal nutrition and feed science research. Advanced synchrotron-based bioanalytical technique can bring a new level of understanding of feed structure on a molecular basis in relation to biological functions in animals.

Figure 9.2. Advanced synchrotron radiation-based Fourier transform infrared microspectroscopy (SR-IMS) at National Synchrotron Radiation Research Center (NSRRC), Taiwan. (A) NSRRC synchrotron facility; (B) two synchrotron radiation-based infrared microspectroscopy at the station BL14.

Figure 9.2. Continued. (C) left: Burker System (SR-IMS); and right: Nicolet system (SR-IMS); (D) left: IR end-station (SR-IMS); and right: endstations in the IR hutch at TLS.

9.2 Working principles of a synchrotron

There are more than 25 synchrotrons world-wide with most of them located in Europe, North America, South America, Asia, and Australia. Most of the synchrotrons are 2nd or 3rd generation which differ in brightness and flux. Synchrotron facilities have also a variety of target energies. For example, the Canadian National Synchrotron Facility-CLS has a 3rd generation synchrotron with 2.9 GeV of target energy. Usually, a 3rd generation synchrotron is at least 1000 times brighter in terms of infrared than a 2nd generation synchrotron. The target energy values of the ESRF synchrotron, Japan SPring-8, USA-APS, TLS and TPS at NSRRC in Taiwan, Australian synchrotron, and Shanghai SSRF are 6.04, 8.0, 7.0, 1.5 (TLS) and 3.0 (TPS), 3.0, and 3.5 GeV, respectively. The very new synchrotron NSLS-II (US Dept of Energy, Long-Island, NY), is also a 3rd generation synchrotron that is presently being commissioned.

A synchrotron has six major components (Australian Synchrotron, 2013; CLS, 2013). The function of each of the components is as follows: The function of the electron gun (EG) is to blast out electrons. The linear accelerator is used to produce electrons

at a relativistic speed. The relativistic electrons are then injected into the Booster ring to further increase their energy to the target energy level, before it will be injected into the Storage Ring. Different synchrotron facilities in the world have different target energy levels from 1.2 to 8.0 GeV. In the Storage Ring, the beam current is further increased. For 1 h, relativistic electrons can travel a distance one billion km. As high energy relativistic electrons pass through a magnetic field, a natural phenomenon occurs, extremely brilliant light is being produced also known as synchrotron radiation or synchrotron light (Australian Synchrotron, 2013; CLS, 2013). A special magnet called the 'undulator' causes electrons to rapidly change course and emitting vast amounts of energy in the form of photons. These photons enter the beamline and are directed to a photon dispersive device called 'monochromator', which selects a specific bandwidth required for each experiment. Finally, light is focused on a sample at the Experimental Station (or end-station) where a spectral image is acquired and data are collected (CLS, 2013; SSRF, 2013).

9.3 Synchrotron-based analytical techniques

Synchrotron radiation is millions of times brighter than sunlight. The light of the sun has a relative brightness of 10^{18} photons/sec/mm^2, with synchrotron radiation reaching 10^{19} photons/sec/mm^2 (CLS, 2013; Synchrotron Radiation, 2013). The extreme brightness allows for matter to be seen at the atomic level. Synchrotron radiation is also very fine, non-divergent and intense, which allows the study a very tiny areas in a sample (Miller *et al.*, 1998; Wetzel *et al.*, 1998; Marinkovic *et al.*, 2002; Yu *et al.*, 2004; Marinkovic and Chance, 2006; Miller and Dumas, 2006; Yu *et al.*, 2019).

Synchrotron radiation has a continuous spectrum and covers the full range of the electromagnetic spectrum from infrared to X-ray. Infrared synchrotron radiation enables the exploration of a very small area and reach diffraction limits of a few microns with ultrahigh spectral resolution, good signal to noise ratio, high precision and broadband (Australian Synchrotron, 2013), making it highly suitable as a spectroscopy technique.

The basic principle of infrared spectroscopy lies in the fact that the energy transition between two vibrational states of a molecule is stimulated by an oscillating dipole of specific vibration motion of a molecule which resonates with the electric field of an electromagnetic wave at a specific oscillation frequency. The resonance energy of vibrational transition of a molecule from vibrational ground state to an excited vibration state is acquired from an infrared electromagnetic wave by increasing the kinetic energy of relative nuclear motion of vibration and increasing the amplitude of a specific vibration motion (Joe and Roth, 1986; Kemp, 1991; Jackson and Mantsch, 1996; Mantsch and Chapman, 1996). When vibration fundamental transition happens, it results in infrared absorptions at specific frequencies in the mid-infrared energy range. So, identification of molecular functional groups is a major application of infrared spectrometry (Joe

and Roth, 1986; Kemp, 1991; Mantsch and Chapman, 1996; Himmelsbac *et al.*, 1998; Marinkovic and Chance, 2006). Synchrotron-based infrared microspectroscopy mainly consists of: (1) infrared synchrotron radiation, (2) microscopy and (3) infrared spectrometry. Other components include computer-controlled XY-stage, a data acquisition and processing program, linked cameras and a computer.

Usually organic molecules have several different characteristic absorption bands (Mathlouthi and Koenig, 1986; Himmelsbach *et al.*, 1998; Wetzel, 1998; Marinkovic *et al.*, 2002; Yu *et al.*, 2003a; Yu, 2012b). For example, protein molecules have relative unique amide A, amide B, amide I and II bands that centre at ca. 3,300, 3,050, 1,650 and 1,550 cm^{-1}. Characteristic absorption bands for phospholipids are at ca. 1,740 cm^{-1} and alkyl bands in the region of 3,000-2,800 cm^{-1} (Wetzel *et al.*, 1998) such as methyl (CH_3) antisymmetric, methylene (CH_2) antisymmetric, methyl (CH_3) symmetric, and methylene (CH_2) symmetric stretching vibration bands.

9.4 Functions of synchrotron-based analytical techniques

The main function of the synchrotron-based infrared microspectroscopy is that it is able to explore molecular structure and chemical make-up or structural conformation within intact tissue at ultrahigh spatial resolution. These techniques can link tissue structural information to chemical information. It can be used to study sample composition, chemistry, environment, and structure at the same time within intact tissue at ultrahigh spatial resolution. This is a huge advantage that traditional wet chemical analytical technique cannot accomplish (Budevska, 2012). In feeds and nutrition studies, synchrotron-based infrared microspectroscopy can provide not only feed chemical composition, but also feed structure and feed matrix (environment) information (Yu *et al.*, 2004; Yu, 2010). These three types of information cannot be obtained with traditional 'wet' chemical analyses (Budevska, 2012; Yu *et al.*, 2004) because they destroy the feed inherent structure during sample preparation such as digestion.

Synchrotron spectral analysis is relatively complex, particularly in a complex feed-based system. Various molecular spectral analyses have to be carried out, which include peak identification, peak component fitting, peak modelling (with Gaussian or Lorentzian functions) (Yu, 2005a), univariate molecular spectral analyses, multivariate molecular spectral analyses (Yu, 2005b) and molecular structural mapping or imaging (Yu, 2011). Although the analysis of spectral data is relatively complex, these advanced techniques have been used in fields other than agricultural science such as animal science (Yu, 2005b; Yu *et al.*, 2009; Yu, 2011).

9.5 Application of synchrotron-based analytical techniques as non-invasive techniques in animal nutrition

9.5.1 Cereal grain as feeds for animals

The synchrotron-based analytical technique SR-IMS has been used in cereal grain research by our team (Liu and Yu, 2010; Yang *et al.*, 2013a,b). In our studies (Yang *et al.*, 2013a,b), we investigated effects of the alteration of carbohydrate traits in hull-less barley (*Hordeum vulgare* L.) on its molecular structure features in relation to its metabolic characteristics in animals. These studies provided a unique molecular means to rank the endosperm of hull-less barley with altered carbohydrate traits in terms of metabolic features of protein and nutrient availability. In this nutrition study of Yang *et al.*, (2013a), four hull-less barley varieties and breeding lines with altered carbohydrate composition (amylose: 1 to 40% DM; β-glucan: 5 to 10% DM) were developed at the Crop Development Centre (CDC), University of Saskatchewan. The hull-less barley varieties included zero-amylose waxy, CDC Fibar; 5%-amylose waxy, CDC Rattan; normal-amylose, CDC McGwire and high-amylose, HB08302. CDC Copeland hulled barley was included as a hulled control.

As a rapid and non-destructive technique, SR-IMS is able to study the molecular chemistry of different botanical parts. The advanced SR-IMS techniques can be applied to evaluate and screen feed quality in ruminant nutrition, detect inherent structure of plant-based feeds in relation to rumen degradation characteristics within intact tissue.

The objectives of the study by Yang *et al.* (2013a) were to (1) reveal molecular structures of the four newly developed hull-less barley varieties using SR-IMS technique, and (2) quantify molecular structural features in relation to rumen degradation kinetics, intestinal nutrient digestion and predicted total true protein supply with the DVE/OEB system (Tamminga *et al.*, 1994, 2007) and NRC Dairy 2001 model (NRC, 2001). The spectra data were collected at tissue endosperm region (ca. 100-600 μm) according to a previous study suggestion (Walker *et al.*, 2009) using the SR-IMS in the mid-infrared region (ca. 4,000-800 cm^{-1}) of the electromagnetic spectrum. The characteristic absorptions were acquired for functional groups of: protein (ca. 1,768-1,475 cm^{-1}), β-glucan (ca. 1,450-1,390 cm^{-1}) and cellulosic compounds (ca. 1,278-1,205 cm^{-1}) and total carbohydrates (CHO) (ca. 1,195-945 cm^{-1}).

Spectral features of β-glucan in hull-less barley varieties are negatively correlated with protein nutrient availability in the small intestine of ruminant including total digestible protein (TDP: r=-0.73, $P<0.05$; r=-0.84, $P<0.05$) in DVE/OEB system and

metabolisable protein (MP: r=−0.71, $P<0.05$; r=-0.84, $P<0.01$). Variation in absorption intensities of CHO among hull-less barley varieties were observed with negative effects on protein degradation, digestion and potential protein supply ($P<0.05$). Hence, molecular structure features of β-glucan and CHO in hull-less barley have negative effects on protein supply to ruminants. Therefore, determination of molecular structure spectral feature provides a means of ranking experimental breeding lines for genetic selection in cereal grains breeding programs.

The results show that SR-IMS can be used for the delicate and fine structure study in cereal grains or other types of seeds or feeds for animal.

9.5.2 Feed and food processing: effects of heat treatments on cotyledon tissues in yellow-type of canola seeds

The synchrotron-based analytical technique has been used in feed and food processing and bioethanol processing studies by our team (Yu and Nuez-Ortin, 2010; Liu *et al.*, 2012; Yu *et al.*, 2013). In the study by Yu *et al.* (2013), we investigated the effects of heat treatment methods on cotyledon tissues in yellow-type of canola seeds and wanted to know sensitivity, penetration and response to different heating treatments by cotyledon tissues in order for us to decide feed processing efficiency.

SR-IMS is able to reveal structural features of biomaterials within intact tissue at both cellular and molecular levels. Heat related-treatments have been used to improve nutrient utilisation and availability of feeds in animals. However, hitherto, there has been no study on the sensitivity and response of each layer in canola seeds to different heat-related treatments. It is not known which layer [epiderm/mucilage, spermoderm, endosperm, or cotyledon] is the most sensitive to heat when heat treatment is applied to the seeds. Traditional wet-chemical analysis is unable to answer such questions.

Therefore, we used SR-IMS with multivariate molecular spectral analyses as a novel research tool to study heat treatment effects on the structural changes in the cotyledon tissues of yellow-type canola (*Brassica*) seeds among raw [treatment code 'A'], wet heating (autoclaving at 121 °C for 60 min) ['B'] and dry heating (dry roasting at 120 °C for 60 min) ['C']. Our results show that different heat-treatments had different heat penetration ability on cotyledon tissues in yellow-type canola seeds.

The multivariate analytical tools principal component analysis (PCA) and agglomerative hierarchal cluster analysis were applied to investigate variance and groupings within the spectral data set [whole spectral range ca. 4,000-650 cm^{-1}; Spectral range ca 1,300-900 cm^{-1} (cellulose or saccharides), ca. 1,800-1,500 cm^{-1} (secondary structures of protein); ca. 1,500-1,300 cm^{-1} (bending motion of methylene and methyl group, this change are consistent with the change in the range of ca. 3,000-2,800 cm^{-1})].

The results (Yu *et al.*, 2013) showed that there were no cluster and groups formed in the cotyledon tissues among the three treatments. There were no distinguished responses of the cotyledon tissues to different types of heat-treatments by using multivariate molecular spectral analyses. The results indicate that the cotyledon tissues were sufficiently penetrated by both heat treatments (autoclaving and dry roasting) under at the specified conditions.

In feed processing studies (Zhang *et al.*, 2012, 2014), we used molecular spectroscopy to study the effect of fractionation processing on processing-induced changes in chemical and nutrient profiles and molecular structure through molecular spectral analysis. In these studies, the co-products from bioethanol processing (called dried distillers grains with solubles (DDGS)) was fractioned into A, B, C and D fractions through the processing. Figure 9.3 shows the multivariate spectral analysis results and we can see fractionation-processing induced-changes in molecular structure among the DDGS fractions A, B, C, and D could be detected using PCA of the 2nd derivative spectra.

Recently bioethanol processing effects on nutrient profile and structural profiles of triticale grains and triticale DDGS for dairy cows has been reported using both a synchrotron based technique and conventional ATR-FTIR spectroscopy (Liu *et al.*, 2012).

Our other studies also show that synchrotron-based techniques combined with Raman molecular microspectroscopy and differential scanning calorimetry can be used to study thermal stability of molecular microstructure in feed grains (Khan and Yu, 2013). The sensitivity and responses of chemical functional groups to feed processing methods on a molecular basis were reviewed recently reported (Yu, 2012c).

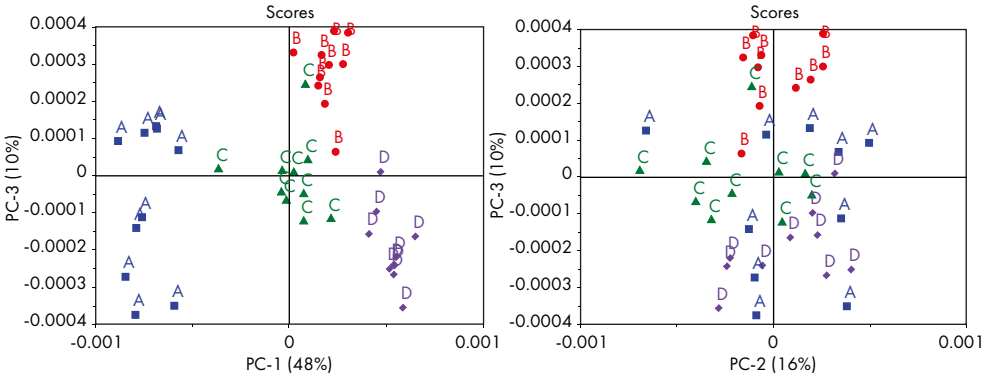

Figure 9.3. Fractionation-processing induced-changes in molecular structure among the dried distillers grains with solubles fractions A, B, C, and D, detect using principal component analysis of the 2nd derivative spectra. A, B, C and D are fractionation processing fractions from co-products from bioethanol production.

9.5.3 Wheat quality: protein structure in hard wheat lines

The synchrotron-based analytical technique has been used to study wheat quality (Bonwell *et al.*, 2008) through studying protein fine structure. The detailed review reported on why we study protein structure and what makes protein indigestible from tissue, cellular and molecular structure aspects (Becker and Yu, 2013). In the wheat quality study, the authors (Bonwell *et al.*, 2008) used SR-IMS to determinate endosperm protein secondary structure in hard wheat breeding lines *in situ* and reveal secondary structural changes in protein films with thermal processing. In this study, Fourier transform infrared microspectroscopy was used to determine protein secondary structure in hard wheat breeding lines *in situ*, providing a molecular means to rank endosperm hardness for the selection of wheat cultivars for a specific end-use. The authors reported that 'Mapping with a single masked spot size diameter of 4.5 μm or confocal 5 μm on beamlines U10B and U2B (NSLS), respectively, produced spectra from the sub-aleurone layer within each wheat kernel using the high spatial resolution available with synchrotron infrared microspectroscopy. This procedure was used for the first four crop years. A focal plane array instrument was adapted for use for the remaining two crop years with a slight reduction of spatial resolution. Deconvolution and curve fitting were applied to the amide I region of spectra selected from the interstitial protein between the starch granules, and the relative amount of α-helix to other protein secondary structures was revealed. Over six crop years, the α-helix to β-sheet ratio of experimental wheat varieties were compared to those of released varieties in 143 mapping experiments. The highest measurable value was 2.50 while the lowest was 1.11. The determination of protein secondary structure provides a means of ranking experimental breeding lines for selection of crop cultivars across specific endues applications.'

9.5.4 Effect of gene-transformation in animal nutrition

The SR-IMS has been used to study the effect of gene-transformation (Yu, 2010; Jonker *et al.*, 2011) or gene knockout (Withana-Gamage *et al.*, 2013). In the study of Withana-Gamage *et al.* (2013), the authors characterised A*rabidopsis thaliana* lines with altered seed storage protein profiles using the SR-IMS. The authors reported '*Arabidopsis thaliana* lines expressing only one cruciferin subunit type (double-knockout; CRUAbc, CRUaBc, or CRUabC) or devoid of cruciferin (triple-knockout; CRU-) or napin (napin-RNAi) were generated using combined T-DNA insertions or RNA interference approaches.

Seeds of double-knockout lines accumulated homohexameric cruciferin and contained similar protein levels as the wild type. Chemical imaging of wild type and double-knockout seeds using synchrotron FTIR microspectroscopy (amide I band, 1,650 cm^{-1}) showed that proteins were concentrated in the cell centre and protein storage vacuoles.

Protein secondary structure features of the homohexameric cruciferin lines showed predominant β-sheet content. The napin-RNAi line had lower α-helix content than the wild type. Lines entirely devoid of cruciferin had high α-helix and low β-sheet levels, indicating that structurally different proteins compensate for the loss of cruciferin. Lines producing homohexameric CRUC showed minimal changes in protein secondary structure after pepsin treatment, indicating low enzyme accessibility. The synchrotron FTIR technique provides information on protein secondary structure and changes to the structure within the cell.'

9.5.5 Feed/seed architecture through molecular chemistry imaging

The SR-IMS has been used in visualising tissue molecular structure of plant seed in a chemical way through molecular imaging (Yu, 2013). The latter study showed that 'the chemical images of protein amides were obtained through the non-invasive imaging technique for the raw, wet and dry heated black-type of canola seed tissues. It seems that different types of the processing have some different impact on protein spectral profile in the black-type of canola tissues. The wet heating had a greater impact on protein alpha-helix to beta-sheet ratio than the dry heating. Both dry and wet heating resulted in different patterns in amide I, the 2nd derivative and Fourier self-deconvolution spectra. Follow-up PCA studies will focus on: (1) to compare the response and sensitivity of canola seeds to various processing methods between the yellow-type and black-type of canola seeds; (2) to develop a sensitive method to compare image difference between tissues and between treatments; (3) to develop a method to link images to nutrient digestion; and (4) to reveal how the structure changes affect nutrient absorption in human and animals.'

Another study was carried out to detect the molecular structural difference between two types of canola seed tissues (Y01-429-2011, Yellow) vs (Y-N07-1374-2011 Black) using PCA of the 2nd derivative spectra collected by advanced SR-IMS. From Figure 9.4, it can be clearly seen that two types of canola tissue differed in chemical make-up. These different chemical make-ups are expected to have different biological functions in a complex biological system.

9.5.6 Feed processing and animal nutrition

The SR-IMS has been used in feed science and animal nutrition studies (Doiron *et al.*, 2009a,b). The objectives of the latter studies were to reveal protein structures of feed tissues affected by heat processing at a cellular level, using the advanced synchrotron technology (SR-IMS), and quantify protein structure in relation to protein digestive kinetics and nutritive value in the rumen and intestine in dairy cattle. The variables assessed included: (1) protein structure α- helix to β-sheet ratio; (2) protein subfractions profiles; (3) protein degradation kinetics and effective degradability, and

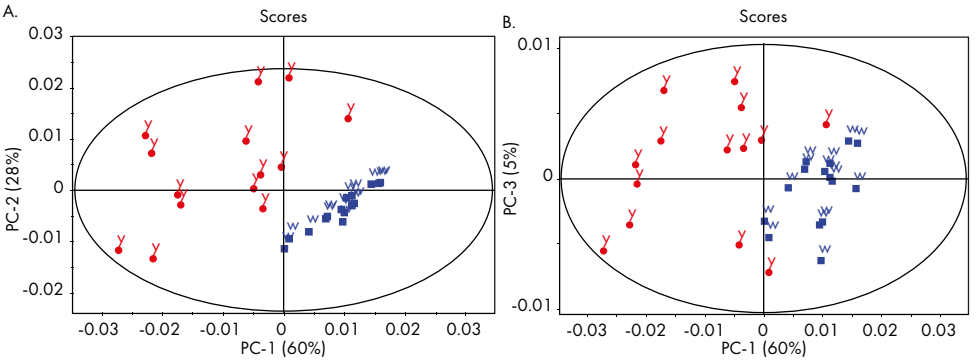

Figure 9.4. Detect the molecular structural difference between two types of canola seed tissues: W: (Y01-429-2011, yellow) vs Y (Y-N07-1374-2011 black) using principal component analysis of the 2nd derivative spectra collected by advanced synchrotron-based IR microspectroscopy (SR-IMS). (A) PC1 vs PC2; (B) PC1 vs PC3.

(4) predicted nutrient supply using the DVE/OEB system in terms of the intestinally absorbed protein supply (DVE) and degraded protein balance (OEB) to dairy cattle. Vimy flaxseed protein was used as a model feed protein and autoclave-heated at 120 °C for 20, 40 and 60 min as treatments T1, T2 and T3, respectively. The hypothesis was that heat-induced protein structure changes affect the protein quality, fermentation and digestion behaviour in the rumen and intestines. The synchrotron-based protein chemistry research was performed at the National Synchrotron Light Source in Brookhaven National Laboratory. Doiron *et al.* (2009a,b) showed that with the SR-IMS, heat-induced protein structure changes could be revealed and identified. The heating at 120 °C for 40 and 60 min increased protein secondary structure α- helix to β-sheet ratio. There were linear effects of heating time on the ratio. The heating also changed chemical profiles, which showed soluble crude protein decreased upon heating with concomitant increases in non-protein nitrogen, neural and acid detergent insoluble nitrogen. The protein sub-fractions with the greatest changes were the rapidly degradable protein fraction in rumen (PB_1) which showed a dramatic reduction, and PB_2 (the fraction fermented in the rumen at a lower rate than buffer-soluble fractions and some of the PB_2 fraction escapes to the lower gut) showing a dramatic increase demonstrating a decrease in the overall protein degradability. *In situ* results showed a reduction in rumen-degradable protein and dry matter without differences between the treatments. Intestinal digestibility by a three-step *in vitro* showed no changes to the rumen-undegraded protein. Modelling results showed that the heating increased total intestinally absorbable protein (feed DVE value) and decreased degraded protein balance (feed OEB value), but there were no differences between the treatments. There was a linear effect of heating time on the DVE and cubic effect on the OEB value. Our results showed that the heating changed chemical profiles, protein secondary structure α- helix to β-sheet ratio and protein sub-fractions, and decreased rumen-degradable protein and rumen-degradable dry matter and increased potentially nutrient supply

(DVE value) to dairy cattle. The protein secondary structure α-helix to β-sheet ratio had a significantly positive correlation with total intestinally absorbed protein supply (DVE value) and negative correlation with degraded protein balance.

In the studies by Doiron *et al.* (2009a,b), the cluster analysis (CLA) and PCA molecular spectral analyses were successfully used to make distinctions between the different treatment spectra and showed enhanced sensitivity upon selection of a smaller spectral window to include only the amide I and II portion of the IR spectrum. Our results indicated that autoclaving had a great enough effect to the mid-IR spectrum of flaxseed to identify the altered α-helix to β-sheet ratio and subsequently differentiated between the treatments using PCA and CLA suggesting greater sensitivity of mid-IR spectral methods in identifying the treatments. Future studies are needed to further quantify the relationship between protein secondary structure and protein functionality.

In another of our recent studies to compare the type of feeds and sources of feeds using cluster analysis, we also found that the CLA analysis can be used to quickly detect the nutrient and chemical profile different through spectral analysis. Figure 9.5 shows different types of feeds (Carinata vs Canola) and different sources (source 1 vs source 2 in Canola; source 1 (beef) vs source 2 (dairy) in Carinata). This method provides an excellent and fast way to detect differences among biological materials.

9.6 Summary and conclusions

In summary, our studies show that the advanced non-invasive synchrotron radiation based analytical techniques are able to investigate feed molecular structure within intact tissue at ultra-spatial resolution. These non-invasive synchrotron techniques can be used to study various aspects in feed/food science, bio-feeds, food/feed processing, animal nutrition, seed science, and molecular biotechnology. These techniques are able detect the processing-induced, enzyme treatment-induced changes of feed molecular structures in relation to nutrient utilisation, nutrient availability and biological functionality in animals. These non-invasive techniques can be used to detect the effect of modification of plant through gene knockout or gene transformation on feed inherent structure and their functionality. These techniques can also be used to image molecular chemistry in a complex plant-based feed system. The non-invasive synchrotron-based analytical technique provides us a molecular means to rank feed, food, seed, and plants. It is believed that the advanced synchrotron-based analytical technique can make significant contributions to advances in feed science and animal nutrition.

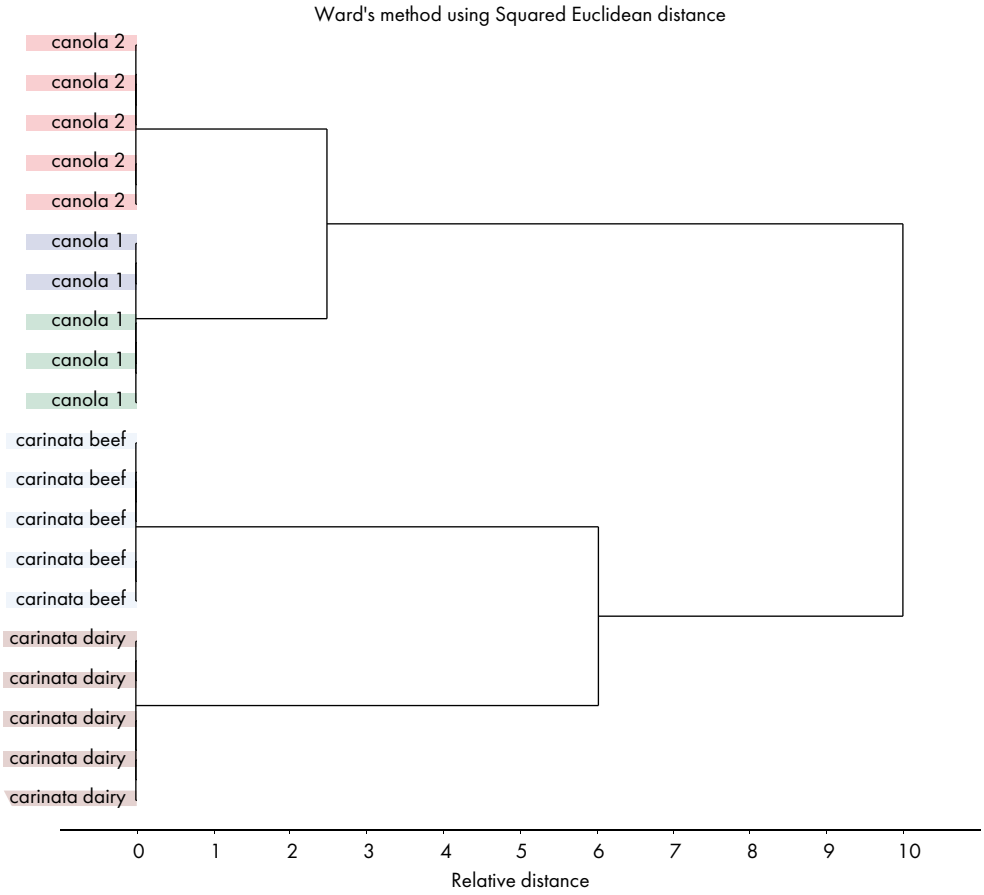

Figure 9.5. Using cluster molecular spectral analyses to detect difference between different types of feeds (Carinata vs Canola) and different sources of feeds (source 1 vs source 2 in Canola; source 1 (beef) vs source 2 (dairy) in Carinata).

9.7 Future perspectives

Current animal nutrition requirement models (NRC, DVE, PDI, ARC, etc) are developed based on 'wet' chemistry analyses methods. These 'wet' chemistry methods fail to reveal feed inherent structure features during processing and digestion. This often results in inaccurate diet formulation. In future, we should consider not only feed chemical composition, but also feed inherent structure. Based on both feed chemical composition and feed inherent structure, we can develop more accurate nutrition requirement estimates and diet formulation models. We can apply the advanced synchrotron based techniques for feed inherent molecular structure study.

Acknowledgments

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10

Biotechnology in the feed industry and animal nutrition: harnessing microbes to provide natural solutions

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Summary points

- Fungi, bacteria, yeast and algae each offer unique, natural solutions for tackling important feed industry challenges.
- Enzymes, probiotics, yeast products and organic minerals help to optimise intestinal function and health and thus enhance nutrient utilisation.
- In the future, algae could play a major role as sources of protein, and long chain essential fatty acids in the feed and food chain.
- Scientific development in feed additive production and animal research will offer new opportunities and accelerate the development of novel tools.

Keywords: animal nutrition, enzymes, yeast, organic minerals, microalgae

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10.1 Introduction

Today's feed industry is working to meet the escalating demand for feed and food by an increasingly urbanised global society. Objectives in meeting this demand include improving feed efficiency and nutrient utilisation to optimise animal nutrition, health and production; enriching foods to better meet human nutritional requirements; ensuring food chain safety; and reducing the agribusiness ecological footprint. Biotechnologies are central to meeting these objectives. Biotechnology is a broad term defined by the Convention on Biological Diversity to mean 'any technological application that uses biological systems, living organisms, or derivatives, thereof, to make or modify products or processes for specific use' (UN, 1992; FAO, 2014). Fungi, bacteria, yeast and algae each offer unique, natural solutions for tackling important feed industry challenges. Although microbes are sometimes referred to as a 'new' frontier, their use in animal nutrition and food production is centuries old. For example, silage production, which involves bacterial inoculation of plant matter followed by anaerobic fermentation, was used to conserve forages as far back as 1500-1000 B.C. (Alonso *et al.*, 2013). Two key contributions of biotechnology to the efficiency of modern animal production have been the manufacturing of essential amino acids and vitamins. In feed formulation for monogastrics, the incorporation of commercial amino acids allows the reduction of the crude protein concentration while meeting the animal's amino acids demand, thus greatly improving protein efficiency, reducing nitrogen excretion and ammonia emissions. Vitamin supplementation is essential to cover the high vitamin requirement of today's high performing breeds protecting their health and allowing them to express their genetic potential.

In this chapter, we review selected organic products and processes that are designed to optimise feed nutritional value and which avoid the risks and controversies that surround genetic modification of feed ingredients (Madan, 2005) or the development of antibiotic resistance. The roles of enzymes, probiotics, yeast products, organic minerals, and microalgae in pig and poultry nutrition and their contributions to sustainable agricultural production are discussed. Other measures to improve animal health and performance, but not discussed herein, include technologies such as pre-treatment of ingredients and feed, acid preservation or the use of organic acids to optimise intestinal health and digestion.

10.2 Enzymes

Plant-derived feedstuffs contain a variety of anti-nutritive factors and forms of essential nutrients (e.g. protein, energy, and phosphorus) that remain unavailable to monogastrics, which lack the enzymatic digestion provided by rumen microbiota (Munir and Maqsood, 2013). For this reason, exogenous enzymes are supplemented to complex feed matrices to hydrolyse non-starch polysaccharides (NSP, i.e. celluloses,

hemicelluloses, pectins and oligosaccharides) or phytate phosphorus during feed processing and animal digestion. Carbohydrases can increase the bioavailability of NSP from plant cell walls, release structurally encapsulated nutrients during digestion, reduce nutrient excretion, and increase animal performance (Adeola *et al.*, 2008; Adeola and Cowieson, 2011; Munir and Maqsood, 2013). Together carbohydrases and phytases account for at least 90% of the global feed enzyme market (Adeola and Cowieson, 2011). In recent years, there has been renewed interest in the use of proteases as well as other enzymes and enzyme combinations in diet formulations. Combinations are of particular interest for use in young animals, such as the weanling piglet, whose digestive function is compromised. Enzyme technologies can help optimise the value of current feedstuffs and can also potentially allow the reformulation of diets with new, alternative, lower-cost, fibre-rich feedstuffs (De Vries *et al.*, 2012). Commercially available feed enzymes are obtained from fermentation systems designed to capture enzymes secreted by bacteria or fungi. Liquid fermentations systems are commonly designed to yield high concentrations of one specific enzyme. Solid-state fermentation technology involves the growth of microorganisms, on complex solid substrates, such as wheat bran (Krishna, 2005; Mienda *et al.*, 2011) to produce a blend of enzymes to maximise digestive efficiency.

The addition of carbohydrases to pig and poultry diets has increased in the past decade as producers strive to improve nutrient utilisation and reduce nutrient waste. Xylanases and beta-glucanases have been effectively used to reduce the anti-nutritional effects of arabinoxylans and beta-glucans, the predominant NSP fraction in wheat and barley (Choct *et al.*, 2004, 2006). By degrading those NSP, the viscosity of the intestinal contents is reduced, thus improving the digestive process and reducing the incidence of wet and sticky droppings (Annison, 1992; Choct *et al.*, 2006). In broilers, wet litter is a key risk factor for breast blisters, hock burns and foot pad lesions. Enzyme supplementation can contribute to better animal health and carcass quality by enhancing litter quality.

Although endogenous proteases are synthesised within the animal gut, nevertheless some protein usually remains undigested, especially in feeds containing plant-derived protein. Exogenous proteases added to layer diets have been shown to increase feed efficiency (Freitas *et al.*, 2011), improve egg-shell thickness, and lower production costs (Yadav and Sah, 2005). A monocomponent protease was shown to increase the digestibility of amino acids in broiler diets with low protein digestibility (Angel *et al.*, 2011). To break down complex feed matrices, a combination of different enzymes can increase efficiency. For example, carbohydrases, protease, and phytase used together have been shown to increase feed efficiency when added to nutritionally marginal diets for broiler chicks (Cowieson and Adeola, 2005). Likewise, protease and xylanase supplementation has been shown to maintain performance in broilers fed distillers dried grains with solubles (Barekatain *et al.*, 2014).

Phytases are supplemented to release phosphorus from plant ingredients, which otherwise remains bound as phytic acid during digestion and is then excreted (Rimbach *et al.*, 2008; Woyengo and Nyachoti, 2013). The use of exogenous phytase lessens the need for the inclusion of inorganic monocalcium or dicalcium phosphate to meet monogastric phosphorus requirements. Adeola and Cowieson (2011), in their review of enzymes for non-ruminants, indicate that zinc and iron are among the nutrients most negatively affected by dietary phytate. Others nutrients similarly affected include calcium, magnesium, copper, and manganese (Rimbach *et al.*, 2008). Unfortunately, trace mineral inclusion rates are typically not adjusted to reflect enzymatic influences.

10.3 Probiotics

Probiotics are live microorganisms, generally bacteria but also yeasts which, when ingested live in sufficient amounts, can have a positive effect on animal health going beyond the nutritional effects commonly known (Anadon *et al.*, 2006). Microorganisms used in animal feed in the EU are mainly bacterial strains of Gram-positive bacteria belonging to the types *Bacillus*, *Enterococcus*, *Lactobacillus*, *Pediococcus*, *Streptococcus* and strains of yeast belonging to the *Saccharomyces cerevisiae* species and *Kluyveromyces* (Anadon *et al.*, 2006). Probiotics are either added to the diets or applied in stress situations via drinking water. They must be present as viable cells in large enough concentrations to improve the digestive process, improve intestinal health and/or the overall immune defence (Guillot, 2003). In livestock, several trials and field experiences indicate the beneficial effects of probiotics, impact on performance characteristics or on the suppression of some aspects of infection with pathogens (Taras *et al.*, 2005; Kabir, 2009). Taras *et al.* (2005) stated that most consistent are reports regarding decreased occurrence and severity of diarrhoea in piglets after application of various probiotic strains. Piglets are commonly weaned between 21 and 28 days of age, a time when the porcine gastrointestinal tract (GIT) is still underdeveloped. Weaning can decrease the absorptive capacity caused by physiological and morphological changes in the small intestine and can thus increase the nutrient flow to the lower GIT (Spreeuwenberg *et al.*, 2001; Boudry *et al.*, 2004). This increased supply can lead to proliferation and overgrowth of enterotoxigenic bacteria such as *Escherichia coli* (Hopwood and Hampson, 2003). Lactic acid bateria can relieve weaning stress and reduce the risk of diarrhoea (Pollmann *et al.*, 2005; Ross, *et al.*, 2010). In addition to improving the intestinal microbiota of piglets, inclusion of *Enterococcus faecium* significantly improved growth and feed conversion after weaning (Chen *et al.*, 2006; Mallo *et al.*, 2010). Positive results have also been reported with the application of cultures combining different species of lactic acid bacteria. A combination of *E. faecium, Lactobacillus acidophilus, Pediococcus pentosaceus* and *Lactobacillus plantarum* has been shown to increase feed intake and weight gain and improve feed conversion (Giang *et al.*, 2010). Several modes of action

have been proposed for lactic acid probiotics. Adhesion of the probiotic strain to the GIT is important for bacterial colonisation, acid production and thus for pathogen exclusion (Lebeer *et al.*, 2008). The interaction with the enterocyte has been shown to stimulate mucin production (Mack *et al.,* 1999) which can protect the epithelial cells by functioning as a physicochemical barrier. In addition, different interactions with the immune system, such as down-regulation of inflammation (O'Hara *et al.*, 2006; Walsh *et al.*, 2008), can lead to improved intestinal health and function. In general, the intestinal microbiota of piglets fed with probiotics are more stable and, therefore, the gut ecosystem can better withstand microbial challenges (Taras *et al.,* 2005).

10.4 Yeast and yeast products

10.4.1 Cell-wall components

Yeast cell walls contain polysaccharides that offer a range of biotechnological applications for feeding livestock. For example, mannan oligosaccharides (MOS) have been shown to alter the microflora in the GIT of pigs and poultry in ways that enhance digestion and immune function. Although not strictly classified as prebiotics by some (Heo *et al.*, 2012), because they do not selectively enhance the growth of beneficial bacteria (Gaggìa *et al.*, 2010), MOS nevertheless can improve intestinal morphology and can bind mannose-specific lectins of gram-negative pathogens that express Type-1 fimbriae, (e.g. *Salmonella* and *E. coli*) so they can be excreted (Thomas *et al.*, 2004; Baurhoo *et al.*, 2009). Immune modulating effects of MOS have been confirmed at the transcriptional level in chickens (Xiao *et al.*, 2012). The health benefits of MOS in pig diets are most pronounced in weaned pigs (Zhao *et al.*, 2012) and in pigs raised in substandard hygienic environments (Halas and Nochta, 2012). Nutritional strategies like MOS supplementation are of increasing importance with the spread of bans on the use of antibiotic growth promoters and pressure to reduce antibiotics prescribed by veterinarians.

Beta-glucans are also present in the cell walls of yeast, bacteria, fungi, and algae. They belong to a group of physiologically active compounds that have been shown to stimulate the innate and adaptive immune systems in both pigs and poultry (Cox and Dalloul, 2010; Ganner and Schatzmayr, 2012). Beta-glucans have been reported to possibly exhibit novel prebiotic properties (Lam and Cheung, 2013) and to interfere with adherence of pathogens to host gut mucosa (Ganner and Schatzmayr, 2012). Yeast beta-glucans have also been reported to benefit intestinal morphology by lengthening villi, shortening crypts and enhancing goblet cell numbers (Kogan and Kocher, 2007; Ganner and Schatzmayr, 2012).

Additionally, beta-glucans have applications for sequestering mycotoxins. Mycotoxins, the secondary toxic metabolites of fungi, universally contaminate the animal feed supply chain (Bryden, 2012). Although the ingestion of high concentrations of mycotoxins is recognised to trigger acute disease episodes, the ingestion of low levels of toxins can be equally problematic, causing an array of metabolic disturbances that reduce pig and poultry productivity (Haschek *et al.*, 2002). One mitigation strategy for addressing naturally contaminated feed has been to supplement animal diets with adsorbing or binding agents. Early efforts involved the addition of adsorptive, inorganic clays (Grant and Phillips, 1998), however these materials tend to reduce the biological value of certain nutrients, are prone to contamination with dioxins and heavy metals, and are associated with adverse environmental effects in manure (Yiannikouris, 2008). More recently, beta-D-glucans from *S. cerevisiae* cell wall have been used to sequester a wide range of mycotoxins in feed (Jouany *et al.*, 2005; Yiannikouris *et al.*, 2006; Yiannikouris, 2008). The affinity of beta-D-glucans for mycotoxins varies between toxins as a function of their unique stereochemistry, with strong affinities to mycotoxins exhibiting aflatoxin-like, deoxynivalenol-like, and zearalenone-like structures (Yiannikouris *et al.*, 2006). The structural conformations of glucans are less conducive to interactions with nutrients, and upon excretion as organic constituents they are biodegradable (Yiannikouris, 2008). Yeast glucans have been shown to counteract reductions in weight gain and to diminish caecal colonisation of *Salmonella* Typhimurium in pigs exposed to T-2 toxin (Verbrugghe *et al.*, 2012). Similarly, yeast glucan supplementation has been shown to improve performance in broiler chickens (Girish and Devegowda, 2006).

10.4.2 Selenium yeast

The trace element selenium is essential to animal health and is a constituent of selenoproteins, glutathione peroxidase enzymes, and deiodinases. The pig and poultry industries supplement selenium to improve animal growth performance, immune response, antioxidant status, reproductive health, and bird feathering (Quesnel *et al.*, 2008; Perić *et al.*, 2009; Heindl *et al.*, 2010; Brennan *et al.*, 2011; Fortier *et al.*, 2012; Surai and Fisinin, 2014). Although traditionally selenium supplements have been from inorganic sources (i.e. selenite, selenate), selenium can be also fed in organic forms, most commonly as selenium-enriched yeast. Yeast cells are capable of synthesising selenoamino acids using the selenium they absorb from fermentation media enriched with selenate or selenite (Lyons *et al.*, 2007). The selenium-containing compounds in yeast are similar to the forms of selenium found in grains and forages to which animals are naturally adapted to absorb in the GIT via the amino acid transport mechanism. Organic selenium supplementation of livestock has been shown to build tissue reserves that can be utilised in times of stress (Surai and Fisinin, 2014). However, measures of nutrient bioavailability must not be limited to tissue retention rates alone, but rather must encompass basic metabolic principles such as nutrient stability under the oxidising conditions of the GIT and its biological activity and

physiological function. Unlike free selenomethionine which is readily oxidised (Le *et al.*, 2008), the selenomethionine in selenium-enriched yeast is protected by the cell wall and bound to proteins or locked in by other molecules. Gene expression profiling has shown distinct differences between selenium forms related to their bioactivity (Barger *et al.*, 2012). In addition to offering animal production benefits, selenium supplementation is increasingly used to create selenium-enriched milk, meat and eggs for addressing global selenium deficiencies in human diets (Fisinin *et al.*, 2009; Bermingham *et al.*, 2014). Enrichment of selenium in products has also been associated with food product improvements. For example, meat quality (i.e. water-holding capacity) has been reported as enhanced in pigs (Li *et al.*, 2011) and chickens (Perić *et al.*, 2009; Wang *et al.*, 2009; Skřivan *et al.*, 2012; Delles *et al.*, 2014) fed selenium-enriched yeast.

10.5 Organic trace minerals

Dietary trace minerals play essential roles in metabolic functions that safeguard animal health and performance (Bao and Choct, 2009; Richards *et al.*, 2010). Traditionally pig and poultry diets have been supplemented with inorganic mineral salts to meet these nutritional needs, with the contributions of minerals inherently present in natural feedstuffs generally ignored, even though the calculated innate micromineral content of conventional diets often exceeds official recommendations. The relatively low cost of inorganic mineral supplements, coupled with unclear dietary requirements, have led to an industry-wide practice of over-formulating diets with mineral salts to ensure their bioavailability (Bao and Choct, 2009). The consequence of this overfeeding is increased risk for antagonistic between-nutrient interactions of free ions released by mineral salts, such as sulphates, carbonates, chlorides and oxides, which can then impair mineral absorption, over concentrate trace minerals in manure, and increase environmental burden (Richards *et al.*, 2010). For example, dietary inorganic copper and zinc are known to have an antagonistic relationship, whereby excessive levels of one can reduce the bioavailability of the other. Using standard industry feeding practices, based on nitrogen content, poultry manure has been shown to contain as much as five and six times the zinc and copper concentrations, respectively, needed by crops (Coic and Coppenet, 1989; Dozier *et al.*, 2003).

To increase their bioavailability in pig and poultry feeds, trace minerals can be alternatively fed as organic complexes (Bao and Choct, 2009). Transition elements, such as copper, zinc, iron or manganese can form chelates with hydrolysed proteins or other organic ligands. Compared with inorganic minerals, organically complexed minerals have been shown to have greater bioavailability as reflected in growth response, accumulation of trace minerals in tissues, and functional assays (Bao and Choct, 2009). Pig dietary requirements for copper, iron, manganese, and zinc were recently re-evaluated in a series of feeding trials utilising organic proteinates. A

study by Martin *et al.* (2011) confirmed the dietary need for the trace minerals iron, selenium and zinc in weanling pig diets. In a subsequent study (Hill *et al.*, 2014), newly weaned pigs were fed diets containing 0, 25, 50, 75 or 100 mg/kg of zinc in organic or inorganic form, or a 50:50 combination of the two sources. Investigators found that growth performance, zinc tissue concentrations, antioxidant activity in the liver, and metallothionein protein in the liver, duodenum and jejunum were best in weanlings fed 75 mg/kg of organic zinc. In another study, newly weaned pigs were fed diets that contained 0, 25, 50 or 100% of NRC (1998) trace-mineral levels as inorganic mineral salts or as organic minerals. Additions of copper, iron, manganese, and zinc in the first 21 days postweaning in either organic or inorganic form did not affect daily gain. However, from 21 to 35 days postweaning, daily gains numerically increased in response to feeding the 25% level. Concentrations of trace minerals greater than 25% did not further improve animal performance. Clearly, newly weaned pigs did not benefit from dietary trace-mineral supplements at the high concentrations recommended by the NRC. In a study of grower-finisher pigs (Gowanlock *et al.*, 2013a) fed corn and soybean meal diets supplemented with organic trace minerals at 0, 50 or 100% of NRC recommendations, no effects of trace-mineral addition on feed intake or feed efficiency occurred. Furthermore, carcass measurements and pork quality also did not differ between trace-mineral treatments (Gowanlock *et al.*, 2013b). In chickens, lower concentrations of organic trace mineral supplements can maintain carcass yield, breast meat pH, drip loss, and meat colour (Taveres *et al.*, 2014) and have no negative effects on bird antioxidant defence systems (Aksu *et al.*, 2010) compared with higher concentrations of corresponding inorganic minerals. Overall, organic minerals offer an effective strategy for improving the efficiency of feeding trace minerals and for reducing environmental burden (Swiatkiewicz *et al.*, 2014). In addition to being in a form of high bioavailability, mineral supplements must also pass quality control standards to ensure the purity, since mineral sources worldwide have been shown to have high risk for contamination (Jarman *et al.*, 2010).

10.6 Microalgae

Growth in the world population has increased the demand for, and cost of, corn and soybean feedstocks. The majority of species of microalgae have nutritional profiles that are comparable or superior to those of conventional feedstuffs (Lum *et al.*, 2013) and thus offer a potential, sustainable (Taelman *et al.*, 2013), alternative source of protein and carbohydrates for animal feed (Austic *et al.*, 2013; Gatrell *et al.*, 2014). Although the crude protein of microalgae varies between species ranging from 6 to 71% (Becker, 2007), their amino acid profiles tend to compare favourably with conventional protein sources (Gatrell *et al.*, 2014). Indeed, studies have shown that high protein microalgae can replace corn and soybean meal at appropriate levels in pig and poultry diets without adversely affecting performance (Gatrell *et al.*, 2014). Partial replacement of traditional feedstuffs with microalgae could translate into direct

savings of corn, soybean meal, and harvestable land (Gatrell *et al.*, 2014). Moreover, whereas feed production contributes to greenhouse gas emissions, autotrophic microalgae production sequesters atmospheric carbon dioxide and thus could help reduce the greenhouse gas effect of animal agriculture (Gatrell *et al.*, 2014).

Similar to those in yeast, the beta-glucans in microalgae cell walls have been shown to have biological activities that stimulate immune response (Lordan *et al.*, 2011) with potential applications for improving livestock disease resistance and stress tolerance. Certain species of microalgae contain high levels of lipids and antioxidants, many of which are essential in the human diet and thus can be especially important as livestock feed supplements for the production of value-added, animal-source foods (Lordan *et al.*, 2011; Lum *et al.*, 2013). The global market for natural fatty acids is projected to reach $13 billion by 2017 (BCC Research, 2013) in response to increased awareness of growing dietary inadequacies in polyunsaturated fatty acids (PUFA), especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (Givens and Gibbs, 2006), which play important roles in human health including modulation of the immune system and inflammation. In recent decades, the DHA content of farmed fish has declined with increased reliance on vegetable feed ingredients in aquaculture and overfishing of the world's oceans. Microalgae offer a sustainable alternative from fish products for meeting this fatty acid demand (Brunner *et al.*, 2009; Yaakob *et al.*, 2014) by providing a natural source for the omega-3 fatty acids DHA, eicosapentaenoic acid, linoleic acid, and the omega-6 fatty acid gamma-linolenic acid. DHA-rich microalgae supplements have been used effectively to enrich chicken and pork products (De Smet, 2012; Nieto and Ros, 2012; Ribeiro *et al.*, 2013), since dietary fatty acids undergo little change during digestion and absorption in monogastrics (De Smet, 2012). Microalgae have been used in chicken diets to enrich meat (Rymer *et al.*, 2010) and eggs (Fraeye *et al.*, 2012; Bruneel *et al.*, 2013; Lemahieu *et al.*, 2013) with omega-3 PUFA. The fatty acid profile of eggs from hens fed heterotrophic microalgae typically has PUFA profiles similar to eggs from hens fed fish oil; their DHA content significantly increases at the expense of n-6 PUFA (Fraeye *et al.*, 2012). Colour intensity of egg yolk is also increased with microalgae supplementation presumably due to transfer of microalgal carotenoids (Bruneel *et al.*, 2013; Lemahieu *et al.*, 2013). Moreover, supplementation of pig diets with omega-3 fatty acids has been shown to improve animal inflammatory response, reproductive performance, and the characteristics of meat and meat products (Rossi *et al.*, 2010).

10.7 Conclusion

The animal industry must continue improving production systems in order to optimally convert feed into edible animal products and to reduce its ecological footprint. Healthy animals with optimal intestinal function are absolutely essential in the push for maximal efficiency. The use of enzymes, probiotics and yeast products

have been shown to optimise intestinal function and health and are thus widely used in the industry. In the future, algae could play a major role as novel protein sources, as a tool to provide long chain essential fatty acids or other nutrients in highly available forms into the feed and food chain.

10.8 Future perspectives

Biotechnology is making key contributions to efficient animal production, animal health and welfare. Major challenges in maximising the impact of biotechnology in the past have been the selection and development of tools and its efficient screening as animal systems are complex and responses vary with production conditions. Novel technologies such as gene-editing may be one of the most significant advancements of this age and can revolutionise the development of new products. Nutrigenomics offer novel approaches to understand the interaction of nutritional with the animal and thus offer new possibilities to precisely understand the effect of nutritional measures on the overall organism and down to the level of the single gene. These approaches will help in optimising nutrition in the field and develop animal and farm specific concepts. However, the trends towards a lifestyle and nutrition closer to nature lead in part of the society to a strong reluctance towards new technologies. This could further separate the nutrition industry in a branch close to nature and a branch which is adopting and implementing the latest scientific technologies.

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11

Co-products in swine nutrition and feed formulation

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Summary points

- As omnivore species, pigs are suited to convert non (human) edible feedstuffs into pork.
- Compared with traditional diets based on a single grain as an energy source and soybean meal as a protein source, feeding high inclusion levels of co-products has a greater risk.
- The risk can be managed using modern feed formulation and feed evaluation to attain predictable swine growth performance, carcass characteristics and pork quality.
- Dietary inclusion of co-products from food and bio-fuel production will improve the human-edible protein balance (edible protein output/edible protein input) of swine production.
- Dietary inclusion of co-products reduces feed cost per unit of pork produced and is part of an effort to create sustainable swine production systems.

Keywords: alternative feedstuff, digestibility, growth, pig, sustainability

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11.1 Introduction

Sustainability is a concept containing 3 dimensions: planet, people and profit (IUCN, 2005) that refer to the area where environmental, social and economic considerations overlap. To follow this concept, sustainable nutrition and feeding of pigs is the area where pig production is profitable, with socially-acceptable practices and a low environmental footprint. For sustainable swine nutrition, co-products are important. First for economic sustainability, co-products have become an important option to control feed cost (Woyengo *et al.*, 2014). The new demand for feed grains for biofuel production has elevated price forecasts for feed grains long-term to higher price plateaus. Using co-products is a risk that should be managed with proper risk management strategies including modern feed quality evaluation. Second, for societal and environmental sustainability, using co-products for swine addresses the argument that pigs compete with humans for food (Nonhebel, 2004). Behind every food product in a supermarket is a useful co-product for swine feeding, is an argument overlooked. The conversion of non-edible residues from food, bio-fuel and bio-processing industries into pork, mitigates the environmental impact of these industries (Van Zanten *et al.*, 2014). Certain global regions already use co-products from the food industry effectively to produce pork and are less reliant on feed grains. Many co-products contain more water than the original grains and liquid feed systems are thus ideal for their inclusion in complete feed (Jensen and Mikkelsen, 1998). Indeed, pigs as an omnivorous species are suited to consume a wide variety of feedstuffs and are thus integral for sustainable livestock production systems (Zijlstra and Beltranena, 2013) by improving the human-edible protein balance (FAO, 2011). Feedstuff selection is thus an important part of sustainable animal nutrition (Makkar and Ankers, 2014).

Using co-products is not new. In North America, inclusion of co-products became routine during periods of price rises for feed grains or soybean meal. Few global regions have a solid logistical system for the swine industry to rely on co-products as main feedstuffs in diets. Introducing co-products is a risk for consistent growth performance and predictable carcass quality that requires management with modern feed evaluation. Co-products have been summarised previously (Chiba, 2001; Myer and Brendemuhl, 2001), so the focus will be on recent developments.

11.2 Feed formulation and risk management

11.2.1 Nutrients

Introducing co-products into swine diets poses a risk that must be managed. Risk factors include nutritional (variability, wider macronutrient range), chemical (residues), biological (mycotoxins, anti-nutritional factors (ANF), microbial contamination) and

reduced pork quality (De Lange, 2000). Countries such as the Netherlands rely heavily on co-products (FEFAC, 2016). The choice of energy evaluation system will alter the relative values placed upon feedstuffs (Noblet *et al.*, 1993). The digestible (DE) and metabolisable energy systems overestimate the energy contribution to support maintenance and growth, while the net energy (NE) system offers a more accurate ranking of feedstuffs (Whittemore, 1997). Energy values for an array of feedstuffs were reported (Sauvant *et al.*, 2004; CVB, 2007; NRC, 2012) and the Dutch feed industry has relied on the NE system since 1970 to manage the risk of a wide ingredient matrix.

Differences in energy evaluation are reflected in research approaches. Regularly, increasing dietary inclusion of new co-products, e.g. corn and wheat distiller's dried grain with solubles (DDGS), was tested by feeding pigs diets that were formulated to an equal DE or metabolisable energy value. Not surprisingly, growth performance was then reduced (e.g. Whitney *et al.*, 2006; Widyaratne and Zijlstra, 2007), because inclusion of high fibre or protein feedstuffs reduces dietary NE value due to increased heat increment. Subsequently, test feedstuffs were blamed for lower growth performance, rather than the evaluation system used to determine the energy value.

Accurate prediction of the NE value of co-products is important to assure equivalent growth performance following dietary inclusion of co-products. The approach to formulate diets to equal NE value may reduce differences in growth performance following the introduction of single co-product, such as canola meal (Landero *et al.*, 2011). Feed intake would then be the major factor affecting growth (Seneviratne *et al.*, 2010). Feed quality evaluation for energy value is important for the successful introduction of new feedstuffs in swine production.

An important risk associated with co-products related to efficiency of production and thus economics, is nutrient variability. For example, a main risk associated with the use of DDGS in swine diets is variability in content of the first-limiting amino acid (AA) lysine due to drying using heat (Zijlstra and Beltranena, 2008). The risk of protein damage by overheating feedstuffs is well understood, and DDGS samples range widely in lysine damage (Fontaine *et al.*, 2007). Likewise, oil extraction using various processing techniques (solvent-extraction, expeller-press and cold press) may results in a range of residual oil and thus variability in energy value of the resulting meal, expeller or cake (Spragg and Mailer, 2007).

11.2.2 Other risks

Residues are a risk associated with co-products, especially of unknown or less reputable sources. A worst-case scenario was the introduction into feed of polychlorinated biphenyls (PCB)/dioxin via contaminated feedstuffs (Bernard *et al.*, 2002). Residues such as PCB can accumulate in pork and poultry tissues (Hoogenboom, 2004) and, thereby, pose a risk for consumers. Likewise, melamine-contaminated swine feed can

affect animal health (González *et al.*, 2009). These incidents point to the importance of prevention (Den Hartog, 2003). As co-products may contain unwanted residues that affect animal health or food safety for consumers, frequent monitoring of residue levels is essential.

Mycotoxins may occur naturally in co-products, because mycotoxins in crops may survive fermentation and drying and are thus not inactivated. Ethanol production from grain concentrates the mycotoxin deoxynivalenol 3-fold in the co-product DDGS (Schaafsma *et al.*, 2009). Mycotoxins should be managed, because deoxynivalenol, even at low concentrations, may reduce swine growth and reproductive performance (House *et al.*, 2002). Knowing the agronomic conditions of the feedstock grain would be beneficial. These conditions relate with deoxynivalenol content in grain used for ethanol production and deoxynivalenol content in the co-product DDGS (Schaafsma *et al.*, 2001).

11.3 Co-products

A range of technologies exists to fractionate crop seeds into components for human food, bio-product or feed application (Zijlstra *et al.*, 2004). Traditionally, crop seeds were dry [without solvent] fractionated to extract a valuable component using physical characteristics for human food application. Examples include oil extraction using a press, milling, sieving and protein and starch separation using air classification. Generated co-products were used as feedstuffs. Advantages of dry fractionation are continuous instead of batch-processing, lower processing costs and the absence of solvents or slurry (Hemery *et al.*, 2007). Disadvantages of dry fractionation are that the fractionation into components is not absolute and that properties of products may not reach the superior value attributes required by some manufacturers of human food or for bio-product applications. Consequently, wet fractionation processes were developed using water, acids, bases, salts or organic solvents to separate valuable components using chemical characteristics (Vasanthan and Temelli, 2008). Advantages of wet fractionation include achieving effective separation of high-value fractions. For example, soluble ANF (e.g. glucosinolates, phenolics) can be washed away in the slurry, and pH or enzymes can dephytinise co-products, thereby, converting phytate-P into available P (Thacker and Petri, 2011). However, wet fractionation is more expensive and drying of the products is often required for long-distance transportation, long-term storage and dry feed application. Although drying using heat may inactive ANF and increase mineral availability, drying may also damage the protein contained in co-products and, thereby, hamper nutritional quality. Spray-drying is far more costly than traditional drying methods, but avoids much of the protein damage in co-products, thereby, maintaining their nutritional and functional properties. Co-products have become increasingly attractive for use in swine diets to

reduce feed costs and, thereby, enhance economic sustainability of the swine industry (Woyengo *et al.*, 2014).

Liquid feeding systems may incorporate wet co-products into swine diets and avoid drying and associated energy-costs. Thus, liquid feeding can be regarded as more environmental and economically sustainable, especially if the swine farm is nearby a processing plant. Liquid feeding may also allow for modification of feed characteristics using steeping enhancing nutrient digestibility (Choct *et al.*, 2004; Niven *et al.*, 2007). Risks associated with the feeding of wet co-products, however, need to be managed to avoid unwanted microbial growth. Fermentation of wet co-products or the inclusion of acids or other additives can reduce this risk. Finally, enzyme addition to co-products or wet feeding systems may enable increased nutrient utilisation.

11.3.1 Bio-fuel industry

Considerable demands exist to replace fossil fuels with renewable fuel sources such as biodiesel and ethanol. Consequently, DDGS, canola cake and crude glycerol have become available as co-products for swine although variability in their nutritional quality is a major concern (Zijlstra and Beltranena, 2008). The use of cereal grains in livestock diets and biofuel production continues to receive considerable attention in discussions around global food supply (Dale, 2008). The biofuel industry directly competes with the livestock and food industry for grain supply, thereby, increasing local grain prices. In turn, the biofuel and food industry also produce co-products that are available for inclusion into livestock diets. If biofuels are produced, markets for co-products are needed. Thus, inclusion of bio-fuel co-products in swine feeds might be cost-attractive to swine producers (Lammers *et al.*, 2010).

Distiller's dried grain with solubles

Of the co-products, corn DDGS has reached global commodity status. The nutritional value of corn DDGS for swine has been reviewed (Stein and Shurson, 2009). Briefly, the fermentation of starch sugars into ethanol results in a co-product with increased density of the other macronutrients and minerals but also contaminants. For corn that contains more oil than wheat, the co-product corn DDGS may reach a similar DE and metabolisable energy value compared with corn grain due to increased density of ether extract, and will reach a higher protein density than the feedstock. Therefore, corn DDGS is an attractive feedstuff for swine as both an energy and AA source. Up to 30% corn DDGS can be included in diets for grower-finisher pigs without changes in growth performance (Xu *et al.*, 2009). However, inclusion of DDGS does not always result in consistent growth performance (Stein and Shurson, 2009). Differences might be related to variability in quality among samples of corn DDGS (Zijlstra and Beltranena, 2008) due to fermentation, drying, and different ratios between distillers grain and soluble and due to differences in dietary energy, macronutrient and AA

profile and mycotoxins among diets. Increasing dietary inclusion of DDGS in corn-soybean meal diets balanced for energy and AA does not affect carcass lean and back fat (Xu *et al.*, 2009). However, increasing dietary DDGS will increase dietary fibre and polyunsaturated fatty acid content that consequently will decrease dressing percentage and increase carcass polyunsaturated fatty acid content, respectively (Xu *et al.*, 2009). Dietary fibre increases gut weight (Jørgensen *et al.*, 1996) and dietary polyunsaturated fatty acid are directly deposited into carcass fat depots (Averette Gatlin *et al.*, 2002). To reduce the negative impact of corn DDGS on pork fat hardness, a 3-wk withdrawal or reduced dietary inclusion of corn DDGS prior to slaughter can be implemented (Beltranena and Zijlstra, 2010).

The oil content of wheat is lower than corn; therefore, wheat DDGS has a much lower energy value than wheat grain (Stein and Shurson, 2009). Wheat DDGS is more useful as a protein than energy source, compared to corn DDGS. For both corn and wheat DDGS, the P content and P digestibility is higher than in the parent grain. Initially, results of feeding wheat DDGS to pigs were not positive. The growth performance of grower-finisher pigs fed 100 g/kg or more wheat DDGS was reduced (Thacker, 2006) even when diets were formulated to equal DE and standardised ileal digestible AA content (Widyaratne and Zijlstra, 2007). However, the wheat DDGS used for these studies had been overheated during drying (Zijlstra and Beltranena, 2008). Recently, ethanol processing plants with improved fermentation and drying technologies produce a wheat DDGS of likely a higher quality. Indeed, 15% of this wheat DDGS could be included in diets fed to weaned pigs with limited effects of growth performance, but 20% wheat DDGS reduced growth performance (Avelar *et al.*, 2010; Figure 11.1). Up to 20% of this wheat DDGS could be included in diets fed to grower-finisher pigs, but reduced performance should be expected at higher

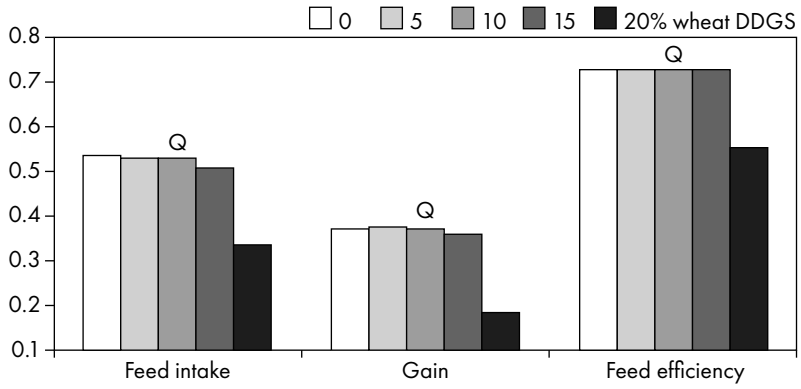

Figure 11.1. Growth performance of weaned pigs fed diets with increasing level of wheat distiller's dried grain with solubles (DDGS) in substitution for soybean meal (Q = quadratic, $P<0.001$) (adapted from Avelar *et al.*, 2010).

dietary inclusion levels (Beltranena and Zijlstra, 2010). Enzymes such as phytase or multicarbohydrase may provide an opportunity to increase nutrient digestibility of wheat-based DDGS (Yáñez *et al.*, 2011; Jha *et al.*, 2015).

Crude glycerol

To produce biodiesel, oil extracted from oilseeds or fat from animal origin is hydrolysed using methanol and a catalyst, thereby, producing methyl esters (biodiesel) and crude glycerol (Kerr *et al.*, 2007) that may serve as an energy source for pigs. Energy digestibility of diets containing 0 to 20% crude glycerol ranged from 89 to 92%, indicating that pigs digest crude glycerol well (Lammers *et al.*, 2008a). Average daily gain of pigs was increased 8% by replacing 10% barley with crude glycerol (Kijora and Kupsch 1996). Replacing up to 6% corn with crude glycerol increased average daily gain of pigs (Groesbeck *et al.*, 2008). Crude glycerol may replace 5 to 10% of cereal grains in swine diets on an equal mass basis (Lammers *et al.*, 2008b).

Concerns exist about feeding crude glycerol. Crude glycerol may contain methanol and NaCl that remain as a residue after processing. Methanol should not exceed 150 mg/kg in glycerol as feedstuff; greater levels may cause metabolic acidosis, vomiting, blindness or gastrointestinal problems (Kerr *et al.*, 2007). Increased NaCl may limit dietary inclusion of glycerol to avoid dietary Na and Cl levels exceeding recommendations. Finally, glycerol is a viscous gel that may present problems for feed mixing and flow (Kerr *et al.*, 2007), but it increased pellet durability and lowered amperage, motor load, and pellet mill production efficiency (Groesbeck *et al.*, 2008).

11.3.2 Food industry

Behind every food product in the supermarket is a co-product. These co-products cover a wide range: canola meal, wheat millrun, citrus pulp, beet pulp, etc. The livestock industry is an ideal platform to convert these low-value co-products into high quality animal protein.

Oilseed meal

The primary reason for producing oilseed meal is oil extraction for human food markets, although biodiesel and bioproducts are increasingly important. Globally, rapeseed oil is the third most important vegetable oil, after soybean and palm oil, and rapeseed meal is the second most important protein meal, after soybean meal. Most of rapeseed in North America and Europe is low in glucosinolates and erucic acid, and is known as canola in North America (Bell, 1993) and 00 rapeseed in Europe. Oil constitutes 40% of the canola seed and is its most valuable component. Solvent extraction, expeller pressing and cold pressing do extract oil to produce raw canola oil and canola meal, expeller and cake, respectively (Leming and Lember, 2005). Historically, reason for limits in dietary

inclusion of canola meal is its lower content of available energy and AA mainly due to less digestible fibre and crude protein (CP) compared to soybean meal (Bell, 1993). Recently, weaned pigs fed diets replacing up to 20% soybean meal with canola meal did not alter growth performance (Landero *et al.*, 2011; Figure 11.2).

Oil from canola seed is mostly extracted in solvent-extraction plants due to high extraction efficiency (~95%), but results in canola meal with a low DE content (Spragg and Mailer, 2007). Expeller pressing without solvents is less efficient in oil extraction (~75%). Hence, the resulting canola expeller contains 10 to 15% oil (Leming and Lember, 2005), and thus has a greater DE value and lower digestible AA content than canola meal (Woyengo *et al.*, 2009; Seneviratne *et al.*, 2010). Compared to canola meal, the greater energy value makes canola expeller a better feedstuff for the energy-dependent phase of growth of pigs (Landero *et al.*, 2012). Using a screw press, canola cake is produced that contains 18 to 20% residual oil (Schöne *et al.*, 2002). Dietary inclusion of 15% canola cake reduced feed intake and weight gain, with residual glucosinolates likely being a contributing factor (Schöne *et al.*, 2002). A maximal content of 2 mmol glucosinolate/kg diet appears a prerequisite for using canola products in pig feeding (Schöne *et al.*, 1997).

Flaxseed (or linseed) meal is a co-product of the flax crushing industry. Depending on oil extraction, flaxseed meal may contain between 3 and 7% residual oil (Bell and Keith, 1993; Farmer and Petit, 2009) and flaxseed expeller may contain 13% residual oil (Eastwood *et al.*, 2009). Due to its low residual oil, feeding of flaxseed meal did not affect fatty acid profiles in plasma and milk of sows (Farmer and Petit, 2009), whereas feeding of flaxseed or flax oil increased α-linolenic acid. Due to its high residual oil, feeding of up to 15% flaxseed expeller increased back fat and loin tissue α-linolenic acid (Eastwood *et al.*, 2009).

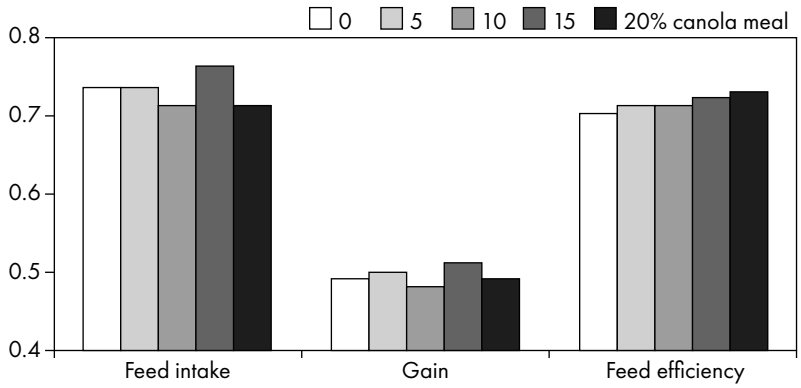

Figure 11.2. Growth performance of weaned pigs fed diets with increasing level of canola meal in substitution for soybean meal (P>0.05) (adapted from Landero *et al.*, 2011).

Wheat co-products

Dry milling of wheat removes grain endosperm to produce flour for human consumption and produces a number of wheat co-products as a residual (Holden and Zimmerman, 1991). Wheat co-products include wheat bran, middlings, shorts and screenings and are also sold in various combinations of these co-products as wheat millrun (Association of American Feed Control Officials, 1988). Wheat co-products from flour milling are thus variable in composition and nutrient profile. Wheat short varies in composition due to different proportions of bran and endosperm (Huang *et al.*, 1999). Increased neutral detergent fibre content among wheat co-products is associated with reduced AA digestibility (Huang *et al.*, 2001). Xylanase supplementation can enhance nutrient digestibility of wheat co-products for growing pigs by removing this negative effect of fibre (Nortey *et al.*, 2008).

Sugar beet pulp

The processing of sugar beets yields as a co-product sugar beet pulp that is high in non-starch polysaccharides (NSP), particularly pectin (Spagnuolo *et al.*, 1999). Sugar beet pulp is fermented well by pigs, even though the rate of fermentation is lower than for rapidly fermentable NSP such as inulin (Awati *et al.*, 2006). Functional properties of fermentable NSP have received interest for 3 sustainability purposes: to alter N excretion patterns, to improve gut health and to influence animal welfare. Fermentable NSP change the flow of nutrients in the digestive tract. Fermentable NSP can shift N excretion from urine to faeces by binding N into microbial protein (Bindelle *et al.*, 2009). Furthermore, fermentation of NSP produces short-chain fatty acids that serve as energy source. The shift in N excretion combined with reduced manure pH can decrease ammonia emission from swine manure (Canh *et al.*, 1998). Sugar beet pulp stimulated growth of the gut and intestine health in high CP diets, but reduced gut health in low CP diets (Hermes *et al.*, 2009). Finally, the lower energetic utilisation of fermented NSP compared with that of starch might be compensated in pigs by reducing their physical activity and altering their behaviour (Schrama *et al.*, 1998).

11.3.3 Fractionation

Dry or wet fractionation of crops, oilseed meal or DDGS may create new crop products with unique nutritional and functional characteristics as feedstuff for livestock with high nutritional demands (Zijlstra *et al.*, 2004). Air classification of pulse grains seems an opportunity, because pulse seeds contain starch and protein that separate well in a stream of air after fine grinding. Dehulling of field pea followed by fine grinding and air classification allowed the separation of fine (mainly protein) and coarse (mainly starch) fractions (Wu and Nichols, 2005). Oil fractionation from, e.g. soybean, rapeseed or sunflower seed, has a long tradition for human food purposes. Fibre

has been fractionated traditionally via dehulling while modern wet fractionation technologies extract fibre fractions with unique functional properties.

Protein fractions

Protein concentrates containing 60% CP and protein isolates containing 90% CP have been developed from soybean meal. Fractionation of field pea has a strong tradition (Bramsnaes and Olsen, 1979), zero-tannin faba bean fractionates well into a protein concentrate (Gunawardena *et al.*, 2010a) and dry fractionation of canola meal and DDGS also creates high protein fractions (Yáñez *et al.*, 2014; Zhou *et al.*, 2015). Such protein concentrates are attractive for specialty protein sources in young pigs (Valencia *et al.*, 2008; Gunawardena *et al.*, 2010b; Zhou *et al.*, 2013; Figure 11.3). Field pea protein isolates can be produced using wet fractionation and may be used as alternative for spray-dried plasma protein. This protein isolate is also highly digestible due to removal of ANF (Le Guen *et al.*, 1995); however, it does not have the functional properties of plasma protein. Therefore, field pea protein isolate will have to be mixed with egg yolk antibodies from hyperimmunised laying hens containing specific anti-enterotoxigenic *Escherichia coli* (K88) antibodies to control an *E. coli* infection (Owusu-Asiedu *et al.*, 2003).

Starch fractions

Starch isolates (90%) are produced for human food purposes, and are rarely included in commercial swine feed. However, starch isolates are used in swine nutrition to study the impact of starch chemistry on rate of starch digestion and glycaemic responses (Van Kempen *et al.*, 2010). Air classification products are highly digestible

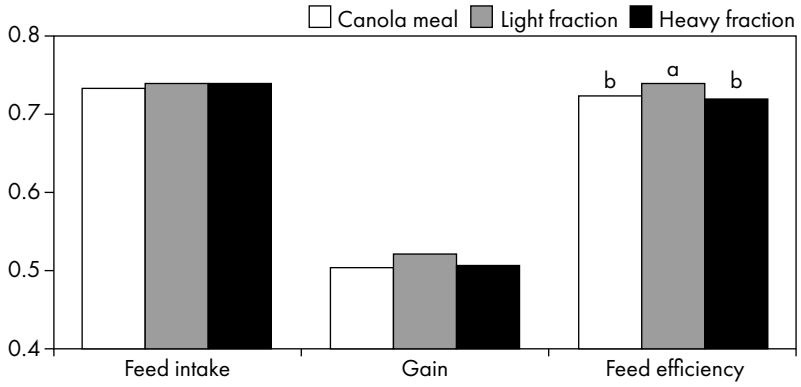

Figure 11.3. Average daily feed intake, daily gain, and feed efficiency of weaned pigs fed diets including 200 g/kg solvent-extracted canola meal or its air-classified light- or heavy-particle fractions ($P<0.05$) (adapted from Zhou *et al.*, 2013).

field pea and zero-tannin faba bean starch concentrate that may serve as a feedstuff for young pigs (Gunawardena *et al.*, 2010b). Cooked rice might be a preferable starch source for young pigs due to enhanced energy intake (Parera *et al.*, 2010). Finally, raw potato starch might be included in diets for young pigs as a source of resistant starch (Bhandari *et al.*, 2009). Resistant starch has prebiotic activity and might be part of a solution to use characteristics of feedstuffs instead of feed additives to facilitate the removal of antibiotics from swine diets. Moreover, dietary resistant starch is fermented similarly as fermentable fibre and might be part of a solution for reducing odour emissions from swine farms (Willig *et al.*, 2005). Finally, dietary resistant starch may reduce skatole formation, and might, thereby, form part of a solution to avoid castration of boars and to reduce boar taint in pork (Lösel and Claus, 2005).

Fibre fractions

Dehulling of cereal grain, pulse seed or oilseed is generally considered advantageous for swine nutrition. Dehulled seed will have a greater energy value and nutrient digestibility than the entire seed. For example, dehulled cereal grain and meal of dehulled canola seed have a greater nutrient digestibility than their hulled counterpart (Kracht, 2004; Hennig *et al.*, 2006). Hull NSP is generally insoluble and has less favourable fermentation characteristics in the porcine digestive tract (Williams *et al.*, 2005). Hull NSP increased diet bulk density, thereby, causing satiety in gestating sows with a restricted access to feed and reduced stereotypic behaviour (Holt *et al.*, 2006). Fibre fermentation characteristics differ among grain samples, and high fermentability may create prebiotic effects (Pieper *et al.*, 2009). The wet fraction may enhance viscosity or prebiotic effects of NSP, for example, wet fractionation of β-glucan from oat or barley yields β-glucan concentrate with a high fermentability, and a specific *in vitro* viscosity depending on chain-length of the β-glucan. These fractions have prebiotic activity (Metzler-Zebeli *et al.*, 2010) and reduce glycaemic responses (Hooda *et al.*, 2010).

Fat fractions

Oil extracted from oil seeds has, after purification, a higher economic value for use as food than as a feed ingredient. Crude plant oil after initial separation has value especially for young pigs with immature gastro-intestinal tracts, because plant oil has a greater digestibility than animal-based fat (Duran-Montgé *et al.*, 2007). However, price or logistical considerations due to impeded material flow can prohibit high inclusion levels of liquid plant oils. Animal-based, saturated fat such as tallow are more cost-effective as an energy source for grower-finisher pigs and may not impact pork fat hardness as unsaturated fatty acids in corn DDGS do (Stein and Shurson, 2009). Opportunities exist to enhance pork omega-3 fatty acid content by feeding flax oil, but doing so may also reduce pork fat hardness unless conjugated linoleic acid is fed simultaneously (Dugan *et al.,* 2004). Feeding flax oil in diets for gestating and

lactating sows will increase α-linoleic acid in sow tissues and milk, thereby, increasing α-linoleic acid in suckling piglets after birth (Boudry *et al.*, 2009). Increased α-linoleic acid in piglets may improve their health status via increased intestinal barrier function (Boudry *et al.*, 2009) and immune resistance (Farmer *et al.*, 2010).

11.4 Future perspectives

Co-product utilisation in swine nutrition is an essential part of sustainable pork production and reduces reliance on cereal grains. The conversion of non-edible co-products into a high quality protein source for human consumption is a story that has been told infrequent and is often missing from public debates. The feeding of co-products may reduce feed costs per unit of pork produced, but also provides challenges to achieve cost-effective, predictable growth performance, animal health, environmental footprint, carcass characteristics and pork quality. Modern feed formulation, rapid diagnostic tools to predict quality of feedstuffs, complete feed and pork produced, and pigs bred for enhanced feed intake are essential tools to mitigate the risks that are associated with the feeding of co-products. Sustained efforts to enhance feedstuff databases that include updated values for existing feedstuffs and new feedstuffs will support efforts to include co-products effectively in swine diets.

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12

Mycotoxins in the feed and animal products

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Summary points

- Mycotoxins are toxins produced by moulds (fungi) in growing crops and stored grains.
- Mycotoxins are usually highly toxic and often carcinogenic, and reduce animal productivity.
- The FAO has estimated that more than 25% of the world's crops are affected by mycotoxins each year.
- The loss due to the impact of mycotoxins in the USA and Canada alone, is estimated at greater than 5 billion USD per year.
- Binders are currently widely used to detoxify mycotoxins, some with poor results. Enzymes, microorganisms, antibodies, aptamers and transgenic crops are new approaches for the control of mycotoxicosis in animals.

Keywords: fungi, toxins, mycotoxicosis, livestock, detoxifiers

12.1 Introduction

Mycotoxins are secondary metabolites produced by fungi. Only some fungi produce mycotoxins, and they are referred to as toxigenic fungi. The fungal toxins are chemically diverse representing a variety of chemical families and range in molecular weights from about 200 to 500 Da (Whitlow and Hagler Jr., 2004). There are hundreds of known mycotoxins but only a few have been extensively researched and even fewer have good methods of analysis. The primary classes of mycotoxins are aflatoxins (B1,

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B2, G1, G2) of which aflatoxin B1 (AFB1) is the most prevalent, zearalenone (ZEA), trichothecenes such as deoxynivalenol (DON) and T-2 toxin (T-2), fumonisins (FUM: FB1, FB2, FB3) and ochratoxin A (OTA) (Chaytor *et al.*, 2011). The major mycotoxin-producing fugal genera are *Aspergillus, Fusarium* and *Penicillium*. Many species of these fungi produce mycotoxins in feedstuffs. Mycotoxins exhibit a variety of biological effects in animals, which include liver and kidney toxicity, neurological, estrogenic and teratogenic effects, to name a few. Some mycotoxins such as AFB1, OTA and FB1 are carcinogenic. Additionally, the consumption of mycotoxin-contaminated feed by animals can cause loss of appetite, decreased feed efficiency, feed refusal, poor weight gain, immunosuppression, and mortality. The analytical methods that are currently available for detection and quantification of mycotoxins include: enzyme-linked immunosorbent assays, thin layer chromatography, high performance liquid chromatography, gas chromatography, near-infrared spectroscopy and liquid chromatography-mass spectrometry. Some of these methods can be used for analysing samples containing multiple mycotoxins. In addition to pre-and post-harvest prevention procedures to control mycotoxin contamination in feedstuffs and feed, there are physico-chemical and biological treatment methods and commercially available products that can be added to the diet that will minimise the harmful effects of mycotoxin-contaminated animal feed.

The FAO has estimated that 25% of the world's crops are affected by mycotoxins each year (Richard and Payne, 2003). The cost of mycotoxins in the US vary with one report estimating this to average $1.4 billion per year while another estimate was $5 billion per year for the US and Canada. The economic impact to the swine industry in the US resulting from mycotoxins in ethanol co-products (dried distiller's grain and soluble) is $18 million per year for fumonisins. Economic losses caused by mycotoxins are due to effects on livestock productivity, crop losses and the costs of regulatory programs directed toward mycotoxins. The regulatory limits for mycotoxins in feedstuffs (feed ingredients) vary based on the toxicity of each mycotoxin and country (Charmley and Trenholm, 2012; Streit *et al.*, 2012; Table 12.1). Thus, it is important to know the various aspects of mycotoxins in animal feed and animal products to understand how they impact the livestock industry directly and human health through animal products such as milk, milk products, meat, meat products, blood/plasma-based products and eggs.

12.2 Mycotoxins in feed ingredients

Moulds can grow and produce mycotoxins in corn, cereals, soybeans, sorghum, peanuts and other food and feed crops or forages in the field and in grains during transportation and during improper storage favourable for the growth of the toxin-producing fungi. Mould growth and mycotoxin production are related to plant stress caused by weather extremes, insect damage, inadequate storage practices and

Table 12.1. Selected examples of regulatory maximum tolerated levels of aflatoxin B1 and guidelines for other mycotoxins in livestock animal feedstuffs/feed.[1]

	AFB1	DON	ZEA	FUM (B1+B2)	OTA
USA[2] (FAO, 2003)	20 µg/kg	10 mg/kg (beef cattle) 5 mg/kg (dairy cattle & poultry) 1 mg/kg (pigs)	1-3 mg/kg (gilts & sows)	10 mg/kg (pigs) 30 µg/kg (cattle older than 3 months) 15 mg/kg (cattle & poultry breeding stock)	N/A
Canada[3] (Charmley and Trenholm, 2012)	20 µg/kg	1 mg/kg (pigs, young calves & lactating dairy cattle)	1-3 mg/kg (gilts) 0.25-5 mg/kg (pigs) 10 mg/kg (dairy cattle)	N/A	0.2 mg/kg (pigs) 2 mg/kg (poultry)
Europe[4] (Streit *et al.*, 2012)	20 µg/kg 5 µg/kg (dairy & young animals)	0.9 mg/kg (pigs) 8-12 mg/kg (in cereals, cereal products & maize by-products)	0.1 mg/kg (piglets & gilts) 0.5 mg/kg (calves & dairy cattle) 3 mg/kg (in maize by-products)	5 mg/kg (pigs) 60 mg/kg (maize & maize by-products)	0.05 mg/kg (pigs) 0.25 mg/kg (cereals & cereal products)

[1] AFB1 = aflatoxin B1; DON = deoxynivalenol; FUM = fumonisins; N/A = not available; OTA = ochratoxin A; ZEA = zearalenone.

faulty feeding conditions. The environmental factors such as temperature range, pH, moisture content, water activity and oxygen levels influence mould growth and mycotoxin production in grains and nuts used as feed ingredients, and in wet feeds such as silage or wet by-products. Rodrigues and Naehrer (2012) reviewed mycotoxin contamination of diverse feedstuff samples from throughout the world for five mycotoxins (AFB1, ZEA, DON, FB1 and OTA; Table 12.2). FB1 was the most frequent contaminant occurring commonly in corn, dried distiller's grain and soluble and finished feeds. Aflatoxins are produced by specific strains of *Aspergillus flavus*, *Aspergillus parasiticus* and *Penicillium* species. About 20 different aflatoxins are known of which AFB1 is the commonest and most toxic. DON is a commonly occurring mycotoxin produced primarily by *Fusarium graminearum* and *Fusarium culmorum* (Rotter *et al.*, 1996). Fumonisins, a family of mycotoxins produced by *culmorum verticilloids* (formerly *F. moniliforme*) and *culmorum proliferatum,* are the main species producing fumonisins B1, B2, B3 mostly in contaminated corn (Cawood *et al.*, 1991).

Table 12.2. Prevalence of mycotoxins (average concentrations in μg/kg) in feed stuffs and feed surveyed worldwide.[1,2]

Geographical regions[3]	AFB1	ZEA	DON	FB1	OTA
North America	8	271	1,947	902	1
Europe	0-3	3-37	88-968	925-3,052	0-9
Oceania	1	50	94	109	1
Asia	8-90	32-219	61-691	380-797	1-15
Central-South America	2-3	0-111	51-237	1,030-3,121	0-9
Middle East	8	14	153	280	4
Africa	42	25	745	855	6

[1] Mycotoxins feedstuffs and feed samples were analysed by high performance liquid chromatography.
[2] AFB1 = aflatoxin B1; DON = deoxynivalenol; FUM = fumonisins; OTA = ochratoxin A; ZEA = zearalenone.
[3] Refer to Rodrigues and Naehrer (2012) for countries included in the geographical regions.

OTA is mainly produced by *Aspergillus ochraceus* and *Penicillium verrucosum* (Pitt, 2000). Zearalenone is produced by *Fusarium* species such as *F. graminearum (Gibberella zeae)* and *F. culmorum*. *A. flavus* growth and aflatoxin production in corn and peanuts are favoured by the heat and drought stress associated with warmer climates (Klich *et al.*, 1994). The *Fusarium* species are associated with economically important diseases, causing ear rot and stalk rot in corn and head blight (scab) in small grains. In wheat, the presence of *Fusarium* species is associated with excessive moisture at flowering and early grain-fill stages. In corn, *F. graminearum* is referred to as a red ear rot and is more commonly associated with a cool, wet growing season and with insect damage. Fusarium ear rots that produce fumonisins are referred to as pink ear rots and vary in their environmental requirements. They are generally associated with dry conditions in mid-season followed by wet weather (Richard and Payne, 2003).

12.3 Mycotoxicoses of animals

Mycotoxins are toxic when consumed by animals or humans. Diseases resulting from the consumption of mycotoxins are called mycotoxicoses (Nelson *et al.*, 1993). A mycotoxicosis outbreak should be considered when the cause of a syndrome is not readily identifiable, the condition is not transmissible and treatment with antibiotics or other drugs has little effect. Outbreaks occur under seasonal conditions conducive for fungal growth and the syndrome appears to be associated with certain lots of feed. Diagnosis of the cause of mycotoxicoses in many cases can only be confirmed by

analysing the feed material for the suspected mycotoxin since only a few incidences of the disease present characteristic pathological signs.

12.3.1 Aflatoxins and aflatoxicosis

Aflatoxins are hepatotoxic, producing parenchymal necrosis and bile duct proliferation in mammals and birds. The young are particularly susceptible. In cattle, clinical effects appear with the commonest signs being production loss, rough hair coat, depressed appetite, and intermittent diarrhoea. In pigs, there is depression, weakness, trembling, ataxia, diarrhoea (often bloody) and icterus. Chronic cases show rough coats and poor feed conversion. Dietary levels of 1 mg/kg or more cause acute aflatoxicosis in adult pigs but concentrations as low as 0.05-0.10 mg/kg affect the weanling pigs. In poultry, acute signs include anorexia, ataxia and depression, and death may occur without any clinical signs being observed. Decreased production of flesh or eggs in poultry, milk in dairy cows, and meat in pigs and beef cattle have also been observed. Reduced growth and productivity may be accompanied by damage to liver, haemorrhaging into the muscles or body cavities, and suppression of natural immunity to parasites and pathogens present in the environment.

12.3.2 Deoxynivalenol toxicosis

The trichothecenes belong to a family of 200-300 related compounds including DON (or vomitoxin), T-2, diacetoxyscirpinol and nivalenol that are commonly found in agricultural commodities (Desjardins *et al.*, 1993). Chronic effects of DON include reduced feed consumption, reduced growth (anorexia and decreased nutritional efficiency), immune function changes and reduced litter size (Pestka, 2004). DON concentrations greater than 1 mg/kg can result in reduced feed intake and lower weight gains in pigs. Field studies have showed that DON is the primary mycotoxin associated with swine disorders; including feed refusals, diarrhoea, emesis, reproductive failure and deaths (Côté *et al.*, 1984). Vomiting has been reported in some outbreaks with high DON concentrations. Other related mycotoxins such as 3- and 15-acetyl DON often co-occur with DON. Berthiller *et al.* (2005) reported on the occurrence of a glucoside of DON in corn and wheat samples. This conjugated DON often escapes detection by routine analytical methods and may account for the toxicity associated with low observed concentrations of mycotoxins. While certain monogastric animals such as pigs exhibit the greatest sensitivity to DON, chickens and turkeys, followed by ruminants, appear to have higher tolerance because of low absorption into tissues and rapid clearance (Prelusky *et al.*, 1994). Layers appear to be more tolerant to DON than are broilers under the stress of rapid growth (Huff *et al.*, 1986). Although poultry can be more resistant to the intake effects of these toxins, some recent evidence points to immune and gut health related effects in poultry at levels below those that may cause clinical outbreaks. Ruminants are relatively insensitive to DON because rumen microorganisms are able to metabolise/detoxify this toxin (King *et al.*, 1984). Studies

have concluded that beef cattle and sheep can tolerate up to 21 mg/kg of DON without obvious deleterious effects. Dairy cows fed 66 mg/kg of DON for 5 days showed no signs of impaired performance or illness (Côté *et al.*, 1986). However, the clinical data appear to show an association between DON contamination of feeds and poor performance in dairy herds (Whitlow *et al.*, 1994).

12.3.3 Fumonisin toxicosis

Fumonisin causes equine leukoencephalomalacia in horses (Marasas *et al.*, 1988), pulmonary oedema in pigs and hepatotoxicity in cattle (Diaz *et al.*, 2000). Equine leukoencephalomalacia toxicosis is characterised by facial paralysis, nervousness, lameness, ataxia and inability to eat or drink. Poultry are apparently more resistant to fumonisins than are pigs and equines. FB1 is toxic to young turkey poults that appear to be more sensitive to the toxin than broiler chicks (Weibking *et al.*, 1993a). While FB1 is thought to be much less potent in ruminants than monogastrics, work by Kriek *et al.* (1981) suggested that FB1 was toxic to sheep. Dairy cattle fed diets containing high concentrations of FB1 (100 mg/kg) for 70 days had lower milk production, which was primarily due to reduced feed consumption.

12.3.4 Ochratoxicosis

OTA is a causative agent of a kidney disease in pigs referred to as mycotoxin porcine nephropathy (Krogh, 1979). The primary toxic effect of OTA is inhibition of protein synthesis. In pigs and poultry, the proximal tubules are mainly affected and the kidney can become discoloured and grossly enlarged. Additionally, fatty liver can be a common occurrence in poultry. The most sensitive indicator of acute ochratoxicosis in chickens is a reduction in the concentration of total serum proteins and albumin, and in pigs, a sensitive and specific indicator is a decrease in phosphoenolpyruvate carboxykinase activity in the kidney (Marquardt and Frohlich, 1992). Exposure to lower levels of OTA in poultry and pigs can result in altered performance including reduced feed consumption and weight gain, and immunosuppression that can lead to higher susceptibility to infection. Other effects in poultry include decreased egg production, compromised bone strength, intestinal damage and carcass discolouration. Clinically, beak lesions and increased water intake in birds can be seen even at low levels of OTA. In cattle, OTA is rapidly degraded in the rumen into harmless phenylalanine and less toxic OTA-α, and thus thought to be of little consequence unless consumed by young pre-ruminant calves (Sreemannarayana *et al.*, 1988). However, mouldy hay containing OTA has been implicated in cattle deaths and abortions. Chronic exposure and acute toxicities are thought to occur in cattle.

12.3.5 Zearalenone toxicosis

Zearalenone mimics the effect of the female hormone oestrogen and at low doses increases the size of the mammary gland or is associated with the early maturity of mammary glands and reproductive organs. At higher doses, zearalenone interferes with conception, ovulation, implantation, foetal development and the viability of new born animals (Jones *et al.*, 1994). Other responses of dairy animals to zearalenone may include reduced feed intake, decreased milk production, vaginitis, vaginal secretions and poor reproductive performance. The animal most sensitive to zearalenone is the pig, in which physiological responses occur at zearalenone levels above about 1 mg/kg of feed. The mammae, ovaries and tubular organs of the female reproductive tract are affected. The usual clinical signs in gilts and sows are mammary and vulvar enlargement. Other signs include rectal or vaginal prolapsed, abortion, small litters, still births, and foetal mummification. Boars show mammary and testicular atrophy, and decreased libido.

The estrogenic effect of zearalenone is one of the principal problems in dairy farming. Consumption of zearalenone results in infertility, reduced milk production, and hyperoestrogenism. Swollen and hyperaemic external genitalia have been observed when feeding higher levels of zearalenone to cows. Other cattle responses may include vaginitis, vaginal secretions, poor reproductive performance and mammary gland enlargement of virgin heifers. The known ruminal conversion of zearalenone to alpha- and beta-zearalenone does not seem to minimise the estrogenic effect of zearalenone in cattle.

12.4 Mycotoxins in animal products

Mycotoxin metabolism involves pathways of bioactivation and detoxification in animals. Detoxification occurs via biotransformation mediated by enzymes in the host cells and in the digestive microflora. Some of the mycotoxins or their metabolites may become fixed in animal tissues. However, most are eliminated in the urine, faeces and milk. The presence of toxic residues in edible animal products (milk, meat, offal, eggs), may have detrimental effects on human health. Aflatoxin B1 metabolites M1 and M2 are of special interest with regard to carry-over, since they can be excreted via milk. AFM1 has been detected in powdered milk, pasteurised milk and milk-based products (cheese, yoghurt, curds, and buttermilk). Evidence of carry-over of AFB1 has also been found in porcine tissues and chicken or duck eggs. It may occur in porcine liver, muscles, kidneys and adipose tissue. In poultry, AFB1 residues have been found in the egg of young laying hens fed diets containing 500 mg/kg body weight for seven weeks (Völkel *et al.*, 2011). In pigs, OTA has been detected in kidney tissue, musculature, but at lower concentrations than in whole blood or blood plasma (Völkel *et al.*, 2011). This might be of special concern for the processing of

specialties such as blood pudding, lunch meat and sausages as many of these products include additives of pig-blood or -plasma. There are reports regarding the transfer of OTA into milk and beef. However, bacterial metabolism in the gastrointestinal tract, especially in the rumen, yields the less toxic cleavage product, OTAα. The level of OTA in milk escaping conversion to OTAα is minute compared to the OTA contamination levels of grains seen during daily feeding practice. Even then, it is sufficient to pose a significant risk to consumers such as children. In pigs, no transfer of ZEA and its metabolites into serum was detected after the administration of 56 μg of ZEA/kg feed (Völkel *et al.*, 2011). Poultry appear to be relatively insensitive to ZEA although the underlying reasons for this have not been identified. One possible explanation might be the naturally high level of oestrogen in poultry blood. No data on ZEA carry-over in commercially produced eggs have been reported. In dairy cattle, transfer of ZEA to milk occurred after ingestion of a very high dosage (20 mg/day) via feed in a single dairy cow for 7 days. In beef cattle, neither ZEA nor its metabolites could be detected in muscles, offals and dorsal fat of a male bovine fed a daily diet containing 100 μg/kg feed (Zinedine *et al.*, 2007). Microbial degradation of DON within the rumen by de-epoxidation will result in a loss of toxicity in ruminants even at the stage of uptake. The concentration of de-epoxy-DON in blood is correlated with the intake of DON (Völkel *et al.*, 2011). The daily excretion of DON in the milk of dairy cows after a daily oral DON intake of 16.6-75.6 mg, varied between 1 and 10 μg DON and 14-104 μg de-epoxy-DON (Völkel *et al.*, 2011). Pigs on both *ad libitum* or restrictive diets that contained up to 6.68 mg of the toxin/kg diet over a period of twelve weeks showed DON and de-epoxy-DON in bile, kidneys, liver, serum, muscle and fat (Völkel *et al.*, 2011). In poultry, the carry-over of DON into eggs was indirectly proven during the 1980s (Prelusky *et al.*, 1987). However, no human diseases due to the carry-over of DON have been reported since the animal probably acts as an efficient filter unit (Völkel *et al.*, 2011). FB1 carry-over in sow's milk and pork meat might only happen after a long-term exposure (Völkel *et al.*, 2011). Pigs fed an FB1enriched diet containing 2-3 mg/kg did not have any accumulation of the toxin in milk and muscles, but liver and kidney turned out to be contaminated.

12.5 Mycotoxin control

While prevention of mycotoxins in feedstuffs and feed involve both the pre- and post-harvest controls, treatment to limit the effects of mycotoxins include: physical, chemical and microbiological methods (Yiannikouris and Jouany, 2002). Pre-harvest control has involved the use of agronomic practices which minimise plant stress and fungal invasion, and thus mycotoxin accumulation in growing crops (Chaytor *et al.*, 2011). These include proper irrigation, insect control, use of resistant varieties, tillage practices, fertilisation, timely planting and avoiding delayed harvest. The best strategy for post-harvest control of mycotoxins is proper storage and handling of feedstuffs to prevent conditions conducive for fungal growth. Temperature, water

activity and insects are the major factors most closely associated with mycotoxin formation in storage. Management strategies also include mycotoxin analysis of feedstuffs, diversion of contaminated lots, treatment to reduce mould growth, dilution and treatments to reduce mycotoxin levels. Also, physical separation by cleaning or screening grains can be helpful. Certain chemical and biological processes may also be of value in reducing mycotoxin effects. Fermentative bacteria may have potential for binding some mycotoxins (Niderkorn *et al.*, 2007). Mycotoxins may be degraded into non-toxic metabolites using microorganisms or enzymes. Ammoniation of grains can destroy some mycotoxins, but this is not a practical method to treat forages. Addition of 0.25 or 0.5% of calcium propionate to feed for detoxification may reduce the effects of AFB1 (Bintvihok and Kositcharoenkul, 2006). Galvano *et al.* (2001) have reviewed dietary strategies to counteract mycotoxins. Furthermore, increasing nutrients such as protein, energy and antioxidant nutrients may be advisable.

The addition of agents (adsorbents/absorbents) able to bind mycotoxins can reduce the bioavailability of these compounds in animals and limit the risk of mycotoxicoses. In the case of AFB1, hydrated sodium calcium aluminosilicates and phyllosilicates from natural zeolites have a high affinity, both *in vitro* and *in vivo,* for the toxin (Yiannikouris and Jouany, 2002). Zeolites, which are hydrated aluminosilicates of alkaline cations, are able to adsorb AFB1 and ZEA (Piva *et al.*, 1995). Bentonites have a lamellar crystalline microstructure, the composition and adsorption properties of which depend on the interchangeability of the cations positioned in various layers. Bentonites have been shown to be effective for the adsorption of AFB1 and T-2 (Ramos *et al.*, 1996). Clays such as kaolin, sepiolite and montmorillonite bind AFB1 less efficiently than hydrated sodium calcium aluminosilicates and bentonites. Activated carbon is also able to bind mycotoxins (Galvano *et al.*, 1996). In addition, resins such as cholestyramine and polyvinyl-polypyrrolidoxynivalenol are able to bind to OTA and AFB1 (Madhyastha *et al.*, 1992; Piva *et al.*, 1995). Glucomannans extracted from the cell wall of the yeast (*Saccharomyces cerevisiae*) are able to bind AFB1, AFM1, FB1, ZEA, T-2, DON and OTA with the binding affinity ranging from 12 to 95% (Yiannikouris and Jouany, 2002). Some of these binders may have adverse nutritional effects. Also, higher concentrations of these binders in the diet can reduce the bioavailability of certain minerals and vitamins.

Enzymes have been found to have potentially useful mycotoxin degrading activity, including protease A or pancreatin (Abrunhosa *et al.*, 2006), carboxypeptidase A or epoxidase (Schatzmayr *et al.*, 2006) and lactonohydrolase (Takahashi-Ando *et al.*, 2002). Microorganisms with potential for mycotoxin detoxification include *Flavobacterium aurantiacum* (AFB1), *Enterococcus faecium* (AFB1), *Eubacterium* strains (DON, T-2) and *Trichosporon mycotoxinivorans* (ZEA, OTA). More recently, Loi *et al.* (2017) have reviewed in detail *in vitro* and *in vivo* studies on the biotransformation of the mycotoxins AFBs, OTA, ZEA, FB1 and the trichothecenes by enzymes from bacteria, fungi and plants. Mycotoxin biotransformation is defined

as 'the degradation of mycotoxins into non-toxic metabolites by using bacteria, fungi or enzymes' (Bouderque *et al.*, 2009). The possibility to use living microorganisms as whole cell biocatalysts for mycotoxins degradation has cost advantages. This represents a valid strategy, especially if multi step reactions are required, or if the microorganism is already implemented within industrial processes. Loi *et al.* (2017) discussed the biotransformation of mycotoxins by native and commercial enzymes, their mechanism of action and their current and possible application in the food and feed industries. One of the main challenges related to the commercial use of mycotoxin degrading enzymes is the co-occurrence of mycotoxins which would require the simultaneous use of different enzyme preparations. This problem appears to be solved at the *in vitro* level as Loi *et al.* (2018) reported that a laccase from *Pleurotus eryngii* and a laccase-mediator systems were able to degrade AFB1, FB1, OTA, DON, ZEN and T-2 toxins individually or all of the mycotoxins when they were present in the same sample. The percent simultaneous *in vitro* degradation of AFB1, FB1, OTA, ZEN and T-2 toxin was by 73, 74, 27, 100 and 40, respectively. They concluded that laccase plus the laccase-mediator systems is a promising approach to degrade most of the common mycotoxin either singly or in combination but that much additional research and development must be carried out before the system will be commercialised. Although, many individual enzymes have also been reported to remove or reduce the contamination of specific mycotoxin in real matrices their application in feed has been very limited. Some of very few commercial biotransforming feed additives that are available are Mycofix®, FUMzyme®, Biomin® BBSH 797 and Biomin®MTV (Biomin Holding GmbH, Getzersdorf, Austria). Only FUMzyme exploits a purified enzyme, an esterase, to perform fumonisin degradation. (European Food Safety Agency, 2014). An alternative approach to using specific enzymes, bacteria or fungi as feed additives to degrade a specific mycotoxin is to use transgenic crops capable of combating mycotoxins. Syngenta has patented trichothecene-resistant transgenic plants (Hohn *et al.*, 2002). The company performed field trials, in Canada, USA, Argentina, and three European countries, proving that the development of self-defending crops is already achievable (Karlovsky, 2011). Presumably, a similar approach can be used to genetically modify crops that are resistant to other mycotoxins.

We proposed the use of both antibodies and aptamers to bind and neutralise the toxic effects of mycotoxins that are present in animal feeds (Madhyastha, and Marquardt, 2018). Aptamers (chemical antibodies) are short and single stranded DNA or RNA oligonucleotides (short strands of nucleic acids) or peptides that have been engineered through a selection process to exhibit exceptional binding affinity and specificity to their target or antigen (Zhou and Rossi, 2017). Our proposal is based on the knowledge that both antibodies and aptamers are widely used for the determination of mycotoxins as they can be designed to specifically bind a wide range of mycotoxins with high affinity (Jo *et al.*, 2015; Urusov *et al.*, 2015). The hypothesis is that both antibodies and aptamers can be used to bind and, therefore, neutralise the effects of mycotoxins when present in the animal diets. Currently no research, as far as the authors are aware of,

have been reported of the ability of aptamers and antibodies to neutralise the effects of mycotoxins when added to diets containing mycotoxins. Mycotox Solutions Inc. has completed preliminary proof of concept studies with zebrafish embryos (ZFE) to determine if a specific AFB1-binding monoclonal antibody (mAb) and a specific AFB1-binding aptamer can protect the ZFE from AFB1 induced lethality. This study involved the testing of ZFE lethality in the presence of AFB1 alone, a mAb or aptamer alone and AFB1 in the presence of either the mAb or aptamer. The ZFE mortalities (lethality) in the antibody study, after the 96 h incubation period, were zero for ZFE administered mAb (0.5 μM) alone, 80% ± 3.3 SD for ZFE administered AFB1 (0.25 μM) alone and zero for ZFE administered AFB1 (0.25 μM) + mAb (0.5 μM) mixture. In the aptamer study, the mortalities after a 96 h incubation period were zero for ZFE administered the aptamer alone (2.5 μM), 82% ± 1.9 SD for ZFE administered AFB1 alone (0.25 μM) and zero for ZFE administered the AFB1 (0.25 μM) + the aptamer (2.5 μM) mixture. These data demonstrate that mAbs and aptamers that are designed to specifically bind AFB1 are capable of completely neutralising the effects of AFB1 in a ZFE model. Thus, as is evident from this study, both specific aptamers and antibodies may be highly effective in neutralising the toxic effects of AFB1 and probably other mycotoxins in animals that are fed diets containing mycotoxins. Additional research with animals is required to determine if antibodies capable of neutralising the toxic effects of mycotoxins. Both mycotoxin binder technologies are patented (PCT/CA2016//051083 & PCT/CA2016/051327). In the future, antibodies will be designed to resist digestion in the intestinal tract, will have a much lower molecular weight and will be inexpensively produced on a large scale in plants (Buyel *et al.*, 2017; Edgue *et al.*, 2017). Aptamers have certain advantages over antibodies as they are much smaller than antibodies (6 to 30 kDa versus 150 to 180 kDa) and, unlike antibodies, can be readily selected against an infinite spectrum of targets. Aptamers, however, may be degraded by nucleases produced by intestinal microorganisms. This problem can be overcome by the use of mirror image DNA or RNA aptamers, called Spiegelmers, that are resistant to all nucleases (Vater and Klussmann, 2015). Spiegelmer aptamers can be produced at a relatively low cost in large quantities. A comprehensive comparison of the advantages and disadvantages of nucleic acid aptamers versus antibodies has been reviewed by Zhou and Rossi (2017). Plant antibodies and Spiegelmers (mirror image DNA or RNA aptamers) both have the potential to specifically and safely neutralise the toxicity of mycotoxins present in animal feeds. A considerable amount of research must be carried out to develop cost effective commercial means of using these compounds to mitigate the effects of mycotoxins present in animal feeds.

12.6 Future perspectives

Mycotoxins are ubiquitously present in feed and feed raw materials. In addition to enforcing compliance regulations, continuous monitoring is needed in order to avoid negative impacts on animal health and performance due to elevated

contamination levels in feedstuffs. Furthermore, the general toxicity, teratogenicity and carcinogenicity of mycotoxins constitute a risk to animal and human health. However, more data are needed about animal toxicity and on interactions with other mycotoxins, nutrients and stress factors, and about the role of mycotoxins in immunosuppression. It is also necessary to emphasise studies dealing with toxin-interactions at the level of absorption and bioavailability. With regard to synergistic effects of co-occurring toxins, monitoring and surveillance should be expanded to mycotoxins and metabolites that are thought to be negligible with regard to transfer into animal tissues.

Although the risk of mycotoxins is currently difficult to evaluate, it is necessary to increase the farmer's and consumer's awareness of their presence in feed and animal products. The development of physical, chemical and biotechnological tools to improve feed quality, and improved cultivation, harvest and storage of forages and cereals is essential to reduce the level of contamination in feeds. Contamination of fungal toxins still occurs even though considerable effort has been undertaken to prevent mycotoxin formation. Therefore, mycotoxin reduction strategies such as the use of binders, biotransformation and biodegradation should be considered for limiting the bioavailability of mycotoxin in animals. Ruminants seem to act as an effective 'filter' against these toxins which contaminate much of the plant world and are found as residues in other animal species. Also, improved screening methods are needed for monitoring mycotoxin occurrence, including the detection of multi-mycotoxin and conjugates of mycotoxins, diagnosing toxicities and prevention as well as treatment of mycotoxicoses. The development and application of multi-mycotoxin liquid chromatography-tandem mass spectrometry methods should be encouraged in order to obtain a more accurate picture of the extent of multi-mycotoxin contamination. It is also necessary to develop newer low-cost mycotoxin detection instruments, which are portable, reliable and easy to handle at field levels.

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13

Novel protein sources in animal nutrition: considerations and examples

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Summary points

- Several strategies are available to fulfil the future protein demand for animals.
- Free amino acids can significantly contribute in reducing levels of dietary protein.
- Food waste can make a significant contribution to future protein supply.
- Novel proteins (e.g. seaweeds, algae, insects, leaf proteins) with a high protein yields per hectare and not competing with current arable land are the most promising.
- Identification of functional benefits of novel protein sources, and application as feed additives should be investigated.

Keywords: future protein, feed, pigs, poultry

13.1 Introduction

The global demand for animal sourced food in 2050 is expected to increase by 70% compared to the 2000 demand, largely as a result of growth of the world population, increased income, and urbanisation, mostly in developing regions (FAO, 2009; Alexandratos and Bruinsma, 2012). As a consequence, the world-wide demand for animal feed is expected to increase to 1,500 Tg in 2050. From 2005 to 2016, global feed

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production has increased from 645 to 1,032 Tg (Alltech, 2017), with Asia and Africa recording the fastest growth.

Dietary protein for animals (production and companion) originates from a variety of sources including forages, grains, legumes, animal meals and various co-products. Of all the dietary components (e.g. protein, energy, fibre, vitamins, minerals), it is especially the protein component which provides the largest challenge to feed formulation in the future. Many countries in the world are not self-sufficient in terms of protein supply and rely heavily on imported soybean meal (SBM). In the EU, for example, the self-sufficiency rate of feed protein is currently only 42%. In order to meet future animal-derived food demands and increase the self-sufficiency level, novel proteins, i.e. proteins that are not currently used as animal feed will become important. This chapter discusses aspects of importance of novel protein sources in order to be considered as feed proteins and uses a number of examples of potential novel protein source as illustration.

13.2 How to meet the increasing feed protein demand?

13.2.1 Need for novel proteins

There are a number of options available to ensure sufficient production of feed, and in particular feed protein, to meet the demand in 2050. Among these are: (1) use of fallow land; (2) increase in the protein yield per hectare of the currently cultivated crops; (3) improvements in feed and protein efficiency of farm animals; (4) prevention of the wasting of resources, e.g. by closing nutrient cycles; and (5) focus on the development of novel protein sources. The FAO expects that up to 2050, the increase in arable land will only amount to 5% (FAO, 2009), thereby providing an indication that the contribution of the extra land to supply feed protein seems to be limited.

The replacement of wheat by soybeans does not appear to be a strategy to increase the protein yield per hectare (Table 13.1). In the EU, wheat protein yields are 1.1 Mg/ha/yr while soybeans would provide up to 1.2 Mg protein/ha/yr. In contrast, cultivation of legumes and grass can contribute to an increased protein production. Cultivation of aquatic proteins, like duckweed, micro and macro algae appear to have, from a yield perspective and their high protein contents (duckweed and several micro-algae), great potential. In addition, these new protein sources do not require high quality arable land for cultivation. The challenges of these novel plant protein sources exist in the large scale cultivation, processing and application as a feed ingredient (Van Krimpen *et al.*, 2013). Besides aquatic proteins, also extracted grass protein (O'Keefe *et al.*,

Table 13.1. Protein content, dry matter (DM) yield, and protein yield of various protein crops cultivated under EU conditions (Van Krimpen *et al.*, 2013).

Feed ingredient	Protein (% in DM)	DM yield (Mg DM/ha/yr)	Protein yield (Mg protein/ha/yr)
Oil seeds – soybean	40	1.5-3	0.6-1.2
Oil seeds – rapeseed	25	3	0.75
Oil seeds – sunflower	23	3	0.7
Legumes (pulses) – peas/beans/ lupine	17-35	4-6	1-2
Legumes (forage) – lucerne	19	13	2.5
Cereals – oat	12-15	3-5	0.4-0.75
Pseudo cereals – quinoa	12-18	3	0.4-0.5
Leaves – grass	12	10-15	1.2-2
Leaves – (e.g. sugar beet leaves)	12	4.5	0.5
Macro algae – seaweed	10-30	25	2.5-7.5
Micro algae	25-50	15-30	4-15
Duckweed	35-45	30-40	10-18
Wheat (as reference)	11	10	1.1

2011a,b) and increased usage of free amino acids (Boland *et al.*, 2013) can contribute to the protein supply of especially pigs and poultry.

The reduction in and efficient use of food waste can also be considered to make a major contribution to future protein supply. Food is wasted at all points along the food chain, with waste at the consumer end constituting one of the largest losses. It has been estimated that 10-50% of the food humans are able to consume is wasted at different points in the food supply chain including harvesting, processing, marketing, and post-consumption (Boland *et al.*, 2013). In the EU, food waste is expected to rise to approximately 126 Mt in 2020 (EC, 2010), originating from households (41.4%), manufacturing (42.8%), the food service/catering industry (11.4%), and retail (4.4%) (Zu Ermgassen *et al.*, 2016). Much of the food waste is safe to feed to animals (e.g. many food by-products, out of specification foods) and already used as a feed ingredient in many countries.

Although practiced in the past, swill feeding of pigs is banned in many countries in the world due to concerns of diseases such as foot and mouth disease, classical swine fever and swine vesicular disease. The safe feeding of food waste is, however, actively practised in countries such as Japan, South Korea, Taiwan, and Thailand. In 2006-2007, Japan and South Korea recycled 36 and 42% of the food waste, respectively into animal feed and the market share of feed from food waste has been increasing in Japan

from 2.5% in 2004 to nearly 6% in 2014 (MAFF, 2014 in Zu Ermgassen *et al.*, 2016). Heat treatment (minimum of 30 min at 70 °C or 3 min at 80 °C) by registered 'Ecofeed' manufacturers (Zu Ermgassen *et al.*, 2016) ensures safe and cost-effective recycling of valuable proteins and other nutrients for use in animal feeds in these countries. The protein content of collected waste food has been reported to be, on average, 23.4% (range 19.8-25.8) on a dry matter (DM) basis (Sayeki *et al.*, 2001), thereby, making a considerable contribution to the total amount of feed protein required. In addition to increased profitability of farmers, swill feeding also has environmental benefits (Zu Ermgassen *et al.*, 2016).

Many agree, however, that an important contribution to future protein nutrition of animals will have to come from novel protein sources, i.e. proteins that are not currently used as animal feed (Boland *et al.*, 2013; Van Huis, 2013; Van Krimpen *et al.*, 2013; Tallentire *et al.*, 2018). The novel protein sources often mentioned are insects, leaf proteins, algae (seaweed and microalgae), duckweed, yeast, and bacterial protein.

13.2.2 Criteria for novel proteins sources as a viable feed source

For novel protein sources to become main stream feed ingredients, a number of aspects inherent to its production, composition and processing are important. Among the nutritional criteria are the composition and quality, nutrient digestibility/availability, presence/absence of nutritionally active factors, palatability and concentrations of contaminations. From a feed manufacturer's perspective, criteria such as inclusion rates, stability, handling and storage and effect on pellet quality and final feed should be considered. Variability of the protein source in terms of composition and quality, its effect on the quality of the final product (meat, milk, eggs) and above all costs need to be considered for successful application of the protein source in feeds. Besides the prices of the feed ingredients, the regular supply of large quantities has to be guaranteed throughout the year as feed manufacturing is a continuous business. Upscaling of the production of novel protein sources not only provides large quantities on a regular basis but also reduces costs. Closely related to the price of protein sources is the nutritional value and in particular the content of amino acids, the amino acid balance and the ileal amino acid digestibility. As can be seen from Figure 13.1, the price of a protein source relative to SBM depends to a large extent on the protein content. Although synthetic amino acids can be supplemented to animal feeds, the more balanced the available amino acid profile compared to the amino acid requirement profile of the animal, still the more valuable the protein source is.

In addition, environmental impacts of the production of feed resources are becoming more important (Van Zanten *et al.*, 2018). The latter authors compared the attributional life cycle assessment, which solely addresses the direct environmental impact of a product of replacing SBM in pork production with either rapeseed meal

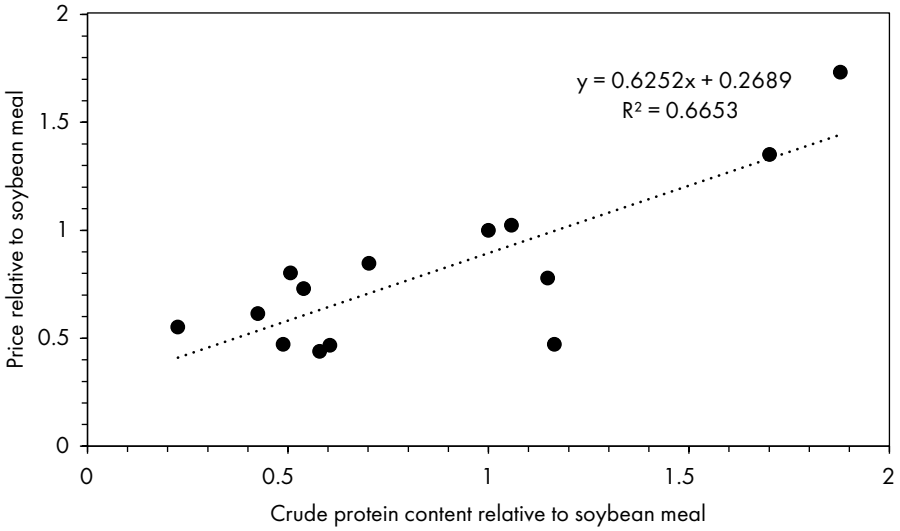

Figure 13.1. Prices of commonly used proteinaceous feed ingredients relative to soybean meal. Feed ingredients with a crude protein content >198 g/kg dry matter from Teekens *et al.* (2016).

or waste-fed larvae meal, with consequential life cycle assessment which describes how environmental flows/processes change within and outside the production cycle. The consequential life cycle assessment contradicted the attributional life cycle assessment results and showed that using co-products and waste-fed larvae meal currently not reduces the net environmental impact of pork production. The latter clearly indicates that the methodology used to assess the environmental impact of a change in the current system affects the conclusions and care should be taken to apply environmental impact analyses to the correct system level. Van Zanten *et al.*, 2018) concluded that 'to gain insight into the environmental impact of feed, animal nutritionists can use an attributional life cycle assessment. If policy makers or the feed industry, however, want to assess the net environmental impact of a potential feeding strategy, it is recommended to perform a consequential life cycle assessment'.

In Table 13.2, the characteristics of the selected conventional and novel protein sources with respect to protein yield, nutritional value, carbon footprint potential, LuLuc (land use, land-use change), N-requirement and applicability in organic diets are presented with wheat gluten meal provided as a reference.

Table 13.2. Characteristics of the selected protein crops under EU conditions (Van Krimpen *et al.* (2013) except for meal worms (De Boer *et al.*, 2014) and black soldier flies).

Feed ingredient	Protein yield/ha[1]	Protein quality[2]	Carbon footprint[3]	Land use, land-use change[3]	Nitrogen requirement[4]	Applicable in organic diets[5]
Reference crop						
Wheat gluten meal	+	+/+	+	+/+	+/-	+
Defatted European oil seed products						
Soybean meal	+	+/+	+/-	+	+	-
Soybean concentrate	+	+/+	+/-	+	+	+
Rapeseed meal	+/-	+/-	+/-	+/+	-	-
Rapeseed concentrate	+/-	+/-	+/-	+/+	-	+
Sunflower meal	+/-	+	+	+	+	-
Sunflower concentrate	+/-	?	+	+	+	+
Grain legumes						
Pea	+	+/+	+/-	+/-	+	+
Pea concentrate	+	+/+	+/-	+/-	+	+
Vicia faba	+	+/-	+/-	+	+	+
Vicia faba concentrate	+	+/+	+/-	+	+	+
Lupine	+/-	-	+/-	-	+	+
Lupine concentrate	+/-	?	+/-	-	+	+
Chickpea	-	?	?	?	?	+
Forage legumes						
Lucerne	+	-	-	+	+	+
Leaf proteins						
Grass protein	+	-	-	?	?	+
Sugar beet protein	-	-	-	+/+	+/+	+
Aquatic proteins						
Algae	++	+/-	-	+/+	?	?
Seaweed	+/+	-	-	+/+	+/+	?
Duckweed	+/+	?	-	+/+	?	?
Cereal protein						
Oat protein	+/	+/-	+	+	-	+
Quinoa protein	-	?	?	?	?	+
Insects						
Meal worms	++	+/-	-	?	+/+	+
Black soldier larvae	++	+/-	?	?	+/+	+

[1] - = <500; +/- = 500-1000; + = 1000-2,000; ++ = >2,000 kg ha.

[2] Ileal apparent protein digestibility; - = <75%; +/- = 75-80; + = 80-85; ++ = >85%.

[3] - = >1000; +/- = >500; + = >250; +/+ = <250 CO_2-eq.

[4] - = >50; +/- = >25; + = >10; ++ = <0 g N/kg yield.

[5] - = no; + = yes; under the condition that the starting material is of organic origin. Oil seed concentrates are only applicable in organic diets if hexane is not used for fat extraction.

13.3 Nutritional value of some novel protein sources

13.3.1 Seaweed

In previous centuries, naturally grown seaweed was relatively commonly used as an ingredient in the diet of animals in coastal regions of Europe (e.g. Norway, Scotland, France) (Chapman, 1970). The historical use of seaweed has been extensively reviewed (Evans and Critchley, 2014; Makkar *et al.*, 2015; Rajauria, 2015). In the present large scale commercial animal production systems, seaweed is not used to any significant extent. Adequate scientific insight in the nutritive value of seaweed is lacking, and the number of published animal studies using intact seaweed is limited. Because of the scarcity of agricultural land, the extension of marine production by large scale cultivation of intact seaweed and co-products from biorefinery processes could significantly contribute to future protein supply.

The chemical composition of seaweeds, and consequently their nutritional value, varies substantially, depending on e.g. seaweed species, origin, and harvesting season (Holdt and Kraan, 2011). Results from our institute (Bikker *et al.*, 2014) also indicated that there is a large variation in nutritive value between seaweed species (e.g. crude protein (CP) 56-311 g, crude fat 7-35 g, crude fibre 28-88 g, starch 1-90 g/kg DM), and relevant dissimilarities within species grown at different locations (Table 13.3). The high contents of non-starch polysaccharides (255-622 g/kg DM), ash (173-445 g per kg DM), potassium, sodium, and heavy metals in some intact seaweed samples is of concern and attention is required in case of application in diets for production and companion animals. Mean *in vitro* ileal (46-80%) and total tract (69-89%) DM digestibility varies drastically, all being lower than the values obtained for SBM as reference ingredient (84 and 98% ileal and total tract) in the Boisen *in vitro* system (Bikker *et al.*, 2014). In our institute, a broiler digestibility study with seaweeds was conducted showing that the precaecal CP digestibility of *Saccharina* silage and *Saccharina* silage residue was 65.9 and 69.4%, respectively (Van Krimpen, personal observation).

In chickens, only relatively low levels of intact seaweed have been included without negative effects on performance characteristics. Ventura *et al.* (1994) determined a very low energy value of the green seaweed *Ulva rigida*, and a negative effect on feed intake and growth performance at an inclusion rate of 10% or higher. Similarly, addition of 10-15% *Ascophyllum nodosum* meal to poultry feed induced diarrhoea in the birds, whereas an addition up to 7% had no negative effects (Guiry and Blunden, 1991). El-Deek and Brikaa (2009) determined a relatively high energy value of dried red seaweed (*Polysiphonia* spp.) in poultry and demonstrated that an inclusion level up to 3% did not adversely influence growth performance of ducks. El-Deek and Al-

Table 13.3. Chemical composition of selected seaweeds on a dry matter basis (Bikker *et al.*, 2014).[1,2]

Seaweed species[3]	DM[4] g/kg	Ash g/kg DM	OM[5]	CP	CFat	CFibre	Sugar	Starch	NSP[6]	NDF	ADF	ADL	HCl-Ash
Laminaria digitata (S)	891.1	275	725	115	16	73	2	2	517	120	200	36	7
Laminaria (I)	923.2	367	633	102	11	69	1	1	449	91	164	28	7
Saccharina latissima (S)	873.8	243	757	92	10	71	6	1	576	122	185	23	11
Saccharina latissima (F)	902.1	273	727	146	12	62	1	1	505	96	171	7	6
Ascophyllum nodosum (S)	882.9	214	786	56	38	54	30	1	606	162	331	180	3
Ascophyllum nodosum (I)	910.1	411	589	71	10	88	1	1	417	152	298	48	11
Palmaria palmata (S)	938.9	209	791	176	12	35	35	17	516	312	50	6	7
Palmaria palmata (F)	948.7	228	772	167	13	28	48	22	492	347	42	5	4
Chondrus crispus (S)	883.1	176	824	123	17	31	6	26	622	392	40	9	2
Chondrus crispus (I)	898.8	445	555	156	7	45	3	90	255	190	53	14	152
Ulva lactuca (S)	841.6	243	757	87	23	76	24	75	472	385	141	70	20
Ulva lactuca (F)	879.7	260	740	209	35	76	12	73	334	329	143	69	8
Ulva lactuca (I)	883.2	173	827	311	21	57	7	42	389	259	135	69	11

[1] ADF = acid detergent fibre; ADL = acid detergent lignin; CFat = crude fat; CFibre = crude fibre; CP = crude protein (N × 6.25); DM = dry matter; NDF = neutral detergent fibre.

[2] Each value in the table is based on one analysis in duplicate.

[3] S = Scotland, I = Ireland, F = France.

[4] Dry matter content of the dried product.

[5] Organic matter calculated as 1000 – Ash.

[6] Non starch polysaccharides calculated as 1000 – Ash – CP – CFat – Starch – Sugars.

Harthi (2009) did not observe a negative effect of up to 12% inclusion of *Sargassum* meal on feed utilisation and egg production in laying hens, whereas El-Deek *et al.* (2011) observed a significant decrease in growth performance and feed utilisation in broilers with incremental inclusion levels of *Sargassum* spp. up to 6%. Abudabos *et al.* (2013) did not observe a negative effect on growth performance of the inclusion of 3% *Ulva lactuca* in broiler diets at the expense of maize.

Relatively much attention has been paid to the use of seaweed in diets of small ruminants, sheep and goats in countries with hot climatic conditions. The brown seaweeds *Sargassum* (Marin *et al.*, 2003; Casas-Valdez *et al.*, 2006; Marin *et al.*, 2009) and *Macrocystis pyrifera* (Castro *et al.*, 2009) have been included up to 30% at the expense of wheat bran, alfalfa meal and other ingredients. The dry matter digestibility was 70-80% and the seaweed inclusion had little or no negative effect on nutrient digestibility, feed intake and growth performance. In lamb diets, up to 20% of *U. lactuca* was included without adversely affecting palatability of the diet (Arieli *et al.*, 1993), whereas inclusion of 20% of the green seaweed *Chaetomorphalinum*, partly at the expense of barley decreased growth performance and gain to feed ratio (Ktita *et al.*, 2010). Seaweed inclusion did not reduce the dry matter digestibility of the rations. In general, the authors concluded that these seaweeds were suitable as an unconventional feed-stuff for sheep and goats, provided that the nutrient composition is taken into account. Greenwood *et al.* (1983) observed a relatively high *in vitro* (Tilley and Terry method) organic matter digestibility (84-97%) of several brown (*Laminaria digitata*, *Saccharina latissima*) and red (*Palmaria palmate*) species and low values for other brown species (e.g. *A. nodosum*), using rumen fluid of Orkney sheep fed seaweed. The digestibility was lower with rumen fluid from grass fed sheep suggesting that adaptation of the rumen microbiota to the seaweed diet is important for optimal digestibility. The majority of studies cited above included intact seaweed, generally sun-dried and ground, into the diet. Van den Burg *et al.* (2013) indicated that the costs of seaweed production is too high for large scale inclusion of the intact product in animal diets. It may be economically more feasible to optimise the use of seaweed via biorefinery processes and use of specific fractions or residues for inclusion in animal diets. High value fractions may be extracted and used for applications in food, pharmaceuticals, chemical engineering and cosmetics (Holdt and Kraan, 2011). Until now this approach has received little attention. As one of the very few studies, Whittemore and Percival (1975) determined the digestibility of the residue of *A. nodosum*, after extraction of alginate, in 40 kg pigs. The dietary inclusion level of the seaweed residue was as high as 50%. This resulted in a large number of pigs with diarrhoea and feed refusals. The authors concluded that the seaweed residue was unsuitable as a major dietary ingredient for the supply of energy or N to pigs. Recent work in our lab with intact *U. lactuca,* and an extracted fraction remaining after enzyme treatment and centrifugation to use the soluble carbohydrates as an energy source, indicated a higher *in vitro* protein and organic matter digestibility of the extracted fraction (Bikker *et al.*, 2016). These results show that the value of

feed ingredients, derived as co-product from biorefinery processes may be promising, but largely depends on the seaweed species used, the processing involved, and the composition of the extracted and residue fractions.

In addition to the nutritive properties, seaweeds may also contain biologically active compounds with a large range of applications as pharmaceuticals, cosmetics, food and animal feed. Seaweeds may contain relatively high amounts of polysaccharides, e.g. alginate, carrageenan, agar, fucoidan, laminarin, mannitol, and ulvan, which have bioactive properties. These polysaccharides have shown to exert anticoagulant, antiviral and antibacterial, anti-proliferative and immuno-modulatory properties (O'Sullivan *et al.*, 2010; Li *et al.*, 2011; Fedorov *et al.*, 2013; Lee *et al.*, 2013). Part of these properties were confirmed in an *in vitro* study conducted at our institute in which effects of polysaccharides from *Saccharomyces cerevisiae* containing β-glucan and mannan (SC, positive control), micro algae containing β-glucan (MA), brown macro algae containing fucoidan and laminarin (BA) and green macro algae containing ulvan (GA) on gene expression in intestinal porcine jejunum epithelial cells (IPEC-J2) were investigated in the presence and absence of the enterotoxigenic bacterium *Escherichia coli* k99 strain (ETEC) as an *in vitro* challenge. Gene expression was measured in IPEC-J2 cells after 2 and 6 h of incubation using 'whole genome' porcine microarrays. Analysis of the generated transcriptomics datasets using bioinformatics programs indicated that 18 acute phase proteins and 49 pathways involved in immune response were modulated in the IPEC-J2 cells by the polysaccharides in the presence of ETEC. Without ETEC challenge, algae modulated 3 (BA) to 13 (MA) pathways involved in immune response, compared to 1 with SC. With the challenge, algae modulated 18 (GA) to 36 (BA) pathways involved in immune response, versus 13 with SC. Other affected pathways included energy metabolism, intestinal carriers, and oxidative response. In this study, the gene expression of acute phase proteins, and regulation of pathways involved in the immune response, energy metabolism and oxidative response were modulated more with brown and green macro algae, and with micro algae than with SC at similar inclusion levels. These data indicated that some non-starch polysaccharides (NSP) from algae may be more effective than NSPs from yeast to alert the immune system in case of infection by *E. coli*. Berri *et al.* (2016) also observed a higher mRNA expression of gut immune response mediators in an *in vitro* system of differentiated porcine intestinal epithelial cells (IPEC-1), because of the use of an aqueous marine-sulphated polysaccharide extract, prepared from the green seaweed *Ulva armoricana*. Moreover, this marine-sulphated polysaccharide was tested as an antibacterial compound against 42 bacterial strains and isolates found in livestock animals, showing that growth of both Gram-positive and Gram-negative bacteria was affected. In a laying hen study, 2 red seaweeds (*Chondrus crispus* and *Sarcodiotheca gaudichaudii,* 0.5, 1 or 2% inclusion level) showed prebiotic effects, as indicated by a higher abundance of beneficial bacteria in the ceca, e.g. *Bifidobacteria*, and a lower abundance of *Clostridium perfringens* (Kulshreshtha *et al.*, 2014). Moreover, villus height and villus surface area were increased as well with sea weeds. Feeding 0, 500

or 1000 mg/kg of an *A. nodosum* extract to challenged young broilers resulted in a reduction in caecal *Campylobacter jejuni* colonisation, while a higher expression of tight-junction genes was observed (Sweeney *et al.*, 2016).

In summary, the chemical composition of seaweeds show a large variation due to species, location, and harvesting season, which provides both opportunities and challenges to ensure a continues supply of a consistent quality of this potential protein source. The high mineral content and sometimes high heavy metal content requires attention. There is limited and variable information regarding the nutritive value of seaweeds for poultry and pigs. Some intact seaweeds showed prebiotic effects or reduced the bacterial load in case of a bacterial challenge. Recent *in vitro* studies with extracted seaweed polysaccharides demonstrated anti-bacterial effects, as well as stimulating effects on the immune system, energy metabolism and oxidative response. These seaweed properties seem to be very promising, but *in vivo* studies should be conducted to validate these findings.

13.3.2 Micro algae

Spirulina (blue-green alga) and *Chlorella* (green alga) are the most prominent protein-rich algae, which are commercially produced. On a DM basis, the protein content of *Chlorella vulgaris* and *Spirulina* ranges from 51 to 58% and from 60-71%, respectively (Lum *et al.*, 2013). The amino acid composition shows a lower concentration of lysine, histidine and phenylalanine in proteins of the Spirulina meal than in SBM, whereas the other essential amino acids are present in higher concentrations in Spirulina meal. In general, protein digestibility varies between 55 and 82%, depending on algae species and method of drying (Becker, 2013). The *in vitro* CP digestibility of a mixture of *Chlorella sorokiana* and *Scenedesmus obliquus* (52.5% CP and 7.0% crude fat), determined in our lab, was 50% (Van Krimpen *et al.*, 2014). In a broiler study, the digestibility of *Spirulina platensis* (58.2% CP, 2.6% crude fat) was investigated and compared to SBM (Alvarenga *et al.*, 2011). The amino acid profile relative to lysine of this micro algae was comparable to the profile of the reference SBM. Protein and gross energy digestibility of the *Spirulina*, however, were low with values of 52.9 and 56.1%, respectively. A dried *Spirulina* sp. (76.0% CP, 4.95% crude fat) was used in studies of Evans *et al.* (2015) and Boney and Moritz (2017) to determine amino acid digestibility and performance in broilers. True amino acid digestibility was high with coefficients ranging between 90 and 95% (Boney and Moritz, 2017). Dietary supplementation of this micro algae up to 16% did not affect broiler performance (3-21 d of age), but feed intake and body weight gain were reduced in birds fed a diet with 21% of this *Spirulina* (Evans *et al.*, 2015). Some micro algae have a very thick and tough cell wall, which hampers the digestibility of the nutrients inside the cells. Therefore, application of mechanical (e.g. bead milling or ultra-sonification) or non-mechanical (e.g. chemicals or enzymes) cell wall disruption methods might increase protein digestibility. More research is required to determine the effect of

cell wall disruption on nutrient digestibility of micro algae. Inclusion of 1% dried *C. vulgaris* to a broiler diet improved body weight gain by 3.5% (1,549 vs 1,603 g) and feed conversion ratio (FCR) by 8.5% (1.52 vs 1.66) (Kang *et al.*, 2013). The effect of inclusion of sun dried *S. platensis* (62.5% CP, 3.0% crude fat) in a commercial broiler diet was examined in a 12 week study, in which dietary fish meal or peanut cake was replaced by algae in concentrations of 140 and 170 g/kg (Venkataraman *et al.*, 1994). Based on a similar feed efficiency, protein conversion rate, and the extent of carcass lean meat content, it was concluded that substitution of fish meal or peanut cake by algae did not affect broiler performance. Moreover, none of the diets affected body composition and histopathology of the various organs of the broilers. Meat quality remained also unchanged. Sensory evaluation of the meat by a test panel showed that broilers fed on the algae-based diets yielded better meat in terms of texture, flavour and colour.

Algae meal from *S. platensis* was evaluated as a poultry feed ingredient in an experiment with broilers (Gongnet *et al.*, 2001). The algae meal contained 344 g total ash and 423 g CP per kg DM. Four diets, containing 0, 50, 100 and 150 g algae meal per kg diet were fed to male broiler chicks from 6 to 34 day of age. Feed intake was reduced in birds fed diets containing 100 and 150 g algae meal, whereas weight gain in birds fed these diets was decreased to less than 80% or the control group. Feed conversion ratio for diets with 0, 50 and 100 g algae meal was within the range of good commercial production, whereas birds fed the diet with 150 g algae meal consumed significantly more feed per unit weight gain. Metabolisable energy content was similar for the four diets, which suggests that the metabolisable energy concentration in the Spirulina meal was not much different from the mixture of maize, SBM, and wheat-starch (Gongnet *et al.*, 2001).

In laying hens, no differences were observed in egg production and egg quality (size, weight, shell thickness, solid content of the egg, albumin index, etc.), and FCR between hens fed diets containing 12% *Chlorella* (cultivated on effluent) and hens fed the control group (Gouveia *et al.*, 2008). In the study of Ekmay *et al.* (2015), a defatted protein rich green (*Desmodesmus* spp., 31.2% CP, 1.5% crude fat) and a full-fatted micro algae (*Staurosira* sp., 13.9% CP, 9.3% crude fat) were included in laying hen diets at 25.0 and 11.7%, respectively and fed from 26 to 40 weeks of age. Despite a lower feed intake, final body weight and bird performance were not affected by the micro algae.

Février and Sève (1975) incorporated dehydrated Spirulina (*Spirulina maxima*) in the diets of 12-day-old weaned pigs. From 12 to 21 days of age, Spirulina was incorporated at a level of 12% (25% of total CP) in the ration, replacing dried skim milk. From 21 to 42 days of age, Spirulina was fed at a level of 8% of the diet, replacing SBM. Although there was some reduction in digestibility of the diet when Spirulina was incorporated, growth was satisfactory and equivalent in all groups. The authors

concluded that the metabolic utilisation of the fraction of absorbed feed was better for the Spirulina group than for the control group, notably during the period between 12 and 21 days, although the supply of lysine in the Spirulina group was 12% lower. Yap *et al.* (1982) replaced one-half of SBM (33% of total dietary protein) in a corn-SBM/dried skim milk starter diet with algal proteins (*S. maxima*, *S. platensis*, and *Chlorella* sp.). The trial was performed with Yorkshire pigs weaned to a dry diet at 4 to 8 days of age. There was no significant difference between control and algal diets during the 15- and 26-day trial periods in growth, diarrhoea, loss of appetite, or toxicity. The researchers concluded that at least one-half of the protein supplied by SBM (one-third of the dietary protein) could be replaced by algal protein without adverse effects. Grinstead *et al.* (2000) performed feeding experiments with dehydrated *S. platensis* and weanling pigs (PIC, L326 X C22; initially 3.7±0.85 kg and 11-12 days of age). From days 0 to 14 after weaning, pigs were fed a control diet or pelleted diets containing 0.2, 0.5, or 2% *S. platensis* replacing SBM on an equal lysine basis. With 2% *S. platensis*, only 3.2 to 3.4% of total dietary lysine was replaced. No differences in pig performance, measured as average daily feed intake and gain, were observed during this interval. In contrast to pelleted diets, meal diets resulted in inconsistent responses to *S. platensis*. Daily supplementation of 2 g of a dried blue algae *S. platensis* positively affected the reproductive properties of sows (Shimkus *et al.*, 2009). The weight of the new-born piglets increased by 19.8% ($P<0.05$), milk yield – by 11.2%, piglet weight on the 21st and 28th day of age by 17.1% ($P<0.05$) and 16.6% ($P<0.05$), respectively and liveability – by 10.1%. The amount of fat in the milk of sows increased by 0.33%, protein by 0.39% ($P<0.05$) and lactose by 0.38% ($P<0.05$).

Besides their protein-providing properties, micro algae have several other functional properties. They are able to enrich poultry meat or eggs with omega-3 fatty acids (Fraeye *et al.*, 2012; Bonos *et al.*, 2016). Moreover, it has been shown that feeding *S. platensis* to broilers challenged with sheep red blood cells upregulated macrophage phagocytic activity, as well as metabolic pathways leading to increased nitric oxide synthase activity (Al-Batshan *et al.*, 2001). It was also demonstrated that *Spirulina* supplementation increased several immunological functions, implying that a dietary inclusion of *Spirulina* at a level of 1% may enhance disease resistance potential in broilers and layer pullets (Qureshi *et al.*, 1996).

Based on results available from literature it can be concluded that micro algae from a nutritional point of view can be considered a useful protein source in diets for pigs and poultry. Inclusion levels between 5 and 15% seem to be applicable without negative effects on performance. The effects of cell wall disruption techniques on nutrient digestibility needs further investigation. Moreover, there are several indications that micro algae contain bioactive components which might contribute to an improvement of animal health and the quality of meat and eggs. As the potential protein yield per hectare of micro algae is very high, algae make a promising novel protein source. The present costs price of micro algae, produced in large scale algae plants, however,

is estimated to range between € 4.50-6.30 per kg DM, depending on the cultivation system (www.enalgae.eu). Therefore, compared to SBM and fish meal, the price of micro algae is currently not competitive as a protein source.

13.3.3 Leaf proteins

In terms of protein yield per hectare, grass cultivation is very efficient in North-West Europe (Van Krimpen *et al.*, 2013). Whole grass is a common ingredient in organic poultry husbandry, and access to a grass-clover pasture can substantially contribute to the protein supply of the broilers. Broilers were able to realise 7% of the recommended amount of protein by the intake of grass-clover from the pasture (Rivera-Ferre *et al.*, 2007).

Protein content and digestibility of fresh grass depend on a number of factors, including stage at harvest. Protein content of fresh grass, harvested at either a young or older (3 weeks later) stage, was 148 and 108 g/kg DM (Van Krimpen *et al.*, 2006). Van der Peet-Schwering *et al.* (2010) observed that protein digestibility of grass silage in sows depended on grass yield/ha, and ranged from 40% at a yield level of 5 Mg DM/ha to 63% at 1.8 Mg DM/ha. In this experiment, protein content decreased and fibre content increased with increasing stage of growth and related grass yield/ha. Therefore, extraction of protein from grass, thereby separating proteins from fibres, might increase their applicability in poultry diets (Chiesa and Gnansounou, 2011). These proteins can be valorised as alternatives to extracted soybeans (Van den Pol-Van Dasselaar *et al.*, 2012).

In recent years, grass processing techniques have been developed. Zhang *et al.* (2014) developed a cost-effective protein separation technique from leaves, based on alkaline extraction. Depending on temperature, amount of NaOH, and extraction time, up to 95% of total protein could be extracted, with a protein content of the extract up to 52%. Pre-treatment, using ethanol or enzymes reduced the alkali consumption by 25%, and further improved protein extraction yield and purity (Zhang *et al.*, 2016). Currently, mobile grass biorefinery equipment is available to produce grass protein on a semi-commercial scale (www.grassa.nl). Grass in the latter biorefinery process is fractionated in different fractions, e.g. a fibre-low/protein-rich fraction (50% CP on a DM base), a fibre-rich fraction, and a sugar-rich fraction. This technique might contribute to valorising protein-rich fresh grass, which in North-West Europe usually has to be harvested in the wet autumn season. A major draw-back for this technique is that freshly harvested green leaves are required, which are usually only available for a few months per year.

Already in 1965, digestibility of different leaf proteins, e.g. from rye, potato, pea, and red clover leaves, after freeze drying was determined in rats (Henry and Ford, 1965). Protein digestibility was reasonable, ranging from 70.6% in red clover leaf protein to

84.8% in rape leaf protein. Until now, however, hardly any information regarding the nutritional value of grass proteins for poultry is available. *In vitro* digestibility studies in our laboratory showed that the DM digestibility increased from 50 (intact grass) to over 90% (extracted grass protein), suggesting that extracted grass-protein can be an interesting protein source for (organic-housed) monogastrics (Van Krimpen, personal observation). Van Kempen *et al.* (2002) found that, following a suitable adaptation period, pigs can digest approximately 40% of the energy in Bermuda grass but none of the nitrogen. Kambashi *et al.* (2014) measured the *in vitro* digestibility of 20 forage plants commonly used for feeding pigs in the Democratic Republic of the Congo, and reported values of 23 to 81%. Laying hens have been reported to be able to consume considerable amounts of fresh grass, which might contribute 12-13% to the total DM intake (Antell and Ciszuk, 2006). Buchanan *et al.* (2007) reported high true amino acid digestibility values ranging from 64.5 for Tyr and 77.3 for Cys of a composite forage sample containing Kentucky bluegrass, tall fescue, white clover, and red clover when fed to 20 week old roosters.

Based on the data available, intact leaf proteins can make a contribution to the protein supply of poultry and potentially pigs, although more research is required on the protein quality of leaf proteins for these species. The fibre content of intact grass, however, may limit its use especially in poultry diets making biorefinery of grass and other leaves to separate the protein and fibre fractions a valuable technology to contribute to the increased requirements of feed proteins in the future.

13.3.4 Insects

Insects are a highly diverse group of animals. A number, including the black soldier fly (BSF, *Hermetia illucens*), house fly (*Musca domestica*), mealworm (*Tenebrio molitor*), and silkworm (*Bombyx mori*) have been proposed to be suitable for upgrading and concentrating protein from waste streams (Van Huis, 2013). Comprehensive literature reviews of the nutrient content of insects have been published (Bukkens, 1997; Finke and Winn, 2004; Raubenheimer and Rothman, 2013; Rumpold and Schluter, 2013; Payne *et al.*, 2016; Khan, 2018). Finke and Oonincx (2017) reported that the protein content of insects is highly variable and ranges between 25 and 75% on a DM basis. In terms of first limiting amino acids, most insects are limiting in Met+Cys to meet poultry and pig requirements. Overall, amino acid digestibility values for poultry of various insect meals (*in vivo*) are high, with mean values reported of adult insects of 93%, larvae of 91% and pupae 82% (Finke and Oonincx, 2017). Additional data reported by the latter authors show that the protein digestibility of insects is generally high with a well-balanced amino acid profile. Khan (2018) concluded that insect meal from e.g. BSF, meal worms, grasshoppers, locust and crickets can partially or totally replace fish meal in poultry diets as no negative effects have been reported on growth of insect meal-fed poultry with most of papers describing similar or even better growth rates in chicks when compared to SBM or SBM plus fish meal.

Spranghers *et al.* (2018) fed weaned piglets diets in which toasted soybeans were replaced by BSF up to 8%. The authors reported no effect on performance parameters and concluded that piglet feed may contain a considerable amount of either full-fat or defatted BSF prepupae without causing adverse effects on performance. However, the authors noted that future research should focus on adding value to BSF, as the current price is not competitive against conventional protein sources when included in a diet for weaned piglets. Potential additive value can be found in insect chitin or chitin-derivatives, which are suggested to induce an immune response (Harikrishnan *et al.*, 2012), acting as antibiotic/prebiotic in rats and chickens (Chen and Chen, 1999; Chen *et al.*, 2002), cited by Khempaka *et al.* (2011), and affecting hypolipidaemic properties in broiler chickens (Hossain and Blair, 2007). Information on properties such as palatability (e.g. taste, texture) is lacking, but should be investigated to add additional value to insects to make it an economical viable alternative for conventional protein sources.

Contrary to this, potential toxic substances, anti-nutrients and pathogens might potentially be present in insects (Rumpold and Schlüter, 2013). Various insect species have been reported to accumulate lead, cadmium, arsenic and chromium (Maroni and Watson, 1985; Kazimirova and Ortel, 2000; Van der Fels-Klerx *et al.*, 2016; Gao *et al.*, 2017). Pupae of the African silkworm *Anaphe* spp. contain a heat-resistant thiaminase while some insects contain toxins originating from the feed and synthesise/metabolise toxins, e.g. cyanogenic or cardiac glucosides, steroids or pederin, as a chemical defence mechanism against insectivores. Consumption of these insects can lead to nausea, vomiting, visual disturbance, or worse (Rumpold and Schlüter, 2013).

13.4 The importance of free amino acids for novel protein sources

Providing diets with a reduced CP content, that are supplemented with increased contents of free amino acids to cover the amino acid requirement, is a cost-effective strategy currently practised in many countries. Moreover, the importance of the use of free amino acids in diets for production animals to balance the absorbed amino acid pattern will increase when novel protein sources are increasingly used as a result of a likely greater amino acid imbalance of these protein sources. The standardised ileal digestible amino acids content of novel protein sources can be considered to be more variable compared to conventional protein source, thereby increasing the need to balance the amino acid pattern.

In order to reduce the CP content of broiler feeds while maintaining growth performance, it is essential to keep the supply and balance of limiting amino acids in line with the broilers requirements by adequate dietary supplementation of free amino

acids. Published studies show that low protein diets allow to achieve a maximum level of performance, provided that the digestible lysine content and the ratio of other essential amino acids to lysine are maintained (Dean *et al.*, 2006; Namroud *et al.*, 2008). However, in low protein diets glycine and serine, that are currently considered as non-essential amino acids (Dean *et al.*, 2006), can become limiting, either because there are not enough metabolic precursors for the respective amino acids available, or because endogenous metabolisation processes are too slow (Aftab *et al.*, 2006; Berres *et al.*, 2010). This could well be the reason why several researchers found decreased performance results with low protein diets supplemented with amino acids, without maintaining the glycine + serine to lysine ratio (Ferguson *et al.*, 1998a; Ferguson *et al.*, 1998b; Bregendahl *et al.*, 2002).

Several studies showed that glycine supplementation prevents adverse effects on broiler performance when low protein diets were provided (Ospina-Rojas *et al.*, 2012; Ospina-Rojas *et al.*, 2013; Ospina-Rojas *et al.*, 2014). In a study in our institute with broiler chickens from 8-35 days of age, a lower growth performance of broilers fed low protein diets was observed, despite essential amino acids (lysine, methionine, tryptophan, threonine, arginine and isoleucine) being supplemented to 10% above CVB (2012) requirements (Veldkamp *et al.*, 2018). The authors suggested that the lack of glycine and serine might explain the reduced growth performance in low protein diets. Because of the importance of glycine in low-protein diets, it is considered as the fourth limiting amino acid after lysine, methionine and threonine (Waguespack *et al.*, 2009). Also according to Ospina-Rojas (2013) glycine is a limiting amino acid in low protein diets, especially in the starter phase but also thereafter. Based on the results in the study of Veldkamp *et al.* (2018) and in literature, it seems to be essential to add free glycine to low protein diets, to maintain the production results of broilers. We conducted a broiler study in which the effects of low CP diets, with partial replacement of SBM by free amino acids, on animal performance, slaughter yields, litter quality, footpad lesions, economic performance and the ecological footprint were evaluated. In this study, dietary SBM content in the grower diets was reduced from 27.3% (control) to 17.3% (-3% CP), while SBM content in the finisher diets was reduced from 25.0% (control) to 14.6% (-3% CP) (Van Harn *et al.*, 2017). The birds that were fed the low CP diets had similar or even better growth performance as broilers fed the control diets. The best overall performance was obtained with the CP-2% diet program. Broilers fed the -2% CP or -3% CP diet program had a significantly improved FCR. Broilers fed the low protein diets had a lower water intake, a better litter quality and less severe footpad lesions compared to broilers fed the control diets. The use of broilers diets with an up to 3% lower CP content while maintaining dietary concentrations of essential amino acids hardly influenced the slaughter yields. Only the breast meat yield (as % of the carcass) of broilers fed the diet with 3% lower CP content was lower, while the breast meat weight did not differ. Overall it can be concluded that supplementation of free amino acids to the diet allows a reduction of the SBM content of broiler diets by 10%, thereby reducing CP content, without

adverse effects on growth performance and slaughter yield provided that diets are supplemented with adequate lysine, and with free methionine, threonine, arginine, isoleucine, valine and glycine in the recommended ratio to lysine. In a follow-up study, in which graded levels of glycine were supplemented to the -3% CP diets in the grower and finisher phase, no responses of glycine were observed (Van Harn *et al.*, 2018). Based on this study, the lowest digestible glycine+serine contents of 12.4 g/kg in the grower diet, and 11.4 g/kg in the finisher diet seem to be sufficient to meet the birds requirement.

From 2000 onwards, a series of papers have been published in which the responses to different CP levels were investigated in body weight ranges of 20-60 kg (Figure 13.2). It must be noted that in these studies, diets were formulated to meet animal requirements on the first six essential amino acids, and if needed, these were added synthetically. Figueroa *et al.* (2012) reported a significant (P<0.05) decrease in average daily gain (ADG) when CP level was reduced from 16.2 to 13.0% whereas Martinez-Aispuro *et al.* (2014) observed a negative trend (P<0.09) when lowering the CP content from 14.5 to 11.5%. For FCR, however, the latter study showed no negative response (P>0.10) to the low level. These studies are very much in line with the current trial in which CP levels below 14.5% also showed to have a negative effect on performance. In the study of Powell *et al.* (2011), 13% CP did not negatively affect (P>0.1) AGD. However, it must be noted that synthetic glycine and arginine were added to the diets which most likely mitigated the negative response to low CP.

In Figure 13.3, results of studies on the effect of reducing dietary CP level on ADG in 40-100 kg pigs since 2000 are presented.

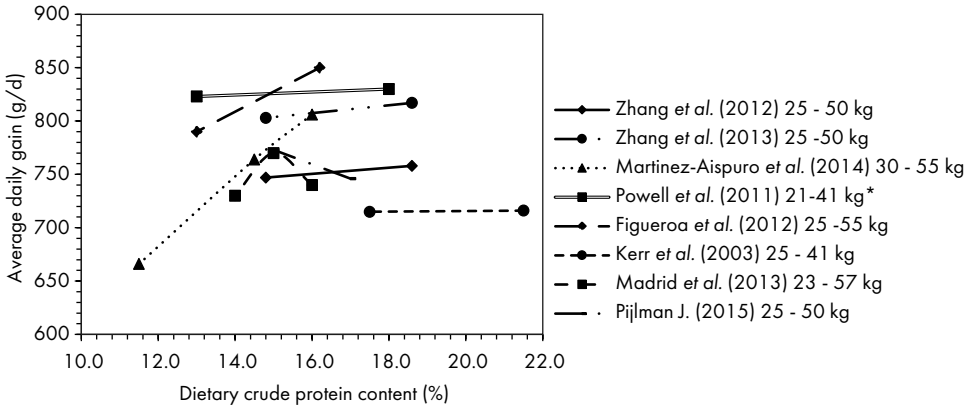

Figure 13.2. Effect of reducing dietary crude protein level on average daily gain in 20-60 kg pigs as reported in various studies.

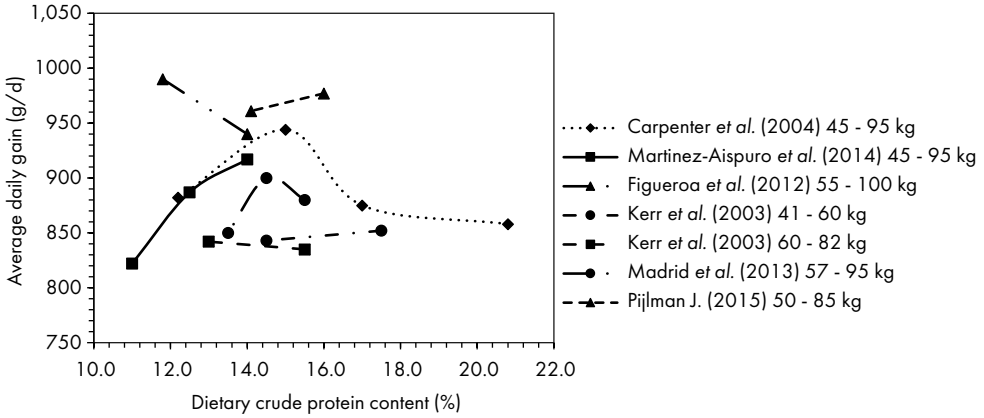

Figure 13.3. Effect of reducing dietary crude protein level on average daily gain in 40-100 kg BW animals as reported in various studies.

Figure 13.3 shows that a CP level of respectively, 11.9 and 12.1% did not negatively affect ADG (both $P>0.1$) in studies of Figueroa *et al.* (2012) and Carpenter *et al.* (2004). Martinez-Aispuro *et al.* (2014) performed a trial in 45-95 kg animals and observed a trend ($P<0.09$) for a negative response when decreasing CP level from 14.0 to 11.0%. In line with these findings, Pluk and Van Krimpen (2018) clearly showed a decreased ADG below a CP level of 12.9%.

In terms of feed efficiency, only in studies from Martinez-Aispuro *et al.* (2014), Kerr *et al.* (2003) and Madrid *et al.* (2013), CP levels below 14.0% did not have a negative effect (all $P>0.1$) on ADG.

13.5 Conclusions

The global demand for animal sourced food in 2050 is expected to have increased by 70% compared to the 2000 demand. Possible strategies to meet this demand are: (1) use of fallow land; (2) increase in the protein yield per hectare of the currently cultivated crops; (3) improvements in feed and protein efficiency of farm animals; (4) prevention of the wasting of resources, e.g. by closing nutrient cycles; and (5) focus on the development of novel protein sources. The protein yield per hectare is rather low for oil seeds, average for legumes and leaf plants, and high for aquatic proteins and insects. In general, limited studies are available to demonstrate the nutritional value, and particular the *in vivo* protein digestibility of novel proteins in non-ruminant species. Besides their nutrient providing properties, micro algae, seaweeds, and insects might positively contribute to the health status of livestock. Studies with low-

protein diets show that up to 40% of the SBM inclusion level can be replaced by free amino acids without compromising animal performance levels.

Based on the current nutritional knowledge we conclude that micro algae and free amino acids currently already can substantially contribute to poultry diets as novel proteins or protein replacers, whereas the potential contribution of seaweeds and leaf proteins needs further investigation.

13.6 Future perspectives

The demand for feed protein is predicted to grow significantly in the future. Development of new protein sources is essential to meet this demand. Much research is currently being conducted to obtain proteins sources from insects, seaweeds, micro algae and leaf plants. In many cases, such protein sources require extensive processing providing potential to damage amino acids. Also, more attention should be paid to the feed safety aspects, e.g. the presence of contaminants, and microbial risks, of these protein sources.

Currently, the costs of novel protein sources is too high to allow inclusion in animal feeds, and a focus to significantly reduce costs to be competitive to conventional sources is essential. In this respect, identification of functional benefits of novel protein sources, and application as feed additives should be investigated. Because of subsequent increased scale of production, costs prices likely will reduce as well, which provides the opportunity to consider these ingredients as competitive protein sources.

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14

Future of animal nutrition: the role of life cycle assessment

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Summary points

- The livestock sector poses severe pressure on the environment via the emissions of pollutants to air, water and soil, and via the use of scarce resources.
- Life cycle assessment (LCA) is a holistic method that enables assessment of environmental impacts throughout the entire life cycle of an animal product, combining information about crop and animal productivity.
- Three main features of LCA are: comparison of products, identification of hotpots, and evaluation of improvement options, of which the latter requires a consequential approach.
- Harmonisation of methods and high quality data are needed to improve interpretation of LCA results of animal products, and to identify improvement options.
- To prevent burden shifting, an LCA should include all relevant environmental impacts, whereas currently, most LCA studies on livestock products focus on climate change only.
- To reduce environmental consequences of deforestation, (crop-) products that are directly related to land use change should be avoided, while land use efficiency should be increased.
- At the individual company or product chain, LCA of feed products may be used in combination with feed optimisation models to improve the environmental sustainability of livestock diets.
- To determine an environmentally sustainable human diet, LCA needs to be combined with other modelling techniques that address environmental impacts of dietary choices at the (inter)national level.

Keywords: feed production and utilisation, environmental impact, LCA methodology, carbon footprint

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14.1 Introduction

Global assessment reports clearly demonstrate that the livestock sector poses severe pressure on the environment via their emissions to air, water and soil (Gerber *et al.*, 2013). The livestock sector also competes increasingly for scarce resources, including land, water, fossil energy, and fossil phosphorus. A main contributor to the environmental impact of livestock systems is the production of feed. In the UK broiler systems, for example, production of feed determines approximately 65 to 81% of the primary energy use, and contributes around 72% to the emission of greenhouse gases, including production stages up to the farm gate (Leinonen *et al.*, 2012). Life cycle assessment (LCA) is a holistic method increasingly used in the agricultural sector to assess environmental impacts throughout the entire life cycle of a product, such as a compound feed, and to identify improvement options without burden shifting (i.e. increasing a certain environmental impact while focussing on another).

The aim of this chapter is to elaborate on the role of LCA to reduce the environmental impact of the pig and poultry sector, with a special emphasis on the production of feed.

14.2 LCA methodology

LCA is defined as the 'compilation and evaluation of the inputs, outputs and potential environmental impacts of a product system throughout its life cycle' (ISO, 2006). The four phases of an LCA include: (1) goal and scope definition; (2) inventory analysis; (3) impact assessment; and (4) interpretation of results.

The goal and scope definition includes a definition of the study objective, the system boundary, the functional unit, and the method to allocate impacts in case of a multiple-functional process. Let us consider that the objective is to assess the environmental impact of a diet of fattening pigs. Ideally, such an assessment should address climate change, acidification, eutrophication, human-, terrestrial- and aquatic eco-toxicity, the use of water, fossil energy, fossil phosphorus and land, and biodiversity loss. The system boundary includes all processes along the chain that determine the environmental impact of a diet for fattening pigs, such as the extraction of raw materials to produce feed, the cultivation and processing of feed, the utilisation of feed for maintenance and growth by the pigs, housing, and emissions from manure during housing, storage, processing and usage. The latter is important because manure composition differs between diets. The functional unit represents the main function of the system and enables comparison of systems or products. In our example, the functional unit would be one kg of slaughter weight produced.

The above described assessment is an example of an attributional LCA (ALCA). An ALCA is used to assess product systems in a status quo situation, and, therefore, describes the environmentally relevant physical flows to and from a product or process. If the objective is to evaluate the environmental consequences of a change in a system, e.g. the consequences of changing from diet A to B, we need to perform a consequential LCA (CLCA). The latter LCA describes how environmental flows change in response to a change in the system, and is most suitable to evaluate the impact of improvement options (Ekvall and Weidema, 2004). Performing a CLCA comprises a prediction of the future consequences of a certain action, and, therefore, requires detailed insight into cause-and-effect chains, which are subject to the uncertainty and complexity of socioeconomic dynamics (Suh and Yang, 2014).

When performing an ALCA, methods are required to allocate environmental impacts in case of a multi-functional process. The latter is a process that yields more than one output. Examples of animal feed products derived from a multi-functional process are beet pulp, a by-product from the production of sugar, and wheat middlings, a by-product from the production of wheat flour. In ALCA studies of livestock products, economic allocation is used most commonly (De Vries and De Boer, 2010). Economic allocation is the partitioning of environmental impacts between (by-) products based on the relative economic value of those products (i.e. a socioeconomic approach; Guinée *et al.*, 2002). Beside economic allocation, mass or physical allocation (i.e. a natural science based approach) and system expansion can be used. Mass or physical allocation is performed in the same way as economic allocation, but uses relative mass or energy values instead of relative economic values of the multiple outputs. System expansion implies that emissions related to processing of by-products (e.g. wheat middlings) are allocated to the main product (e.g. wheat flour), whereas emissions related to the product that is replaced by the by-product (e.g. barley) are subtracted. When performing a CLCA, the environmental consequences of a change in a system are assessed, which indirectly implies that system expansion is applied.

In the inventory analysis, the inputs and outputs of each process included in the system boundaries are defined. This implies that hundreds of emissions and resources are quantified. To gain insight into the full environmental impact of a product, and to prevent burden shifting, all relevant environmental impacts should be addressed. Most LCA studies, however, do not include all environmental impacts, but focus on one or several environmental impacts only. In LCA studies of livestock products, climate change (generally referred to as carbon footprint assessment), energy use, eutrophication, acidification and land use are most commonly assessed (De Vries and De Boer, 2010). Within the assessment of climate change one should account for greenhouse gas emissions related to land use change. Land use change relates to the conversion of land (e.g. forest or scrubland) into cropland used for feed production.

The impact assessment encompasses classification and characterisation of the emissions and resources used. Emissions of carbon dioxide, methane, and nitrous oxide, for example, all contribute to the impact category climate change (classification), and can be summed up based on their impact in terms of carbon dioxide-equivalents (characterisation). A next step can be to weigh the different impact categories into so called endpoint categories such as human health, ecosystem quality, and resource depletion. Endpoint categories can facilitate decision making by policy makers and can help to create understanding by a broader audience, but involves subjective judgement, increases uncertainty, and enables compensation of poor results for one of the impact categories (Hellweg and Canals, 2014).

Interpretation of results and answering the study objective make up the final step in LCA. Interpretation of environmental impacts of, for example, dietary changes, however, is currently hindered by variability in methods. Differences in system boundary (i.e. which processes to include and which not), method of allocation, and impact assessment method can have an important influence on the results. Methods to account for land use change, i.e. an important element to determine the impact of feed production on climate change and biodiversity loss, contain high levels of uncertainty and variability (Persson *et al.*, 2014). There are two approaches to allocate environmental impacts from land use change to products: a direct versus an indirect approach. The direct approach allocates impacts from land use change to the products that are produced on the land that is cleared. Such a method can stimulate individual companies to invest in sustainable production. This could lead to a combined demand of many actors for more sustainable products (e.g. no deforestation), and reduce land use change in the long term. An indirect approach allocates impacts from land use change to different products based on their relative contribution to the expansion of crop land (e.g. Leip *et al.*, 2010) or to total cropland area (e.g. Audsley *et al.*, 2009). This latter method stimulates efficiency and increasing crop yield, and will favour feed crops from regions were the growth potential is highest due to agro-ecological circumstances, because reducing land use requirements is the only option to reduce environmental impacts related to land use change. Increasing land use efficiency is an important way to reduce land use requirements, but it does not provide a strong direct incentive to reduce land use change such as deforestation. To reduce land use change related to feed production, therefore, products directly related to land use change should be avoided, while land use efficiency should be increased.

Harmonisation of methods, such as allocation methods, impact assessment methods, and methods to account for land use change, is required to improve interpretation of environmental impacts of livestock systems, and to assess the potential of improvement options. In addition to a harmonised method, high quality inventory data are needed for each activity in the production chain. Feed products, for example, can be produced all over the world; information on the precise location of production, however, is often lacking. If the location is known, production data such as crop yield, soil type, type

and amount of fertilisers applied, and rotation techniques are often scarce or subject to a high level of uncertainty. Improving the availability of high quality data on crop cultivation and feed processing is required to improve interpretation of LCA results, and evaluation of improvement options.

14.3 The role of LCA in animal nutrition

LCAs in the livestock sector demonstrated that the production and utilisation of feed are the dominant factors determining environmental impacts of pig and poultry production. Improving the production efficiency of crops and animals, therefore, has been a major focus for reducing environmental impacts related to livestock products. An LCA implicitly combines information regarding crop productivity (i.e. crop yield per ha) and animal productivity (i.e. feed efficiency along the chain, including breeding, rearing and producing animals). In this way, an LCA creates understanding of the interaction between processes, and the environmental impact of the entire supply chain.

Current applications of LCA in the pig and poultry sector are mainly attributional, and focus on the status quo of a process or product system (e.g. Basset-Mens *et al.*, 2007; Dolman *et al.*, 2012; Leinonen *et al.*, 2012; Meul *et al.*, 2012; Dekker *et al.*, 2013; Reckman and Krieter, 2015). Analysing the status quo of a pig or poultry system creates understanding regarding the environmental impact of the current situation, and can be used to compare contrasting systems, to identify hotspots, and to identify potential improvement options. In animal nutrition, ALCA has been used to evaluate environmental impacts of single ingredients and complete diets. An example of a database that is used to model environmental impacts of feed ingredients is FeedPrint (Wageningen UR, 2012). FeedPrint contains data to determine the carbon footprint of more than 150 ingredients based on an ALCA approach, including the production and utilisation of feed. The database was updated to also provide information on eutrophication and acidification potential, on land- and energy use, and on fossil fuel depletion. At the level of the individual company or product chain, databases such as FeedPrint may be used in combination with feed optimisation models to ensure that nutritional and economic requirements are fulfilled, while aiming for a reduced environmental impact. It should be recognised, however, that to evaluate the impact of improvement options, such as changes in dietary composition, CLCA is most suitable (Plevin *et al.*, 2013).

To illustrate the importance of CLCA for the evaluation of improvement options, the example of by-products is considered here. Feeding livestock mainly by-products from arable production or the food processing industry offers potential to reduce the environmental impact of livestock products, such as pork, chicken meat and eggs. The amount of by-products available, however, is limited and dependent on the

production volume of the determining product (e.g. the amount of wheat middlings depends on the production volume of wheat flour). This means that when company A decides to increase its use of by-products in livestock diets, fewer by-products are available for company B, which has to adapt its production plan. Based on an ALCA, which does not take into account the consequences for company B, increasing the amount of by-products is a promising strategy to reduce the environmental impact of company A. However, taking into account the consequences for company B, might provide a different outcome: the environmental benefit of increasing the use of by-products in company A will depend on the current application of the by-product in company B. Besides livestock feed, by-products can be used for human consumption or biofuel production. By performing a CLCA, information will be provided on the environmental change in comparison with the current situation. So, if the current application of a by-product is biofuel, and the new application will be livestock feed, the consequences related to the decrease in biofuel production will be taken into account. The difficulty of performing a CLCA on feed production, however, is that it requires insight into the impact of the improvement option on world food and feed markets, which is subject to numerous socio- and economic aspects (Suh and Yang, 2014).With the right information available, CLCA can be used to identify improvement options in animal nutrition and livestock production, without compromising the environmental impact of the agricultural sector as a whole.

Although a CLCA provides information on the environmental consequences of a certain mitigation strategy outside the production chain of the investigated product, it does not answer questions at a higher aggregation level. Questions like: 'What is the best application of a certain by-product?' or 'What is the role of livestock in formulating an environmental sustainable diet?', for example, remain unanswered. Consider the use of cereal grain in livestock diets. Due to an improved feed efficiency of animals, the amount of human-edible plant products, like cereal grains, in livestock diets has increased. Direct consumption of those cereals by humans, however, is ecologically more favourable than consumption of food produced by animals fed with these cereals (Foley *et al.*, 2011). An LCA does not account for this competition for food products between humans and animals. To answer questions at a higher aggregation level, LCA has to be combined with land-use optimisation models and other types of modelling techniques that address the impact of dietary choices at the national or international level. Such integrated models can be used to determine an environmentally sustainable human diet, and to address the role of livestock to (global) food security.

14.4 Conclusions

To conclude, LCA has been used generally to assess and improve product systems. Three main features of LCA are: (1) the comparison of products; (2) the identification of hotpots; and (3) the evaluation of improvement options. To determine an environmentally sustainable human diet, LCA needs to be combined with other modelling techniques that address environmental impacts of dietary choices at the national or international level.

14.5 Future perspectives

To improve LCA results on the production and utilisation of livestock feed, and to assess the impact of improvement options, feed evaluation systems should be developed that facilitate the collection and analysis of high quality data. Besides, harmonisation of methods is required to improve interpretation and comparison of results across studies. To fully understand the consequences of a change in feed composition, the feed industry should collaborate with the food- and biofuel industry to optimise the allocation of biomass streams between sectors. Similarly, to investigate how the livestock sector can contribute to an environmentally sustainable human diet, LCA needs to be combined with other modelling techniques that address environmental impacts of dietary choices at the national or international level.

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15

Nutrition and environmental sustainability

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Summary points

- Diet formulation has a direct impact on the efficiency of use of nutrient and energy by pigs and poultry and, consequently, it affects nutrient and waste flow at farm level.
- All the compounds found in the manure, or emitted into the air, originate from the fraction of the diet which is not retained by the animals.
- The improvement of efficiency of nutrient retention by pigs and poultry is an efficient way to reduce excretion and emission from the animals.
- Changing the composition of the feed is efficient to modify the chemical properties of excreta in order to reduce the gaseous emissions and the production of odours.
- Changing the composition if the feed may allow to better adapt the composition of excreta to their future use for biogas production or as fertilisers.
- Innovations in the development of enzymes and amino acid production have been and are still important levers for the development of low environmental impact feeding strategies for pigs and poultry.
- The development of feeding strategies for reducing N and P excretion by pigs and poultry requires a good knowledge of nutrient bioavailability in feed ingredients and a precise evaluation of requirements.
- Phase feeding is required for practical application of improved feeding strategies. It is facilitated by the use of computerised feeding systems with the perspective of precision feeding.

Keywords: pig, poultry, nutrition, environment

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15.1 Introduction

For a sustainable pig and poultry production, emission of pollutants and use of non-renewable resources should be decreased over the whole production process, as much as possible. Nitrates and phosphates originating from pig and poultry manure contribute to eutrophication of freshwater or seawater. In addition, the world reserves of mineral phosphates are limited and should be preserved. The accumulation of Cu and Zn in soils may impose a medium or long term toxicity risk on plants and soil micro-organisms, and increases the risk of their transfer to water ecosystems. Ammonia (NH_3) emission from manure is involved in acidification and eutrophication, with recognised detrimental effects on forests, soils, and biodiversity. Ammonia also contributes to the emission of small particles to the air, with possible detrimental effects on human and animal health. Although lower than in ruminants, the raising of monogastric animals also contributes to direct emissions of greenhouse gas, especially methane (CH_4) from enteric fermentation in pigs, and CH_4 and nitrous oxide (N_2O) emissions from slurry and litter.

Diet formulation has a direct impact on the efficiency of use of nutrient and energy by the animals and, consequently, it affects nutrient and waste flow at a farm level (Petersen *et al.*, 2007). All the nutrients found in the manure, or emitted into the air, originate from the fraction of the feed which is not retained by the animal, indicating that the manipulation of the diet can be an efficient way to control the amount and chemical composition of manure produced, and the emissions of pollutants to the environment.

Over the last decades, different ways to reduce the environmental impact of pig and poultry production have been investigated. The nutritional approach has received great attention from researchers and legislative decision makers (Jongbloed *et al.*, 1999b; Aarnink and Verstegen, 2007). It mainly relies on improvements in our knowledge of the physiology of pigs and poultry in order to achieve a better agreement between supply and requirement and improve nutrient bioavailability in feedstuffs. The main strategies that have been investigated are: (1) the improvement of efficiency of nutrient use in order to reduce excretion and emissions from the animals; (2) the modification of chemical properties of excreta in order to reduce the gaseous emissions and the production of odours from manure; (3) the better adaptation of excreta to their future use for energy production or as fertilisers in different strategies of valorisation with or without treatment.

The aim of this chapter is to provide an overview of the scientific knowledge regarding the nutritional possibilities to reduce N, P, Cu and Zn excretion by pigs and poultry, as well as emission of NH_3 and greenhouse gas and odours, and to describe the means that could be or are already implemented in practice. The environmental impacts related to the production of feeds and feed ingredients are not considered in this chapter but are discussed elsewhere.

15.2 Improvement of efficiency of use of protein

15.2.1 Efficiency of protein in pigs

The efficiency of protein utilisation by pigs depends on the dietary composition and the physiological status or the growth stage of the animals. In growing-finishing pigs fed a cereal-soybean meal diet, approximately 32% of the N intake is retained (Dourmad *et al.*, 1999b). Faecal N excretion which consists of the undigested protein fraction of the feed and endogenous losses amounts to approximately 17% of the intake depending of the protein sources used. Digested proteins are absorbed as amino acids which can subsequently be used for protein synthesis. Obligatory losses of amino acids relate to digestion, protein metabolism (synthesis, degradation and turnover), and renewal of skin and hair. The remaining digestible amino acids, after protein deposition and obligatory losses, are catabolised and excreted mainly as urea. With conventional diets this latter fraction is often the most important. Average efficiency of N retention is lowest in sows (20-30%), intermediate in growing pigs (30-40%), and highest in weaners (45-55%) (Dourmad *et al.*, 1999a).

Two complementary nutritional approaches can be used to improve the efficiency of protein utilisation in pigs and, consequently, to reduce N excretion. The first approach is to ensure adequate protein/amino acid supply over time according to the growth potential of the animals or their physiological state. This requires a joint fitting of daily supply of energy and protein (amino acids), depending on genetic potential and stage of production. In sows, N excretion is reduced by 20 to 25% when different diets are allocated for pregnancy and for lactation instead of a single diet. This is now implemented on most farms. Further improvements could be achieved with the use of two- or multi-phase feeding during pregnancy. This requires a precise evaluation of requirements, which can be achieved using modelling tools. Dourmad *et al.* (2014) calculated that two- and multi-phase feeding strategies during pregnancy resulted in 10 and 14% reduction of protein intake, 15 and 20% reduction of N excretion over the whole reproductive cycle, and 6 and 8% reduction of feeding cost, respectively. Although such feeding strategies can be implemented in practice with the use of automated feeding stations that allow the mixing of two feeds differing in their nutrient contents, they are not yet commonly used in practice. In the same way the distribution of tailored diets resulting from the mixing of two feeds could also be considered for lactating sows in the future. In fattening pigs, phase feeding is also an efficient way to improve protein efficiency. Latimier and Dourmad (1993) reported an approximate 10% reduction in slurry N when different diets were applied during the growing and finishing periods, compared to feeding the same diet during both periods. A further 5 to 12% reduction in N excretion can be achieved by increasing the number of diets used over the fattening period or with weekly or daily multi-phase group feeding (Bourdon *et al.*, 1997; Pomar *et al.*, 2014). With the use of individual

precision feeding in the future (Chapter 18, Pomar *et al.*, 2019) each pig will receive the exact amount of nutrients according to its own expected performance and housing conditions allowing to tackle the between pig variability in protein requirements. Compared to a three-phase feeding strategy, precision feeding allowed the reduction of protein intake by 14% and N excretion by 22% (Andretta *et al.*, 2014).

The second nutritional approach to increase the efficiency of protein retention in pigs is to improve the dietary digestible amino acid balance, which allows reducing crude protein (CP) content of the diet. This can be obtained through a combination of different protein sources and/or the substitution of protein by inclusion of feed-use amino acids in a free form. In fattening pigs, Dourmad *et al.* (1993) measured a 35% reduction of N excretion after improvements in the dietary amino acid profile without affecting feed intake, average daily gain, feed efficiency and carcass composition. In the past there has been some controversy regarding the effect of reducing CP content on animal performance and especially on carcass composition with some studies indicating an increase in fat deposition with low protein diets. This was often related to the formulation of diets on a metabolisable or digestible energy basis, resulting in a higher net energy content for low protein diets, and consequently lower amino acid to net energy ratio. Moreover, diets were often formulated on crude and not digestible amino acid contents, increasing the risk of deficiency in second amino acids and higher fat deposition. The development of net energy and ileal digestible amino acid systems is thus an important step to secure the formulation of low protein diets for pigs.

The ultimate reduction of N excretion can be reached when multi-phase feeding is combined with a perfect balance between essential amino acids (close to the ideal protein), and with an optimisation of the supply of non-essential amino acids. Such a feeding strategy has been evaluated experimentally in fattening pigs by Bourdon *et al.* (1997). In that study, the use of a single diet (17.5% CP) over the whole growing-finishing period was compared to a 'multi-phase' strategy which consisted of the mixing of two diets (13.0 and 10.7% CP, re-equilibrated with free amino acids) in proportions that were optimised each week. Growth performance and carcass quality were similar, and N excretion was reduced by approximately 50%. With this feeding strategy, N excretion represented only 50% of N intake. This can be considered to be close to the technically maximal attainable reduction in N excretion for fattening pigs.

It must be pointed out that the development of such feeding techniques for reducing N excretion by pigs requires good knowledge of the amino acid availability in feedstuffs, and of the changes in amino acid requirements according to growing stage or physiological state. This is now within reach with the use of modelling techniques for predicting requirements (Dourmad *et al.*, 2008; Van Milgen *et al.*, 2008; NRC, 2012) together with a better knowledge of variations in amino acid availability in feedstuffs (CVB, 2000; Sauvant *et al.*, 2004; NRC, 2012). Moreover, more numerous amino acids

are now available for feed use (lysine, methionine, threonine, tryptophan and valine) which allows a further reduction in dietary protein content. This can also be achieved in practice by using computerised blend feeding systems which allow adapting the diet composition on a daily or weekly basis (Feddes *et al.*, 2000; Pomar *et al.*, 2007).

The reduction in dietary protein content results in a lower proportion of N excreted in urine relative to faeces, which might affect the utilisation of manure N after field application (Sørensen and Fernandez, 2003). In the study of Portejoie *et al.* (2003), the ratio NH_3-N:total N in fresh manure decreased from 0.79 with the 20% to 0.63 with the 12% CP diet. However, according to Gerdemann *et al.* (2000) and Sørensen and Fernandez (2003), the availability of slurry N for the plant was not affected by the dietary protein content.

Feeding strategies with reduced protein content are already implemented in practice in many countries. Different strategies are compared in Table 15.1. The first two-strategies correspond to the hypothesis used for the official calculation of N excretion in France (Corpen, 2003). Compared to the one-phase feeding strategy without any constraints on protein content, used as reference, the two-phase feeding strategy with limited CP contents, reduces N intake and N excretion by 10 and 15%, respectively. A further reduction in dietary CP and consequently N excretion, could be achieved in a relatively short term by incorporation of more amino acids in free form, which means in practice to relax the minimum constraint on CP, and using multiphase feeding for fattening pigs (Garcia-Launay *et al.*, 2014, Table 15.1). However the development of such techniques is dependent on technical and economic considerations.

15.2.2 Efficiency of protein retention in poultry

In the same way as for pigs, protein retention in poultry depends on the dietary protein level and the physiological stage of the bird. Amino acids from digested feedstuffs are absorbed and used for protein metabolism. Excess of digestible amino acids and obligatory losses related to metabolism are catabolised and excreted as uric acid. Undigested proteins as well as the endogenous urinary and faecal protein losses are also excreted. As a consequence, N excretion and retention depend on the quality of consumed raw materials (protein digestibility), the amino acid balance of the diet and the difference between birds' amino acid requirements and their supply. Thus, improving efficiency of protein retention requires a perfect knowledge of animals' requirements and raw material compositions. When this is not the case, as often is in practice, safety margins are taken which increase losses and reduces the efficacy of production.

To reduce N excretion and improve N retention efficiency, CP content of the diet is adjusted to physiological stage of the bird. For instance modern broilers are generally fed four different diets, or more, throughout their life, according to phase feeding

Table 15.1. Effect of protein feeding strategy of sows and fattening pigs on crude protein (CP) and nitrogen (N) excretion per slaughter pig produced (Corpen, 2003; Garcia-Launay *et al.*, 2014).

	Actual (Corpen, 2003)		Perspectives (Garcia-Launay *et al.*, 2013)	
	'One-phase'	Two-phase	Two-phase	Multi-phase
CP (g/kg)				
Gestation	170	140	120	120
Lactation	170	165	155	155
Pre-starter	210	200	180	180
Starter	190	180	160	160
Grower	175	165	150	150
Finisher	175	150	130	110
Average	177	158	140	131
N per pig (0-115 kg BW) (kg)				
Intake	9.10	8.16	7.23	6.78
Retention	2.89	2.89	2.89	2.89
Excretion	6.06	5.13	4.22	3.78
% of standard	100	85	70	63

strategies. Reducing protein content of the diets can be achieved by adding feed-use amino acids such as L-lysine, DL-methionine, L-tryptophan, or amino acid analogue such as hydroxyl methylthio butanoic acid, in order to reach an optimum profile of digestible amino acids (Mack *et al.*, 1999). Meda *et al.* (2011) calculated that each 10 g/kg reduction in dietary CP in layers and broilers resulted in 10% reduction of N excretion. A similar reduction was observed in ducks by Baeza *et al.* (2012) and Van Cauvenberghe and Burnham (2001) in broilers. However, poultry seem to be more sensitive than pigs to a reduction in dietary protein. For instance, Leclercq (1996) observed that a reduction of 10 g CP/kg feed significantly reduced N excretion, but increased feed conversion ratio by 0.021 kg/kg. Aftab *et al.* (2008) concluded that above 10% reduction of CP (compared to NRC recommendations), broilers performance might be reduced. Inadequate essential/non-essential amino acids or net energy/metabolisable energy ratios were suggested by these authors as possible reasons to explain these effects. This is confirmed with the recent study of Belloir *et al.* (2017) in which dietary protein content was reduced by 20-30 g CP/kg feed in finishing broilers without any negative consequences on growth performance. Nitrogen excretion was reduced by 13% for each 10 g decrease of CP/kg feed, resulting in an increase of 3.4 percentage points of retention efficiency. In turkey, Travel *et al.* (2005) found that an

important reduction in dietary CP during the finishing period reduced N excretion by 37%, did not affect growth performance but tended to reduce breast meat yield.

Efficiency of protein retention in poultry is also affected by the type of production (Corpen, 2003). The lowest efficiency is observed for laying hens (28%) and the highest (54%) for conventional broilers. Efficiency is reduced in slow growing/high meat quality and free range broilers (36 and 33%, respectively). The efficiency of turkey is intermediate (46%). The lowest efficiency for protein retention is generally found in organic poultry production systems, mainly because industrially manufactured amino acids are not allowed to be used.

15.3 Improvement of efficiency of use of minerals

15.3.1 Phosphorus

To reduce P excretion, P supplied to pigs and poultry should be adjusted to their requirement, and strategies to improve P bio-availability should be implemented (Poulsen, 2000; Knowlton *et al.*, 2004). This relies on an accurate knowledge of P requirement according to the physiological status of pigs and poultry, and of feed P bio-availability, including the effect of the addition of enzymes. Indeed with diets based only on cereal, soybean meal and minerals, the efficiency of P retention is rather low. For instance, with such diets in growing-finishing pigs, approximately 45% of the P intake is absorbed, 30% is retained, and the remaining 15% is excreted via urine (Poulsen *et al.*, 1999). In growing chickens, the absorption of P intake is approximately 57% and retention 50% (Plumstead *et al.*, 2008).

The most efficient approach to increase P retention efficiency and reduce excretion is to improve digestibility of P. This can be achieved with use of highly digestible mineral P supplements. For example, monocalcium rather than dicalcium phosphate can be used because of its much higher digestibility in pigs and poultry (Sauvant *et al.*, 2004). However, most strategies implemented to reduce P excretion by pigs and poultry refer to improvements in phytic P utilisation (Jongbloed *et al.*, 1992). Indeed, monogastric animals do not produce phytase, and this enzyme is required for the hydrolysis in the stomach of phytic acid which is the main form of P in cereals and meals. Some feed ingredients (wheat and wheat by products, rye, triticale, etc.) contain significant amounts of natural phytase, but this phytase is sensitive to high temperature and may be inactivated in the pelleting process. This is why two P digestibility value are proposed (Sauvant *et al.*, 2004) depending if the feed is pelleted or not. However, microbial phytase which is currently used in many countries, appears the most efficient to increase P digestibility. Total P supply may be decreased, resulting in reduced P excretion up to 40 to 50% (Jongbloed and Lenis, 1992; Latimier *et al.*, 1994;

Frapin, 1996, Juin *et al.*, 2001; Lelis *et al.*, 2010). This strategy proved to be efficient in most poultry species: growing and finishing broilers (Létourneau *et al.*, 2010a; Rousseau *et al.*, 2012; Bougouin *et al.*, 2014), laying hens (Ahmadi and Rodehutscord, 2012), turkeys (Maguire *et al.*, 2005) and ducks (Adeola, 2010). Different types of phytase are available on the market, their efficiency and sensitivity to temperature may differ (Igbasan *et al.*, 2000). However, for all of them, the response of digestible P to graded levels of microbial phytase is curvilinear, and the maximum P digestibility never exceeds 60-70%, even at high levels of phytase supplementation. Based on literature reviews, equivalency equations of digestible P for microbial phytase have been established (Kornegay, 2001; Johansen and Poulsen, 2003) and can be used for diet formulation. The adjustment of the Ca:P ratio in combination with phytase is recognised as a valuable strategy to improve P retention in pigs and poultry (Plumstead *et al.*, 2008; Létourneau *et al.*, 2010b).

In the same way as for protein and amino acid supply, the second approach to reduce P excretion is to ensure adequate supply over time according to the growth potential of the animals or their physiological status. This requires a precise evaluation of P requirements, as well as P availability in feed ingredients. In pigs, this can be achieved in practice by the use of a feeding system relying on, P apparent digestibility (CVB, 2000; Sauvant *et al.*, 2004) or bioavailability (NRC, 2012), and the factorial determination of P requirements (Jongbloed *et al.*, 1999a; Jondreville and Dourmad, 2005; NRC 2012). In poultry, different systems of available P coexist based on phytate and non-phytate P feed content (NRC, 1994; GfE, 1999), P relative bioavailability (Sauvant *et al.*, 2004) or retention (CVB, 2000). Although a factorial approach was previously proposed to determine P requirements (Sauveur, 1985), more global methods based on the maximisation of growth performance or bone mineralisation are generally employed. Currently, a common European P system is under consideration. Finally, this allows the lowering of safety margins when formulating diets, resulting in a decrease in P excretion.

Feeding strategies with reduced P content are already implemented in practice in many countries. Different strategies are compared in pigs (Table 15.2) and broilers (Table 15.3). In pigs, the first two-strategies correspond to the hypothesis used for the official calculation of P excretion in France (Corpen, 2003). Compared to the one-phase feeding strategy used as a reference, the two-phase feeding strategy with limited P content reduces P intake and P excretion by 13 and 19%, respectively. A further reduction in dietary P and consequently P excretion could be achieved in a relatively short term by using higher levels of phytase and by using multi-phase feeding for fattening pigs (Table 15.2, *perspective*). In broilers, the use of 750 phytase units (FTU) microbial phytase results in 36% reduction of P excretion (Table 15.3).

Table 15.2. Phosphorus (P) feeding strategy and P excretion of pigs.

Item	Corpen (2003)		Perspectives
	'One-Phase'	Two-phase	
P, g/kg diet			
Gestation	6.5	5.0	4.5
Lactation	6.5	6.0	6.0
Pre-starter	7.5	6.8	6.0
Starter	6.5	5.8	5.0
Grower	5.8	4.8	4.2
Finisher	5.8	4.4	3.8
Average	6.0	4.9	4.3
P per (0-115 kg), kg			
Intake	1.91	1.54	1.35
Retention	0.60	0.60	0.60
Excretion	1.31	0.94	0.76
% of standard	100	72	58

Table 15.3. Influence of dietary microbial phytase addition on phosphorus (P) excretion of broiler chickens.

Item	Standard	Standard +750 phytase units/kg microbial phytase
P, g/kg		
Starter	7.5	6.5
Grower	6.5	5.5
Finisher	6.0	5.0
P per chicken (g)[1]		
Intake	19	16
Retention	11	11
Excretion	8.5	5.4
% of standard	100	64

[1] Final body weight: 1.9 kg.

15.3.2 Copper and zinc

Copper (Cu) and zinc (Zn) are involved in many metabolic functions, and their provision in sufficient amounts in feed is indispensable to ensure good performance and animal health (Jondreville *et al.*, 2002; Revy *et al.*, 2003). However, because they are used as growth promoters at pharmacological levels (Poulsen, 1995), or because large safety margins are applied, Cu and Zn are often oversupplied in diets. Consequently, these elements are highly concentrated in manure and accumulate in soil, where they may impose a medium or long-term toxicity risk to plants and micro-organisms (Jondreville *et al.*, 2003). Moreover, when a treatment is applied to the slurry, Cu and Zn will follow the solid fraction where their concentration often exceeds the maximal values allowed for the utilisation of these products as organic fertilisers. The only way to decrease the concentration of trace element in manure is to restrict their incorporation in the diet.

The incorporation of 150 to 250 mg/kg Cu in pig diets has been employed for a long time because of its growth promoting effect (Braude, 1980). Accordingly, Cu is routinely fed commercially to broiler chickens at high pharmacological levels. This practice is currently authorised in the EU allowing diets containing a maximum of 170 mg/kg Cu for pigs up to 12 weeks. After 12 weeks of age, the use of Cu as a growth factor is no longer allowed within the EU, and the maximal level of incorporation is 25 mg/kg as for all poultry species. Compared to the former allowed inclusion (175 mg/kg up to 16 weeks of age and 100 mg/kg thereafter (Table 15.4, 'former'), this results in a drastic reduction of Cu in manure by almost 60% (Table 15.4, 'actual'). Nevertheless, the practical supply for pigs and poultry remains higher than the usually published requirements (less than 10 mg/kg), and average retention efficiency is still less than 1% for pigs and 6% for chickens.

Supplementing weaned piglet diets with 1,500 to 3,000 mg/kg Zn as ZnO has also been reported to prevent or overcome post-weaning diarrhoea and stimulate their growth (ANSES, 2013; Sales, 2013). In 2003, the maximal allowed Zn incorporation in pig diets was reduced to 150 mg/kg, compared to 250 mg/kg before. More recently, EFSA (2014) pointed out the high potential for Zn reduction in animal feed and recommended a reduction from 150 to 100 mg/kg of Zn for pigs for fattening and most poultry species except for turkeys for fattening (120 mg/kg). Consecutively, a 31% reduction in Zn emission could be achieved for pigs for fattening that could reach 53% when phytase is used (EFSA, 2014). These levels are much closer to the published requirement, which varies between 45 and 150 mg/kg for pigs and from 35 to 120 mg/kg for poultry species depending on growing stage and authors (NRC, 1994; Gfe, 1999; IFZZ, 2005; Revy *et al.*, 2005; MTT, 2013). However, in some EU countries supplementation with 2,500 mg/kg Zn is still allowed as medication, resulting in an drastically increased excretion.

Table 15.4. Estimates of copper and zinc balance[1] according to different scenarios of supply (former EU regulation, actual EU regulation and perspectives) in pig feeding.

Item	Copper			Zinc			
	Former	Actual	Persp.	Former	Actual	'Actual'[3]	Persp.
Concentration (mg/kg)							
Prestarter	175	170	10	250	150	2,000	70
Starter	175	170	10	250	150	150	50
Fattening pigs	120	25	10	250	120	120	30
Sows	100	25	10	250	150	150	70
Balance (0-110 kg BW)							
Intake (g/pig)	40.4	13.0	3.1	78.3	40.1	53.0	12.2
Excreted (g/pig)	40.3	12.9	3.0	75.8	37.6	50.6	9.7
Slurry (mg/kg DM)	1,060	348	80	1,995	1000	1,390	255
Delay, years[2]	50	167	1,040	99	175	125	1,160

[1] Calculated according to Jondreville *et al.* (2003), expressed per slaughter pig, including intake and excretion by sows.
[2] Delay to reach 50 mg copper or 150 mg zinc/kg soil DM.
[3] In case of use of 2,000 mg/kg of zinc in pre-starter diet as allowed in some EU countries as medication.

As for P, the main approach to reduce Cu and Zn in pig manure is to adjust the supply to the requirement, and to improve the availability to the animal. Zinc requirement of weaned piglets was evaluated to be approximately 90 mg/kg diet (Revy *et al.*, 2005) which is consistent with the former recommendations and below the usual level in practice. In broilers, the Zn requirement for maximal growth is approximately 30-40 mg/kg and ranged from 50 to 90 mg/kg for maximal plasma and bone Zn content (Schlegel *et al.*, 2013). In most cases, values used by the feed industry remain higher than dietary requirements. When microbial phytase is incorporated in the diet, the Zn supply may be reduced because of increased bio-availability. In weaned piglets, incorporation of 500 phytase units/kg diet was evaluated to be equivalent to the supply of 30 mg/kg of Zn as Zn sulphate (Jondreville *et al.*, 2005). In broilers, 500 FTU was equivalent to 5 mg of Zn as sulphate (Jondreville *et al.*, 2007). Consequently, in a maize-soybean meal based diet formulated to contain 60 mg/kg of Zn, Zn excreted would be reduced by 10% when adding 500 FTU of microbial phytase per kg. In broilers, compared to the present situation (100 mg/kg Zn/kg feed), Zn excretion by broilers would be reduced by 36% in case of a reduction to 100 mg/kg of feed, and by 77% in case of feeding according to the animal's Zn requirement (Table 15.5).

With the present EU regulation, Cu and Zn contents in pig manure dry matter (DM) (~ 350 and 1,250 mg/kg DM, respectively) are below the maximal concentration

Table 15.5. Estimates of zinc balance according to different scenarios of supply (current EU regulation, EU regulation perspectives and dietary requirements) in chicken feeding.

Item	Current	Perspectives	Dietary requirements
Concentration (mg/kg)			
Starter	120	100	60
Grower	120	100	50
Finisher	120	100	40
Balance[1] (mg/bird)			
Intake	370	309	134
Retention	38	38	38
Excretion	332	270	96
Relative excretion (%)	100	80	29

[1] Final body weight: 1.9 kg.

generally allowed in sewage sludge (1000 and 3,000 mg/kg DM, respectively), but they exceed the concentration allowed for organic fertilisers (300 and 600 mg/kg DM, respectively). Assuming that 170 kg N/ha are spread out each year, it will take 160-170 years for the soil to reach 50 mg Cu or 150 mg Zn /kg soil DM (Table 15.4). This is much longer than with the previous regulation (50 to 100 years). But although the situation has been drastically improved by this regulation, Cu and Zn inputs to soil with a manure application rate of 170 kg N/ha still exceed the export by crops. In the future, further reductions in Cu and Zn excretion should be possible (Table 15.4, *perspective*), resulting in a better agreement between spreading and export by plants. However, this will require a better understanding of the factors that affect Cu and Zn availability and a more precise evaluation of the requirements.

15.4 Effect of feeding on ammonia emissions from manure

According to the EMEP/EEA (2013) air pollutant emission inventory guidebook, NH_3 emission during storage of animal manure can be calculated as a proportion of N excreted or its NH_3 content. According to this approach, reduction of N excretion, and especially of the urinary fraction, will thus result in reduction of NH_3 emission. By changing feeding practices, it is possible to influence urea or uric acid concentration of excreta and the pH of slurry, which both affect NH_3 release (Van de Peet-Schwering *et al.*, 1999). When pigs are fed low CP diets, urinary urea concentration and pH decrease (Canh *et al.*, 1998; Portejoie *et al.*, 2004). When water is available *ad libitum*,

feeding low CP diets also results in lower urine production due to decreased water consumption (Pfeiffer *et al.*, 1995; Portejoie *et al.*, 2004). These changes in slurry characteristics result in lower NH_3 losses during housing, storage and following application of slurry (Canh *et al.*, 1998; Hayes *et al.*, 2004; Portejoie *et al.*, 2004; Jarret *et al.*, 2011). For instance, in the study of Portejoie *et al.* (2004) NH_3 emissions over the whole period from excretion to field application, was decreased by 63% when dietary CP was decreased from 20 to 12% in finishing pigs (Table 15.6).

The electrolytic balance, calculated as $(Na^+ + K^+ - Cl^-)$, is often used in pig and poultry feeding to evaluate the acidogenicity of the diet, a decrease in the electrolytic balance resulting in a decrease in urinary pH. When dietary CP content is reduced, electrolytic balance decreases generally because of the high potassium (K) content of most protein sources (INRA-AFZ, 2004). This partly explains the effect of CP on urinary pH. However, as shown by Canh *et al.* (1998), more drastic changes in urinary pH and NH_3 volatilisation can be obtained by inclusion of the calcium (Ca) salts $CaSO_4$ or $CaCl_2$ instead of $CaCO_3$. The addition of Ca-benzoate (Canh *et al.*, 1998) or benzoic acid (Guiziou *et al.*, 2006) was also effective in reducing slurry pH and NH_3 volatilisation (by 25 to 40%), because these products are metabolised into hippuric acid which is rapidly excreted in urine. A similar effect (25% reduction in NH_3 emission) was observed with adipic acid (Van Kempen, 2001) which is partially excreted in urine. Some feed ingredients with a high content of sulphur have also a

Table 15.6. Effect of protein feeding of fattening pigs on slurry characteristics and ammonia volatilisation (Portejoie *et al.*, 2004).

Item	Dietary crude protein content (%)		
	20	16	12
Slurry composition			
Amount (kg/pig/d)	5.7	5.1	3.6
Dry matter (%)	4.4	4.6	5.9
Total nitrogen (g N/kg)	5.48	4.30	3.05
Total ammonia nitrogen (g N/kg)	4.32	3.13	1.92
pH	8.92	8.61	7.57
Nitrogen balance (g/pig/d)			
Retention	23.2	23.5	21.9
Excretion	40.7	27.6	15.0
Ammonia-N volatilisation[1]	17.4	13.8	6.4
Available to plants	23.3	13.8	8.6

[1] Cumulated N-NH_3 volatilisation in housing, during storage and during spreading.

high acidogenic potential. This is for instance the case for dried distillers grain with soluble because of the use of sulphuric acid in the production process of bioethanol.

Urea N excretion by pigs can also be reduced by including fibrous feedstuffs in the diet. With more fermentable non-starch polysaccharides (NSP) in the diet, some of the N excretion is shifted from urine to bacterial protein in faeces (Canh *et al.*, 1998; Kreuzer *et al.*, 1998; Sørensen and Fernandez, 2003; Jarret *et al.*, 2011, 2012), while total N excretion is not affected. Moreover, slurry pH is decreased with the use of fermentable NSP due to volatile fatty acid formation in the hindgut of the pig and in the slurry (Table 15.7). Canh *et al.* (1998) reported a linear relationship between NSP intake and slurry pH as well as NH_3 volatilisation; for each 100 g increase in NSP intake, the slurry pH decreased by 0.12 units and the NH_3 emission from slurry decreased by 5.4%. This is consistent with the recent results obtained by Jarret *et al.* (2012) who compared two diets differing in their fibre content (Table 15.7).

The utilisation of pig manure N after field application may also be affected by the level of NSP in the diet, because a greater proportion of N is excreted in faeces in more complex organic forms. Availability of slurry N was reduced after the inclusion of dietary fibre with low fermentability (Sørensen and Fernandez, 2003), whereas it was not affected when the dietary content of fermentable structural carbohydrates increased (Gerdemann *et al.*, 2000; Sørensen and Fernandez, 2003), although in all cases the proportion of N excreted in urine decreased. Combined with the proportion of urinary N, the fibre content of faeces provides a good prediction of the short term availability of slurry N to plants (Sørensen and Fernandez, 2003).

Table 15.7. Effect of fibre content in fattening pigs diet on composition of excreta, nitrogen balance and ammonia emission (Jarret *et al.*, 2012).

Item	Control	High-fibre[1]	
Crude fibre, g/kg	29.4	49.0	
Nitrogen balance, g/d			
Intake	55.9	55.5	
Excretion	28.7	30.3	
% in faeces	26.3	40.0	***
pH urine	8.28	7.15	***
pH faeces	8.39	8.11	***
VFA[2] in faeces, mg/l	62.6	260.0	***
Ammonia emission (%)	17.9	12.4	***

[1] In the high fibre diet soybean meal was replaced by wheat dried distillers grain with soluble and rapeseed meal.
[2] Volatile fatty acids analysed according to Peu *et al.* (2004).

Effects of dietary manipulations on NH_3 production from poultry production have been described by Meda *et al.* (2011). In broilers, Ferguson *et al.* (1998) measured that litter humidity was reduced by 6%, N excretion by 16.5% and NH_3 volatilisation by 31%, when dietary CP content decreased from 21.5 to 19.6%. It is noticeable that the effect is more marked on NH_3 volatilisation than on N excretion. The effects of dietary sodium (Na), K and electrolytic balance on water consumption of broilers and humidity of litter have also been investigated in poultry and indicate that humidity of excreta and litter increase linearly with dietary Na, when fed above the physiological requirements. The recent results of Belloir *et al.* (2017) confirmed that the reduction of dietary CP content in finishing broilers decreases N volatilisation, due to two effects acting in synergy: (1) the direct reduction of N excretion (Section 15.2.1.); and (2) the lower proportion of excreted N to be volatilised (minus 4-5 points per CP point reduction) due to a lower moisture content in the litter.

Feed viscosity appears to be another important criteria affecting water excretion of poultry (Francesch, 2005). In turkey, Carré *et al.* (1994) showed that water excretion was significantly correlated with feed viscosity, but the best prediction of water excretion was obtained by combining feed viscosity and K content. This would explain the poor quality of litter when poultry are fed cereals with a high NSP content associated with a high viscosity. In this context, the use of enzymes may contribute to improve litter quality as shown in different studies (Francesch, 2005). The incorporation of some other feed ingredients such as some co-products or leguminous grain may also alter litter quality. The effects of these changes in litter quality on NH_3 emission have not been evaluated, but it may be expected that the increased humidity should result in higher emissions.

15.5 Effect of feed composition on direct emissions of greenhouse gas

Nutrition may affect both emissions of N_2O and CH_4. According to IPCC (2006), emissions of N_2O are calculated from N excretion and specific emission factors that depend on manure management. This means that according to this procedure of calculation, N_2O emission will be proportional to N excretion, and all strategies that will reduce N excretion will also affect direct the calculate N_2O emissions, in the same proportion, as well in pig and poultry. However, it should be confirmed by experimental results since, to our knowledge, this has not yet been done.

Methane has mainly two origins: enteric fermentations, which are negligible in poultry, and fermentation from collected and stored manure. Enteric fermentations in pigs vary according to age of animals and the amount of digested fibre ingested. The latter is the difference between digested organic matter (OM) and digested protein,

fat, starch and sugar (Sauvant *et al.*, 2004). The loss of energy as CH_4 ($E(CH_4)$) can be obtained by multiplying digested fibre by 670 or 1,340 J/g for growing pigs and sows, respectively (Noblet *et al.*, 2004). Methane production (CH_4 Enteric, kg) can then be calculated from $E(CH_4)$, considering a methane calorific value equal to 56.65 MJ/kg (IPCC, 2006). This is illustrated in Table 15.7 from the results of Jarret *et al.* (2012) who compared two diets with different fibre contents in fattening pigs.

According to the IPCC (2006) Tier 2 methodology, CH_4 emission from stored manure can be calculated as:

$$CH_4 \text{ Manure (kg)} = VS \times B_0 \times FCM$$

with VS = volatile solids in excreta, roughly considered as amount of OM excreted (kg), B_0 = maximum CH_4 producing capacity (m^3/kg OM) and MCF = a CH_4 conversion factor for the management system considered, expressed as a percentage of maximum potential. Volatile solids excreted depend on the digestibility of feed OM and is mainly affected by dietary fibre content. Volatile solid can be calculated from OM digestibility values in feed tables (Sauvant *et al.*, 2004). This means it is mainly the indigestible OM content of the diet that will affect CH_4 emission from manure. This is illustrated in Table 15.7 from the results obtained in pigs by Jarret *et al.* (2012) who compared a conventional and a higher fibre diet. Volatile solid excreted per pig per day was significantly increased with the high fibre diet (by 64%) whereas B_0 of excreta did not differ between treatments. This resulted in a 76% higher CH_4 emission per pig during a simulated storage of 100 d, for the high fibre diet. In the same way, when comparing diets with different types of high fibre feed ingredients (rapeseed meal, sugar beet pulp and dried distillers grain with soluble), Jarret *et al.* (2011) observed only limited differences in B_0 values, but a significant increase of excreted VS, resulting in a much higher potential of CH_4 emission per pig for the three high fibre diets (+60%). When the excreta were fermented in a mesophilic anaerobic digester, the high fibre diet resulted in a 70% increase in the production of biogas per pig. This indicates that it should be interesting to adapt the composition of the diet according to the chain of waste collection and treatment in order to minimise uncontrolled emissions of NH_3 and CH_4, and conversely in case of anaerobic digestion to maximise the controlled production of CH_4.

Composition of the diet also affects the dynamic of CH_4 emission. Although B_0 values of excreta from pigs fed a high protein diet (471 l CH_4/kg OM) tended to be higher than from a low protein diet (449 l CH_4/kg OM), the emission during storage was accelerated for the low protein diet, in relation with a lower initial pH and a lower NH_3 concentration (Jarret *et al.*, 2011). In the same way, CH_4 emission started much faster in effluent from pigs fed a high fibre diet which had also lower pH and lower NH_3 concentration. These results indicate that MCF is affected by duration of storage and depends on the type of diets. For instance in the study of Jarret *et al.* (2011) for

the 50 days of storage in mesophilic conditions, MCF varied from 2% for the high protein diet to 54% for the high fibre diet (from 18 to 75% after 100 days of storage), the value for the low protein diet being intermediate.

15.6 Effect of feed composition on odours

Odours are mainly associated with volatile compounds that animals excrete, or which are released during manure storage (De Lange *et al.*, 1999). These volatile compounds are generated by the microbial conversion of feed in the large intestine of pigs, in manure pits or in the litter.

Few studies have evaluated the direct effect of diet manipulation on odour production, mainly because it is difficult to assess odours objectively. As mentioned previously, protein nutrition affects NH_3 production, but the NH_3 production is not well correlated with odour strength (Le, 2006). Using olfactometry, Hayes *et al.* (2004) showed a significant reduction in both NH_3 and odour emissions when CP content was reduced, but this was not observed in all studies. Hobbs *et al.* (1996) reported that the concentration of nine out of ten odorous compounds in the air was significantly reduced when low CP diets were fed to the pigs. Le (2006) also found a reduction by 80% in the odour emission, as determined by olfactometry, when dietary CP was reduced from 18 to 12%. Moreover, the results from the same author suggested an interaction between effects of CP and fermentable carbohydrates (FC) on odour production suggesting that odour production depends also on the balance between dietary CP and FC. This is in line with the literature review from Le *et al.* (2005) suggesting that CP and FC would play a major role in the production of odour nuisance from pig production. The manipulation of gut fermentation could also be a way to alter the production of odorous compounds such as skatole (De Lange *et al.*, 1999).

Using a different methodology for assessing 'pleasantness', 'irritation' and 'intensity' scores of odours, Moeser *et al.* (2003) were able to significantly discriminate between diets differing in composition. The diets that yielded manure with the worst odour were high in sulphur (rich in garlic or feather meal), whereas a purified diet mainly based on starch and casein presented the lowest score (most pleasant). This is in agreement with the >700% increase in odour emission measured by Le (2006) with diets supplemented with a sulphur-containing amino acid (methionine) at high levels.

15.7 Conclusion

Improving the efficiency of nutrient utilisation through feeding appears a very efficient way to reduce excretion in slurry and emissions by the animals. In a whole-farm perspective, this is also an efficient way to decrease the import of nutrients, especially N, P and trace elements, from outside the farm, reducing the nutrient load per hectare. Moreover, gaseous emissions from livestock housing and during storage and spreading of manure are also affected whenever animal nutrition is modified, mainly due to changes in chemical composition of the effluents. These changes have significant effects on emissions of greenhouse gas and N compounds and to some extent on odours. This contributes to lowering the environmental impacts of animal production, and also improving nutrient recycling at whole system level. Modifying diet composition also offers opportunities to increase the potential of energy production from effluents in case of biogas production.

However, in a whole production chain approach, not only the emissions and resource use occurring during the raising of animals should be considered, but also those occurring during the production of the feed ingredients and the use of effluents. This can be achieved using life cycle assessment (Chapter 14, Van Middelaar *et al.*, 2019). With this approach it appears that the step of production of feed ingredients has an important contribution to many of the environmental impacts of pig and poultry production, especially for energy use, emission of greenhouse gas and eutrophication (Basset-Mens and Van der Werf, 2005). This means that diet composition also indirectly affects environment impact of animal production through the choice of feed ingredients. For instance, decreasing dietary CP content may be an efficient way to reduce N excretion and consequently NH_3 emission, but it may also reduce the emission of greenhouse gas due to the reduced incorporation of soybean meal that may be associated with deforestation (Garcia-Launay *et al.*, 2014). Moreover diet formulation also affects the type of crops to be produced locally or away from the farm with effects on use of land, crop rotation, biodiversity, and capacity of the system to recycle nutrients.

15.8 Future perspectives

Intensive research has been conducted in the last three decades with the aim to increase the efficiency of nutrient use in pigs and poultry, and reduce excretion and emissions. The scientific knowledge on nutrient utilisation by animals has been drastically improved, allowing a much more precise evaluation of their requirements. At the same time, new knowledge on nutrient values of feed ingredient and new feed evaluation systems have also been provided. Moreover many innovations in production of diverse enzymes and free amino acids have been developed. This knowledge has been progressively integrated in mathematical models that can be used in practice.

Although great progress has been achieved in knowledge of pig and poultry nutrition, there is still room for further improvements. This is the case for instance for efficiency of use of amino acids which is still poorly evaluated for most of them. The role of dispensable amino-acid in low protein diets is also not well elucidated, as well as interactions among amino-acids. In the same way, mechanism controlling the digestive and metabolic utilisation of calcium, phosphorus and trace elements are not well understood, neither quantified. Improving this knowledge will allow to improve our prediction models and secure the use of low protein and low mineral diets whilst reducing security margins. Moreover, the recent and drastic progress of knowledge on gut microbiota also offers new opportunities for innovation in the development of enzymes and additives, in order to improve energy and nutrient digestibility.

The drivers for the future of pig and poultry nutrition are also changing with an increased consideration of the societal demand towards production systems with reduced environmental impacts, but also less in competition with human food demand, taking better benefit of by-products and food waste, more local and less dependent on imported feed ingredients, especially of protein sources associated to deforestation, without GMO. This will require new scientific knowledge and the development of innovations for the production of alternative protein sources, such as for example insects of protein extracted from forages. It can also be expected that these changes could result in an increased variability of feed quality over time that will be necessary to manage in practice.

The development of precision feeding is another important aspect to consider for the future of sustainable pig and poultry nutrition. A large diversity of sensors is now available that may give almost real time information on feed composition, housing conditions and animal performance. At the same time feed dispensers allow to control the amount and/or the composition the feed distributed each day to each room/pen/animal, allowing to better adapt supplies to requirement, which has been shown to be a major diver for reducing excretion. This should also allow to better take account of the expected increase in variability of feed quality over time. This will however require the development of new types of models and decision support systems able to handle and learn from 'big data' and to take decision at real time to control feeding devices.

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16

The role of nutrient utilisation models in precision animal management

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Summary points

- Tremendous progress has been made in improving our understanding of the biology of nutrient utilisation for production in pigs, poultry, and other farm animals. Integration of this knowledge into dynamic and stochastic nutrient utilisation models allows for reasonably accurate predictions of nutrient requirements as well as feed intake and performance responses to varying dietary nutrient levels when animals are managed in a relatively stress-free environment.
- The accurate mathematical representation of interactive effects of animal genotype with both its infectious environment and social stressors on nutrient utilisation continues to be a challenge.
- There is opportunity for further model development, including the representation of the dynamics of nutrient digestion and absorption, the prediction of carcass quality, body fat distribution and fatty acid profiles in different fat depots, and the control of animal end-product quality.
- Increasing model complexity does not equate to improved predictive accuracy; therefore, models should be constructed for a defined purpose (i.e. increased understanding of complex biological processes vs increased predictive accuracy of animal productivity under varying conditions) and in collaboration with targeted model users.
- In the future, nutrient utilisation models are likely to become integrated with sensors, to monitor animals and their environment, and robotics, for automated animal management including feed preparation and delivery, allowing optimisation of precision animal management strategies in real-time, i.e. precision animal management.

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- The use of advanced mathematical and statistical methods, such as self-organising artificial neural network-genetic algorithms, should be carefully considered when optimising animal management, especially when animal production cycles are relatively short and environmental conditions can be closely controlled.

Keywords: animal management, decision support systems, modelling, nutrient utilisation, optimisation

16.1 Introduction

The rapidly growing global demand for food protein of animal origin, the increasing competition between humans and animals for limiting feed and food resources, and impact on the environment (soil, water, atmosphere, ecosystem) are key challenges that must be considered as we manage and further develop food animal production systems that are environmentally sustainable and economically viable. In addition, and largely driven by consumer demands, there must be increased emphasis on the well-being of animals, as well as animal-product quality, safety, and traceability. These challenges demand effective integration of our cumulative knowledge and innovative technologies in decision support systems that are important tools for optimising animal management on individual animal production units. For these decision support systems to be most effective, they must be robust under varying conditions, include technologies for rapid automated data collection (i.e. animal and environmental sensors), have substantial computing capacity for data analyses and system optimisation, and be reasonably easy to operate by trained users. Ideally and when feasible, these systems should function in real time and be integrated with automated systems to control the animals' environment. Mathematical models representing utilisation of nutrients for growth and reproduction will likely continue to be the core of meaningful decision support systems. Moreover, nutrient utilisation models will continue to be used in education and research to improve understanding of nutrient utilisation for growth and reproduction. In this chapter, a brief history of the development of nutrient utilisation models is provided, followed by a discussion about model components that may be developed further for supporting precision animal management, and some future perspectives. The main emphasis is on growing pigs and poultry, while there is only limited discussion on nutrient utilisation to support reproduction. It is beyond the scope of this chapter to discuss the main model components in detail; however, key references that deal with specific model components are listed where appropriate.

16.2 Evolution of nutrient utilisation models

16.2.1 Representing the biology of nutrient utilisation

The mathematical representation of nutrient utilisation for growth and reproduction in food animals was first explored in the late 1970s and early 1980s (Whittemore and Fawcett, 1976; Emmans, 1981; Black *et al.*, 1986; Moughan *et al.*, 1987), and was initially driven by rapidly evolving computing capacity. The first models were deterministic and focussed primarily on the use of dietary energy and amino acid intake to satisfy maintenance requirements, that were determined rather empirically (i.e. without considering the underlying biology), and to support retention of protein and lipid in body weight gain and products of reproduction (i.e. milk, eggs and foetus) in animals managed under relatively stress-free conditions. Over time, these models have evolved to become more mechanistic in representing animal biology, in order to more accurately predict animal and environmental effects on animal productivity under a wider range of conditions (Moughan *et al.*, 1995; Kyriazakis, 1999; Gous, 2007; Dourmad *et al.*, 2008; Van Milgen *et al.*, 2008; Rivera-Torres *et al.,* 2011; NRC, 2012). Key aspects to improve understanding of nutrient utilisation include: characterisation of animal genotypes in aspects of nutrient partitioning (Black *et al.*, 1995; Schinckel, 1999; Gous, 2007), nutrient needs for biological processes associated with body maintenance functions and supporting animal production (Emmans, 1994; Moughan, 1999; Birkett and De Lange, 2001; NRC, 2012; Letourneau-Montminy *et al.*, 2015), representing effects of environmental stressors (i.e. social, thermal, disease; Black *et al.*, 1995; Wellock *et al.*, 2006; Gous, 2007), representing the dynamics of nutrient digestion and absorption (Bastianelli *et al.*, 1996; Chapter 2, Moughan, 2019), and the prediction of feed intake (Torrallardona and Roura, 2009; Richards *et al.*, 2010; Chapter 3, Everaert *et al.*, 2019).

Based on our improved understanding of the biology of nutrient utilisation in animals, a generalised framework has evolved that can be used to represent nutrient partitioning for growth and reproduction aimed at predicting animal performance (Figure 16.1). Key elements of this framework are the characterisation of: (1) the animal's performance potential in aspects of nutrient partitioning; (2) the animal's capacities to deal with environmental constraints; (3) available (dietary) resources; and (4) environmental constraints imposed by available resources. The animal's performance potential determines nutrient needs of animals managed in a non-limiting environment. Examples of animal capacities are upper limits to ingesting and processing feed, or maximum heat loss to the environment. Important environmental resources include diets that supply bio-available nutrients and alternative micro-environments that are available to animals. Examples of environmental constraints are the bulkiness of available diets, feed delivery systems, and the thermal environment that will determine whether energy is required for thermoregulation. These four key

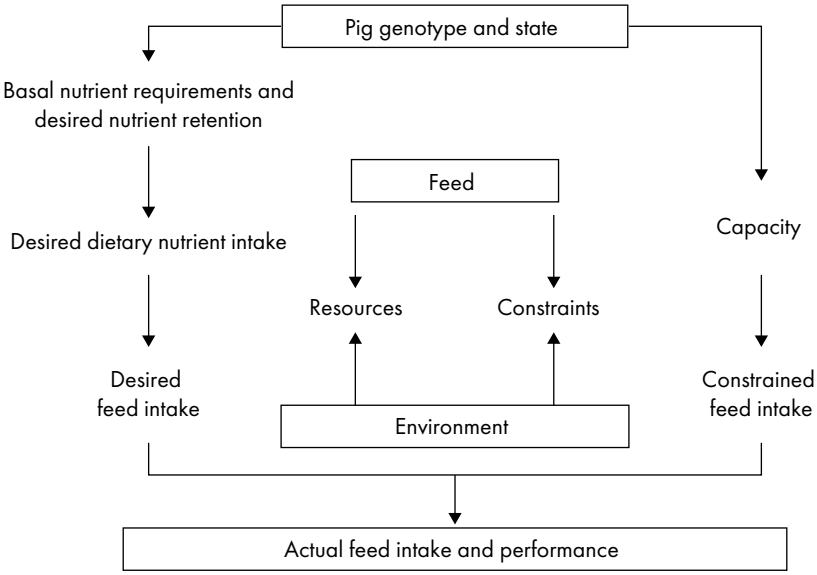

Figure 16.1. Conceptual framework representing a generalised approach to predicting performance of *ad libitum* fed animals (adjusted from Emmans 1981, 1987).

elements will allow estimation of the animal's realised feed intake and achieved level of performance under defined environmental conditions.

Core to nutrient utilisation models are the interactive effects of intake of protein (i.e. essential and semi-essential amino acids, nitrogen) and energy (i.e. energy yielding nutrients) on whole body protein and lipid deposition. Underlying principles and various approaches to mathematically represent these interactive effects are described in detail elsewhere (Gous *et al.,* 1987; Moughan *et al.*, 1995; Kyriazakis, 1999; Möhn *et al.*, 2000). In general terms and provided that animals are managed in a relatively stress-free environment and consume sufficient amounts of essential micro-nutrients, minerals and essential fatty acids, the achieved rate of whole body protein deposition is determined by either (1) the animals' (genetically determined) upper limit to body protein deposition, (2) energy intake, or (3) the intake of the first limiting dietary amino acid (Figure 16.2). It is a formidable challenge to actually determine the animals' (genetically determined) upper limit to body protein deposition; a working definition may be the observed rate of body protein deposition that cannot be improved further by increasing energy and nutrient intake or reducing environmental stressors. Obviously, this upper limit to body protein deposition will change with stage of maturity and requires that upper limit to body protein deposition curves are established. The slope of the (linear) increase in body protein deposition with increasing energy intake (Figure 16.2) is determined primarily by the minimum ratio between whole body lipid deposition to whole body protein deposition. This

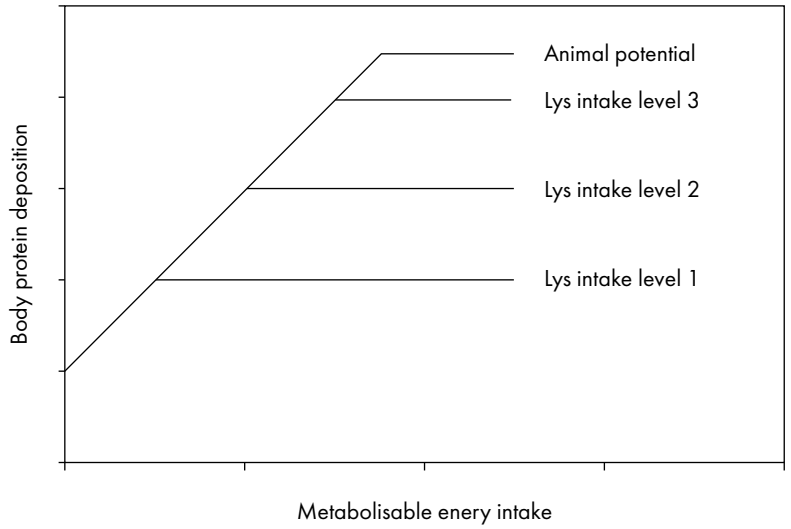

Figure 16.2. Typical body protein deposition response of growing animals fed varying levels of energy and amino acid intake. Lysine (Lys) intake levels 1, 2 and 3 represent increasing daily intakes of the first limiting dietary amino acid. Animal potential is the rate of body protein deposition that cannot be increased further by increasing intake of either energy or Lys.

ratio reflects that it is generally not physiological to utilise incremental amounts of energy intake only for supporting extra body protein deposition, even when energy intake limits expression of the animals' upper limit to body protein deposition. This minimum ratio can be represented mathematically using various approaches and is known to be affected by animal genotype, environmental conditions, stage of maturity, level of energy intake and nutritional history (De Lange *et al.*, 2008). It is generally accepted that energy intake over and above maintenance energy requirements that is not used for body protein deposition and associated minimum body lipid deposition is used for deposition of (excess) body lipid.

The generalised framework (Figure 16.1) and the partitioning of energy and amino acid intake (Figure 16.2) can easily be expanded to accommodate the representation of additional concepts, such as effect of environmental stressors (i.e. infectious, thermal and social environments) on maintenance energy and nutrient requirements or reductions in the animal's (operational) upper limit to body protein deposition. In a similar manner, the contribution of various animal and environmental constraints relative to the animal's capacity to deal with these constraints can be integrated. It can also be easily expanded to represent nutrient losses into the environment, simply by applying mass balances and calculating the difference between intake and retention in animal products of nutrients or elements (e.g. carbon, nitrogen, phosphorus; NRC, 2012).

16.2.2 Integration of stochastic and dynamic elements

As nutrient utilisation models have evolved, they have become dynamic in representing changes in nutrient utilisation over time, including effects of (nutritional) management history. For example, nutrient requirements and compensatory growth following a period of amino acid intake restriction may be represented dynamically (e.g. Eits *et al.*, 2003; Martínez-Ramírez and De Lange, 2007). Moreover, models are increasingly becoming stochastic in representing between-animal variability within groups of animals, by imposing variation and co-variation among animal genotype-determined aspects of nutrient partitioning and animal-environment interactions. These dynamic and stochastic elements are critical when models are used to optimise management of groups of animals on individual production units with their unique environments and constraints, resulting from variation in genetically determined animal performance potentials, available (feed) resources, environmental and economic conditions, and payment schemes for animal products (Pomar *et al.*, 2003; Morel *et al.*, 2008).

Optimising the production system requires that we move away from meeting the animal's requirements for nutrients and optimal environmental conditions towards characterising the dynamic response of (groups of) animals to varying dietary nutrient levels and environmental conditions. For example, in order to optimise profits and nutrient losses into the environment for animals that are managed as groups, it not feasible to meet the dietary nutrient requirements of all animals and at all times, and the extent of between-animal variability should be considered for establishing the optimum dietary nutrient levels. Between-animal variability is one of the main contributors to the diminishing marginal efficiency of amino acid utilisation for production when dietary amino acid levels approach requirements for maximum performance in groups of animals (De Lange *et al.*, 2001; Pomar *et al.*, 2003). This is illustrated in Figure 16.3, where the typical growth responses of groups of pigs to varying amino acid intake levels (Figure 16.3A) is contrasted with the response to varying threonine intake levels observed in individually housed pigs (Figure 16.3B; De Lange *et al.*, 2001). A diminishing marginal efficiency of nutrient utilisation implies that profit is not maximised when feeding dietary amino acid levels required for maximising growth performance of groups of pigs. For maximum profits, the optimum dietary amino acid level should be chosen based on maximising the difference between variable costs (determined by levels of expensive amino acids, such as lysine, in the diet) and generated value (determined by changes in growth rate, feed efficiency, and carcass quality).

An example that supports the need for dynamic representation of nutrient utilisation is to balance effects of feeding regimen on gut and animal health with meeting the animals' nutrient requirements for expression of animal performance potentials. Given the negative effects of feeding high dietary levels of relatively inexpensive plant protein sources that contain various anti-nutritional factors to young animals with

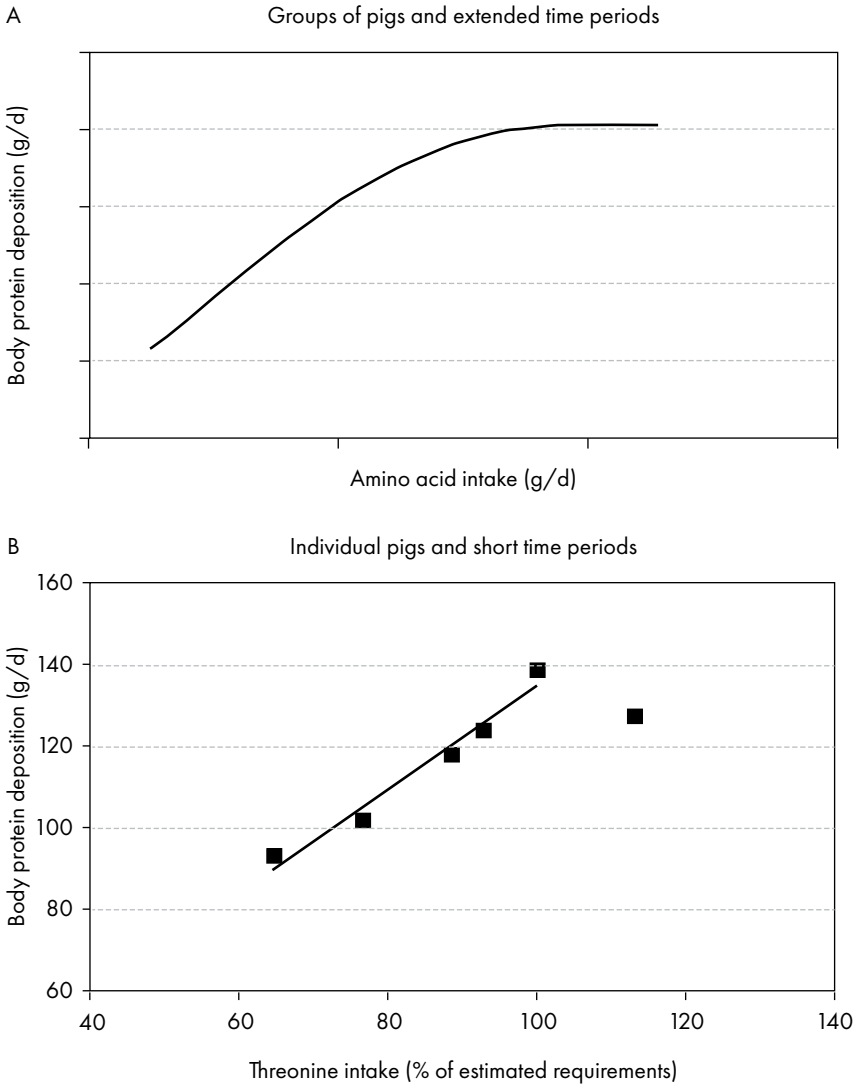

Figure 16.3. Typical relationship between dietary nutrient levels and growth responses observed in (A) groups of animals and (B) a dose-response study were pigs were housed individually (De Lange *et al.*, 2012).

immature digestive and immune systems (Chapter 4, Pluske and Zentek, 2019), it is appropriate to feed young animals temporarily below their amino acid requirements for maximum growth performance, and to capitalise on the concept of compensatory growth, to optimise long-term animal health and productivity, as well as profitability. The latter requires that the dynamic effects of temporarily feeding animals below their amino acid requirements, as well as the extent and rate of compensatory growth,

on long-term animal performance is represented mathematically (De Lange *et al.*, 2012). It has been well established that young growing animals have the capacity for compensatory growth (e.g. Eits *et al.*, 2003; Martínez-Ramírez and De Lange, 2007; Skinner *et al.*, 2014).

16.2.3 Current state-of-the art of pig and poultry nutrient utilisation models

In general, carefully developed and well-tested nutrient utilisation models are now sufficiently accurate to represent, on a whole animal basis, utilisation and dietary requirements for bio-available energy, amino acids and phosphorus of pigs managed in a relatively stress-free environment and fed diets of varying nutrient composition (e.g. NRC, 2012). In contrast, poultry nutrition utilisation models appear not as accurate as pig models in predicting whole animal responses, especially when feeding diets with rather extreme nutrient profiles (Gous, 2007; Applegate and Angel, 2014). The latter may reflect the shortage of experimentation to explore the biology of nutrient utilisation in poultry, as well as the interactive effects of feed ingredients on the rate and extent of nutrient digestion, absorption and utilisation, which are more likely to occur in poultry than in pigs (e.g. De Lange *et al.*, 2012). Based on these considerations, further improvements in accuracy of representing nutrient utilisation for growth and reproduction are likely to come from enhanced understanding of animal-environment interactions and the dynamics of nutrient digestion, absorption and utilisation. These aspects, as well as modelling animal product quality, are addressed in more detail in subsequent sections in this chapter.

While nutrient utilisation models have become more mechanistic in representing the biology of nutrient utilisation, they have also become more complex and require more detailed characterisation of the animals and their environment for accurate prediction of animal productivity and nutrient use. Increasing model complexity does not necessarily improve accuracy of prediction and may make models less robust. For example, body protein deposition represents the net balance between protein synthesis and degradation. Given the relatively large energy cost associated with body protein turnover (NRC, 2012), it is of greater interest to represent mathematically the control of both protein synthesis and protein degradation than to simply predict body protein deposition as the difference between these two processes. However, our understanding of the control of protein turnover under varying conditions appears insufficient for a robust prediction of body protein deposition from protein synthesis and protein degradation (Moughan, 2018). Moreover, the direct prediction of whole body protein deposition appears reasonably robust under varying conditions and at various stages of growth (e.g. Schinckel, 1999). This short discussion stresses the need to carefully balance model complexity with predictive accuracy, ease of collecting data that are required model inputs, and efforts required to identify optimum animal management strategies. When developing nutrient utilisation models it is thus

becoming increasingly critical to define the purpose for which the model is to be used (i.e. improve understanding of complex biological processes vs improved accuracy of predicting animal productivity under varying conditions) and to specify how the model will be integrated with animal and environmental monitoring systems.

The accurate prediction of feed intake of individual animals that are housed in groups and exposed to a number of external stressors remains one of the main challenges in practical animal nutrition (Kyriazakis, 1999; Torrallardona and Roura, 2009; Richards *et al.*, 2010; Westerterp-Plantenga *et al.*, 2012; Chapter 3, Everaert *et al.*, 2019). This accurate prediction requires a highly detailed characterisation of the animal's performance potentials, the animal's capacities to deal with environmental constraints (including disease challenges), diet characteristics that are known to influence feed intake, as well as diurnal changes in environmental conditions and feeding behaviour. To improve understanding of the complex interactions between all animal, diet and environmental factors that affect feed intake, it is imperative that we continue to refine models that are aimed at predicting feed intake under varying conditions. However, for the application of nutrient utilisation models in practice and given the difficulty to routinely and accurate quantify all factors that affect feed intake, it appears more meaningful to accurately measure feed intake of animals managed under commercial conditions and to consider – in real-time – the achieved levels of feed intake for the optimisation of feeding and management strategies. In a similar manner, the rate of body weight gain, and even changes in body composition, may be measured in real-time to continuously calibrate nutrient utilisation models in order to match observed with model predicted performance and assess implications of model predicted changes on actual animal productivity.

During model development, the intended model users should be given careful consideration. Ideally model users should be part of the model development team, and be closely involved in defining the purpose for which the model is developed, including the specifications of model boundaries and limitations. Models, especially those intended for use in practice or in education programs, must be complemented with clear user-guides that may include case studies to enhance the user's understanding of the model and its applications.

16.3 Modelling interactions between animals and their environment

It has been well established that environmental conditions can limit animals from expressing their genetically determined performance potential, as outlined in several chapters (e.g. Chapter 4, Pluske and Zentek, 2019; Chapter 5, Bouwens and Savelkoul, 2019; Chapter 8, Babinszky *et al.*, 2019). For example, Williams *et al.* (1997) showed

that exposure of growing pigs to various diseases that are common on commercial pig units with a compromised health status can depress the rate of lean tissue (i.e. muscle) growth by as much as 30%. Animals that are exposed to pathogens generally eat less and, when standardised to a constant energy intake level, have higher ratios of body lipid to body protein deposition than healthy animals (Black *et al.*, 1995; Sandberg *et al.*, 2006). In terms of the animals' social environment and housing conditions, it has been shown that pigs managed in individual pens achieve higher productivity than pigs that are housed in groups (Chapple, 1993), and that animal productivity is reduced when floor space per animal is restricted too severely (NRC, 2012). Finally, when animals are exposed to heat stress, their levels of performance are effectively reduced, which is largely, but not entirely, mediated through reductions in feed intake (Black *et al.*, 1995; Verstegen *et al.*, 1995; Black *et al.*, 1999).

These environmental effects on nutrient utilisation and animal performance have been represented in animal performance and nutrient utilisation models in a rather empirical manner. For example, level of disease or social stress may be related statistically to effective reductions in the animals' performance potentials, while the extent of lung damage in animals at slaughter is a practical measure of 'level of disease' (Black *et al.*, 1995, 1999). The effects of heat stress and floor space allowance per animal may be related directly to changes in feed intake, and thus indirectly to nutrient utilisation and animal performance (NRC, 2012). Important limitation of these empirical relationships are that they only apply to conditions under which they were developed, and that they will not adequately represent likely interactions among the various environmental factors, as well as known interactions between environmental conditions and animal genotype (Black *et al.,* 1995; Nyachoti *et al.*, 2004). Therefore, continued efforts should be made to improve our understanding of the underlying biology to identify causal relationships between environmental stressors and changes in nutrient utilisation for growth or reproduction.

16.3.1 Modelling the impact of the infectious environment

Modelling the impact of disease on animal performance and nutrient utilisation continues to be a challenge (e.g. Sandberg *et al.,* 2006). The animal's immune system is a highly complex system that involves many tissues, cell types, as well as short- and long-term signals (Reeds and Jahoor, 2001; Colditz, 2002; Johnson, 2012). It is, however, well established that the pro-inflammatory cytokines – interleukin (IL)-1β, IL-6 and tumour necrosis factor-α – are key mediators that link inflammation to changes in nutrient partitioning from supporting animal productivity towards mounting an immune response (Rakhshandeh and De Lange, 2011; Johnson, 2012; Litvak, 2012; Figure 16.4). These cytokines have direct effects on the central nervous system as well as muscle, liver and fat tissues. These cytokines cause changes in circulating levels of factors affecting growth, such as insulin-like-growth factor-1 and insulin. They also influence, either directly or indirectly, expression of genes that

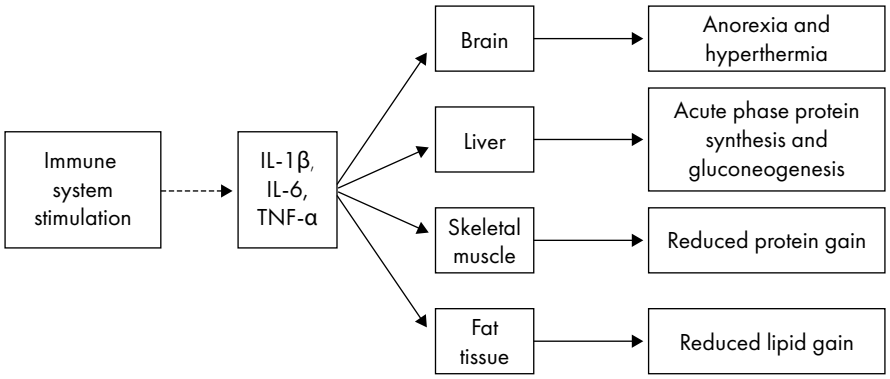

Figure 16.4. Schematic representation of the effects of immune system stimulation on feed intake and nutrient utilisation mediated by the pro-inflammatory cytokines interleukin (IL)-1β, IL-6 and tumour necrosis factor-α (TNF-α; adjusted from Reeds and Jahoor, 2001; Litvak, 2012).

reduce muscle growth, such as the myostatin gene in muscle (Escobar *et al.*, 2004). It has been shown that by manipulating the immune response of growing pigs during a disease challenge, the negative impact of disease on carcass protein can be reduced by blocking receptors to IL-1β (Dionissopoulos *et al.*, 2006). The latter indicates substantial opportunity to reduce the impact of disease on animal productivity. Also, there appears potential to use circulating levels of IL-1β as a bio-marker to predict disease-induced reduction in animal performance (Johnson, 2012). Alternatively and given the rapid dynamic changes in plasma levels of IL-1β, hepatic expression of the IL-1β gene may be used as a bio-marker. In a controlled study and when pigs were exposed to porcine reproductive and respiratory syndrome virus, feed intake, body weight gain and whole body protein deposition were all negatively correlated with circulating levels of IL-1β (Escobar *et al.*, 2004). However, in a small scale epidemiological study across a number of commercial swine operations and that were exposed to varying levels of disease, a relationship between hepatic expression of the IL-1β gene and growth performance of individual nursery pigs could not be identified (Slifierz *et al.*, 2013). A better understanding and modelling of the relationships between the immune system and the control of growth and reproduction, will lead to the identification of (nutritional) means to reduce the negative effects of exposure to pathogens (Chapter 5, Bouwens and Savelkoul, 2019) and of the known interactions between animal genotype and exposure to pathogens (e.g. Knights *et al.*, 2014). It may also lead to bio-markers for estimating the level of disease or extent of immune system stimulation in animals (Johnson, 2012).

16.3.2 Modelling the impact of the thermal and social environments

Extensive reviews have been written about modelling effects of the thermal environment on feed intake and performance of pigs and other farm animals, with a large focus on exchange of heat between the animals and their environment (e.g. Bruce and Clark, 1979; Verstegen *et al.*, 1995; Black *et al.*, 1999; Knap, 1999; Aerts and Berckmans, 2004; Yahav *et al.*, 2005; Renaudeau *et al.*, 2012). In these models, an important concept is the prediction of upper and lower temperatures to characterise the thermo-neutral zone; within this zone environmental temperature has minimal impact on feed intake and animal productivity. Exposure to thermal stress, when animals are outside their thermo-neutral zone, leads to changes in feed intake, activity levels, as well as in partitioning of retained energy between body protein and body lipid deposition or products of reproduction. In animals under cold stress, feed intake is increased until some maximum physical feed intake capacity is reached, while in animals under heat stress, feed intake is driven by the (maximum) heat loss from the animal's body to the environment. Thermal environment-induced changes in body protein and body lipid gain can be attributed largely to changes in the amount of energy available for production (i.e. energy intake minus energy requirements for body maintenance functions including temperature regulation). According to Verstegen *et al.* (1995), pigs under cold stress will slightly reduce the animal's upper limit to body protein deposition, while Black *et al.* (1999) and Knap (1999) indicate that only severe heat stress will reduce the animal's performance potential.

In these models, there has been very limited emphasis on the animals' ability to adapt to cold or hot conditions, indicative of a lack of data about the dynamic nature of the physiology of animals that are exposed to thermal stress. Recent data indicate that animals are capable of adjusting to heat stress conditions, based on the gradual recovery in feed intake following the initial rapid reduction when animals are first exposed to heat stress (Renaudeau *et al.*, 2015). Alternatively, it has been shown that, in order to maintain the effect of heat stress on voluntary feed intake, environmental temperature must be gradually increased (Kellner *et al.*, 2015). This gradual adaptation to heat stress appears mediated primarily by a reduction in circulating levels of the thyroid hormones, triiodothyronine and its prohormone thyroxine, indicating thermal environment-induced changes in the animals (basal) metabolic rate (Renaudeau *et al.*, 2015), and remains to be explored further.

Capturing the effect of the thermal environment on a mechanistic and dynamic (diurnal) representation of heat exchange between animals and their environment, will lead to a more robust prediction of the effects of the thermal environment on feed intake, animal activity, nutrient utilisation, and animal productivity (Knap, 1999; Yahav *et al.*, 2005, Renaudeau *et al.*, 2012). As mentioned earlier, such complex modelling approach should be weighed against the need for a detailed characterisation

of the animals' environment. For practical animal management it may be more effective to directly monitor the animals' response to its thermal environment by observing animal behaviour or measuring both eye temperature as a measure of core body temperature, and body surface temperature (e.g. Andersen *et al.*, 2007; Giloh *et al.*, 2012). The posture and distribution of resting animals within pens provides a stockperson with behaviour observations to assess whether animals are under cold or heat stress. Body temperature measurements can serve as objective indicators of heat exchange between the animal and its environment and may be connected directly to environmental control systems.

Group housed animals will establish social hierarchies that influence behaviours of individual animals, such as feeding and activity (e.g. De Haer and de Vries, 1993; Morgan *et al.*, 1999; Estevez *et al.*, 2007). The animals' social environment can add to stress and will contribute to between-animal variation in body weight, especially when environmental resources such as floor space or feeding space are limiting or when the thermal environment is not sufficiently controlled. In extreme situations, abnormal behaviours (e.g. belly nosing and tail biting in pigs; feather pecking in birds) may be induced, which affects animal welfare as well as animal productivity (Moinard *et al.*, 2003; Dixona, 2008). Based on a review of the literature, Chapple (1993) concluded that group housed growing pigs have lower daily feed intakes and growth rates than individually housed pigs, and that the reduction in nutrient intake only explained part of the observed reductions in body weight gain. Moreover, observed reductions in feed intake and body weight gains that are observed in group housed animals cannot be fully explained by changes in the physical environment (e.g. floor space, feeding space, thermal environment; Morgan *et al.*, 1999). These findings indicate that social interactions among animals have a direct effect on the expression of the pigs' growth potentials and, as a result, an indirect effect on feed intake. There is increasing recognition of the effect of social ranking of animals within groups on (synchronised) behaviours and animal productivity. There appear to be opportunities to mathematically represent and genetically select animals based on their (positive) effects on productivity of pen mates (Ellen *et al.*, 2014). Social stress may be mediated through stress related hormones (e.g. catecholamines and glucocorticoids such as cortisol and corticosterone; Möstl and Palme, 2002). The measurement of cortisol in various tissues and pools (e.g. plasma, saliva, faeces, hair) may provide insight in short and long term stress and even stress in specific tissues (e.g. Palme, 2012). It should also be noted that part of social stress may also be mediated by reducing the animals' ability to cope with disease stress based on compromised immune system responsiveness (e.g. Couret *et al.*, 2009).

16.4 Modelling dynamics of nutrient absorption and utilisation

Dynamics of nutrient digestion and absorption affect the rate of appearance of nutrients that are available for metabolism and may be manipulated to synchronise supply of different nutrients to the animal (De Lange *et al.*, 2013; Chapter 2, Moughan, 2019). The importance of considering the dynamics of nutrient absorption has been shown in broiler chickens (Weurding *et al.*, 2003) and growing pigs (Van den Borne *et al.*, 2007). For example, when feeding different types of starch of similar digestibility to broiler chicks, more slowly digested starch yielded higher efficiencies of amino acid utilisation for growth (Weurding *et al.*, 2003). It was hypothesised that rapid digestible starch reduced glucose supply to the lower part of the digestive tract, forcing intestinal tissue to use increased amounts of amino acids as an energy source, and reducing amino acid availability to support growth. Alternatively, dynamics of nutrient absorption may influence the animals' endocrinology (e.g. plasma levels of insulin) and, thereby, the physiological control of nutrient utilisation (Zijlstra *et al.*, 2012). Feeding frequency and feed ingredient choice may thus be used to control the dynamics of nutrient digestion and absorption and, ultimately, to manipulate post-absorptive nutrient utilisation.

Various attempts have been made to model nutrient digestion and absorption, whereby the gastro-intestinal tract has been divided in the various compartments (e.g. stomach, various segments of the small intestine and large intestine; Usry *et al.*, 1991; Bastianelli *et al.*, 1996; Bastianelli and Sauvant, 1999; Rivest *et al.*, 2000; Strathe *et al.*, 2008). In these models, the rate of passage of (undigested) nutrients between subsequent compartments is represented, as well as the rate of digestion and release of nutrients that are available for absorption. In their current form, these models are rather conceptual, and require large amounts of data to properly represent effects of chemical and physico-chemical properties of feedstuffs on rates of passage, endogenous secretions into the digestive tract, digestion and absorption. It is of interest to note that these models indicate that among the main nutrients (starch, fibre, protein and fat), fat digestion and absorption represents the biggest challenge, based on the prediction of experimentally determined fat digestibility coefficients (Strathe *et al.*, 2008). In these models, potential interactions among nutrients and feedstuffs should be considered, especially among feedstuffs with high contents of fat or soluble or fermentable fibre (e.g. Bakker *et al.,* 1995; Smits and Annison, 1996). Data required for further model development may be obtained from multi-compartmental dynamic simulation systems (Minekus *et al.*, 1995, 1999). Digestion and absorption models may be tested *in vivo* by monitoring the appearance of nutrients in portal blood (Yen *et al.*, 1989; Mansilla *et al.,* 2017). However, in that case, the high rate of nutrient metabolism in the portal-vein drained visceral organs should be carefully considered (Yen *et al.*, 1989; Burrin *et al.*, 2001).

To mathematically represent the dynamics of nutrient absorption and post-absorptive utilisation in *ad libitum* fed animals, feeding frequency and meal size must be estimated. This will require iterative mathematical procedures, as the initiation and cessation of feeding can be determined by levels of metabolites and hormones in tissues (e.g. intestinal lumen, blood, liver, brain, physical gut fill, as well as heat production and heat losses from the animal's body; Torrallardona and Roura, 2009; Richards *et al.*, 2010).

For the prediction of whole animal performance, digestion and absorption models must then be integrated with dynamic post-absorptive nutrient metabolism models that represent the exchange of nutrients between free pools in blood and intracellular space in various tissues, short term storage of nutrients (e.g. blood albumin, liver glycogen), use of nutrients for body maintenance functions or as source of energy (i.e. nutrient catabolism), conversions among nutrients (e.g. gluconeogenesis, endogenous synthesis of fatty acids and non-essential amino acids) and nutrient retention in animal products.

16.5 Modelling animal product quality

Given the increased emphasis on animal product quality, we should pursue opportunities to improve animal product consistency and manipulate various quality attributes, such as the size of dissectible muscle (i.e. meat cuts), content and fatty acid profile of fat in dissectible muscle, and where appropriate, tenderness, water holding capacity and colour, among other meat attributes.

There has been considerable effort to mathematically model the physical body composition of growing animals (Emmans, 1987; Gu *et al.*, 1992; Emmans and Kyriazakis, 1999; Gous *et al.,* 1999; De Lange *et al.*, 2003). In these models, physical body composition (i.e. gut fill, size of viscera, blood, bone, integument, dissectible muscle and fat) is predicted from chemical body composition, which is largely driven by whole body protein and whole body lipid mass. The latter implies that body water mass and body ash mass are closely related to body protein and body lipid mass and that these four chemical body constituents determine empty body mass. In these relationships, the effects of body weight, feeding regime (i.e. feeding level; time off feed pre-slaughter; feed composition, especially diet fibre characteristics), animal genotype, and possibly the thermal environment and health status on the distribution of body protein and body lipid mass over these physical body components should be considered (De Lange *et al.*, 2003). Except for extreme animal genotypes, the distribution of body protein mass over the main carcass cuts is relatively constant. In contrast, there are meaningful animal genotype and environmental effects on the distribution of body lipid over body fat depots (Rook *et al.*, 1987; Baeza and LeBihan-Duval, 2013). The latter implies, for example, that the manipulation of intra-muscular

fat (i.e. marbling) may occur somewhat independent from the manipulation of total body fatness (Hocquette *et al.*, 2010). Relationships between chemical and physical body composition deserve careful consideration in the practical application of nutrient utilisation models, especially when body fat measurements such as back fat thickness are used to assess carcass value. Obviously, the accurate prediction of whole body protein and whole body lipid deposition is a prerequisite for the accurate prediction of physical body composition, as discussed in earlier sections in this chapter.

In monogastric animals, there is a close relationship between the dietary fatty acid profile and the fatty acid composition of retained body lipid, representing opportunities to minimise the negative effects of relatively large amounts of unsaturated fatty acids in animal products, such as reduced shelf life, separation of fat from muscle tissue and reduced consumer acceptance (Wood *et al.*, 2008). At the same time, value-added animal products may be generated by increasing the content of health promoting ω-3 fatty acids (e.g. eicosapentaenoic acid [C20:5], and docosahexaenoic acid [C22:6]) in animal products (Kouba and Mourot, 2011). Various attempts have been undertaken to mathematically model the fatty acid profile in animal products (Danfaer, 1999; Lizardo *et al.,* 2002; Kloareg *et al.,* 2007). Key aspects of fatty acid utilisation may be represented in a manner that is consistent with the utilisation of dietary amino acids, as summarised by Birkett and De Lange (2001) and Moughan (1999) and shown in Figure 16.5:

1. Bioavailability (estimated as true ileal digestibility) of fatty acids.
2. The need for (essential) fatty acids for body maintenance functions, including endogenous fatty acid losses into the gut and with integument losses, synthesis of non-lipid compounds, and minimum fatty acid catabolism associated with body lipid turnover.
3. The maximum marginal efficiency of retaining absorbed fatty acids in body lipid (inversely related to the rate of inevitable fatty acid catabolism, which is considered to be low; Chwalibog *et al.*, 1992; Danfaer, 1999; Kloareg *et al.*, 2007; Martínez-Ramírez *et al.*, 2014).
4. The need to catabolise fatty acids when the supply of energy from other dietary nutrients is insufficient (preferential fatty acid catabolism; generally negligible as fat intake generally represents a small fraction of total energy intake; Kloareg *et al.*, 2005, 2007).
5. The profile of de novo endogenous fatty acid synthesis (largely palmitic acid [C16:0], stearic acid [C18:0] and oleic acid [C18:1]; Kloareg *et al.*, 2007) to achieve the target rate of body lipid deposition based on energy intake partitioning (Birkett and De Lange, 2001).

Additional considerations are:

6. The distribution of total body lipid over the various fat tissues pools.
7. The (tissue specific) conversion among fatty acids, including effects of the thermal environment on fatty acid (de-) saturation as well as the elongation and desaturation of ω-3 and ω-6 fatty acids (Kloareg, *et al.*, 2005; Martinez-Ramirez *et al.*, 2014).

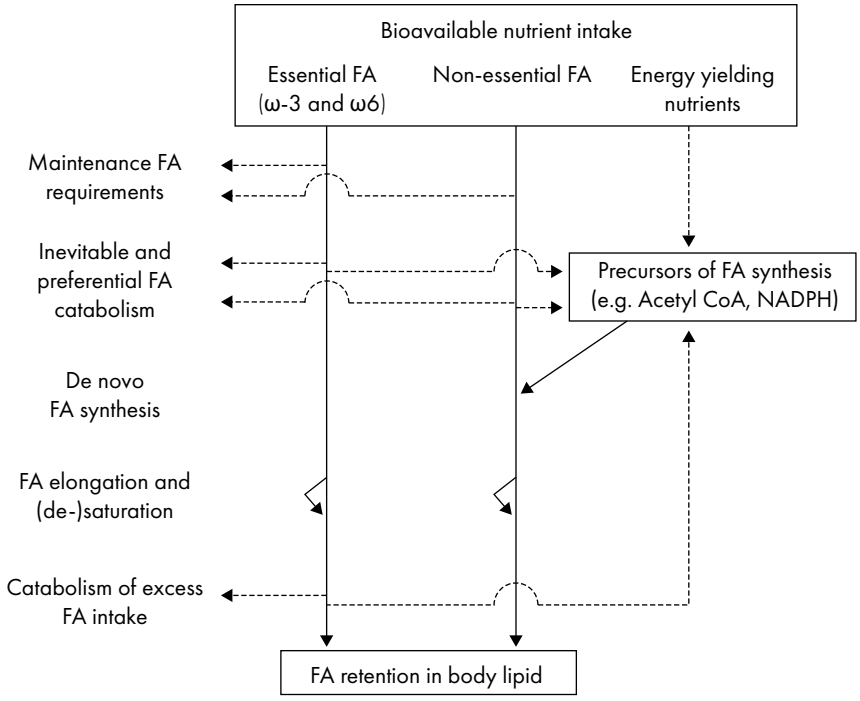

Figure 16.5. Schematic representation of key aspects of fatty acid (FA) utilisation that are required to predict the FA composition of body lipid deposition.

Combined, these aspects of fatty acid utilisation contribute to changes in the size and fatty acid profiles in the various fat tissues and body lipid pools. For the prediction of fatty acid profiles within specific fat tissues, changes in the relative contribution of structural lipids (e.g. phospholipids) and storage lipids (i.e. triglycerides) and the difference in lipid turnover between these fractions may be considered as well (Kloareg *et al.*, 2007). Animal and feed effects on most of these aspects of fatty acid utilisation remain to be quantified, and require the application of a combination of mass-balance and isotope tracer studies (e.g. Chwalibog *et al.*, 1992; Bruininx *et al.*, 2011; Martinez-Ramirez *et al.*, 2014). It is, however, of interest to note that there appears to be negative feedback from end products on enzymes controlling elongation and desaturation of ω-3 and ω-6 fatty acids, in addition to competition between ω-3 and ω-6 fatty acids for these metabolic pathways (Martinez-Ramirez *et al.*, 2014). This has important implications for feeding strategies to manipulate the content in consumable animal products. The mathematical representation of the dynamic accumulation of docosahexaenoic acid and eicosapentaenoic acid in consumable animal products remains to be fully explored.

As the need for improving consistency and quality of meat products continues to improve, the demand for decision support systems to manage meat quality will increase (Ouali *et al.*, 2006; Huff-Lonergan *et al.*, 2010), including relatively simple means to monitor or predict various aspects of meat quality (e.g. Brondum *et al.*, 2000; Vermeulen *et al.*, 2015). The conversion of muscle to meat is a complex process (Ouali *et al.*, 2006; Simons *et al.*, 2006; Faustman *et al.,* 2010; Huff-Lonergan *et al.*, 2010) and involves a number of physiological and biochemical processes that are influenced by muscle characteristics (e.g. muscle fibre types, contents of fat, connective tissue and glycogen), pre-slaughter management (e.g. feeding regimen and animal handling) and post-slaughter management (e.g. cooling, electrical stimulation, ageing, injection of salts). For example, muscle glycogen stores and the amount of stress imposed prior to slaughter will affect the rate of anaerobic muscle glycolysis which yields primarily lactic acid. Lactic acid accumulation in muscle will directly affect muscle pH, which in turn impacts directly the denaturing of muscle proteins, and indirectly muscle colour and water holding capacity. Moreover, proteolytic enzymes such as cathepsins and calpeins that are present in muscle may be activate around the time of slaughter and improve muscle tenderness by degrading some the myofibrillar structures (Huff-Lonergan *et al.*, 2010). In addition, oxidative stress in muscle will contribute to muscle protein denaturing and fat oxidation, which can lead to unfavourable meat colour and flavours (Faustman *et al.*, 2010). Largely because of these many interacting variables, as well as known animal genotype effects on meat quality (e.g. Suzuki *et al.*, 2005), there are opportunities to integrate our knowledge of processes involved in converting muscle to meat to optimise handling of animals, carcasses and meat around the time of slaughter in a quantitative manner (e.g. Thompson, 2002; Simons *et al.,* 2006). For example, Simons *et al.* (2006) discussed the development of a predictive meat quality model that can be used to calculate quantitative measures of meat quality outcomes – tenderness, colour, colour stability and water-binding capacity – from any choice of processing options. In that model the focus is on characterising changes in muscle temperature and pH in the immediate post-slaughter period.

16.6 Future perspectives

Tremendous progress has been made in improving our understanding of the biology of nutrient utilisation for production in pigs, poultry, and other farm animals. This allows reasonably accurate predictions of nutrient requirements as well as feed intake and performance responses to varying dietary nutrient levels when animals are managed in a relatively stress-free environment. These predictions, however, require an accurate characterisation of available nutrient levels in the diet, animal performance potentials in aspects of nutrient partitioning and, in cases of predictions of feed intake, an accurate characterisation of the animals' environment. Given the continued improvements in the animals' genetic performance potentials, there is a continued need to characterise the animals and interactions with their environments.

The quantitative representation of the impact of the animals' infectious environment and social stressors on nutrient utilisation remains a challenge, especially as there are animal genotype effects on the animals' ability to deal with these stressors. When models are used in practice to assess the environmental and financial impacts of alternative animal management strategies, then these environmental stressors should be considered, as well as effects on animal product characteristics that affect animal product value (e.g. carcass yield, carcass muscle content, fat content and quality, and sensory characteristics).

A key limitation of effectively applying nutrient utilisation models in practice is the time lag between obtaining data required as model input (i.e. characterisation of diets, animals and their environment) and implementing changes to animal (feeding) management. Currently and when nutrient utilisation models are used in practice, optimal management strategies are generally based on historical rather than current performance, or on predictions for data required as model inputs (e.g. considering seasonal changes and trends). Ideally data collection, data processing and identification of the optimal management strategies should be conducted in real-time and be highly responsive to changes in economic and environmental conditions. In this context the advancement in sensors and robotics presents real opportunities (Frost *et al.*, 1997). Sensors may be used for real-time monitoring of feed, animals and their environment, while robotics may be used for preparation and delivery of feed and managing the animals' environment. These systems may be integrated with nutrient utilisation models and serve as decision support systems for real-time animal management, i.e. precision animal management. Examples of development toward such integrated systems are precision feeding of growing-finishing pigs (Pomar *et al.,* 2011) and controlled feeding of broiler breeder pullets (Zuidhof, 2014). An additional advantage of such systems is that they may reduce the need for a highly detailed monitoring of the animals' environments. If sensors are capable of accurately monitoring feed intake and animal performance (e.g. rate and composition of body weight gain) for real-time integration of performance monitoring with nutrient utilisation models, then this reduces the need for modelling the impact of these external stressors on feed intake and animal performance. In other words, nutrient utilisation calculations may be based on 'operational' animal performance potentials and feed intakes that are influenced by the animals' environment, rather than based on the true genetically determined animal performance potentials and a quantitative understanding of the effect of environmental stressors on expression of the animals' genetic performance potentials. In the future, physiological measures such as circulating levels of cytokines, other indicators of immune system stimulation and stress, key growth regulating hormones such as insulin-like-growth factor-1, or even circulating levels of RNA to monitor expression of a large number of genes and in various tissues (Choi *et al.,* 2014) may be used as bio-markers and integrated in real-time decision support systems. As an example, for the management of dairy cows, robots are already available for automated milking and feeding; these systems are equipped with sensors to measure

the amount of milk that is being produced as well as monitoring levels of a selected numbers of biomarkers in milk including lactate dehydrogenase to estimate somatic cells counts in milk (mastitis) and β-hydroxybutyrate to estimate ketosis (DeLaval, 2015).

During the further development of nutrient utilisation models there will be continued debate about the optimum level of complexity, i.e. the level of detail in representing nutrient utilisation within individual tissues and the coordination of nutrient use across the main tissues involved in nutrient utilisation (i.e. gut, liver, muscle, fat). In this debate, the purpose for model development should be clearly stated, as well as the amount of detail required to obtain data that serve as model inputs. It can be argued that for the accurate prediction of whole animal performance there is no need to accurately represent nutrient utilisation in individual tissues; in that case models should merely represent the control of nutrient utilisation at the whole animal level and the representation of the coordination of nutrient utilisation among tissues leads to unnecessary complexity and a loss of model robustness. When the purpose of the model is to gain a better understanding of nutrient utilisation and to support orchestration of research efforts, then it is more appropriate to mathematically represent nutrient utilisation in individual tissues and the accurate prediction of whole animal performance is less critical.

The rapid development in computing power and advancement in mathematical or statistical methods should be considered as well. For example, when animal production cycles are relatively short and environmental conditions can be closely controlled, as is the case in broiler production, the use of advanced mathematics or statistics can be used to relate key factors to animal productivity. In various instances it has already been shown that approaches such as central composite design, response surface methodology and (self-organising) artificial neural network-genetic algorithms can be used effectively to interpret results of multi-factorial broiler experiments that involve a large number of experimental treatments (e.g. Mottaghitalab *et al.*, 2010; Ahmadi and Golian, 2011). It should be noted, though, that in these studies the established relationships are of empirical nature, and apply only to the conditions under which these relationships were developed. It remains to be demonstrated that these approaches can be used to integrate information obtained across experiments that explore different variables (e.g. diet composition vs environmental conditions), or that self-learning algorithms will be effective in analysing complex and continuously changing animal and environmental conditions that are observed on commercial animals units, in order to function as part of effective decision support systems for optimising animal management.

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Future technologies in pigs & poultry nutrition

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Summary points

- The economic room for technologically and nutritionally more advanced diets will increase in the future.
- Currently, feed mills have a large diversity of equipment and systems.
- On-farm processing of feed and purchase of concentrates will likely become more important in the future.
- Other ingredients and processing technologies are becoming more important.
- A new business model may emerge called 'sustainable precision husbandry'.

Keywords: economics, particle size reduction, mixing, pre-conditioning, pelleting, ingredients, formulation, fermentation, enzymes, sensors, analytical methods

17.1 Introduction

When an animal is fed a nutritionally well balanced diet performance is optimal. This is reflected in a healthier animal with a better feed and mineral conversion (in particular nitrogen and phosphorus) and thus a decreased environmental footprint. Traditionally in large part of the world, the compound and premix industry provides the farmer with the whole or a part of the nutrients to meet the animal's requirement. In 2016 the world compound feed production exceeded 1 billion Mg (IFIF, 2017). The total amount of feed fed to farm animals is larger as can be seen in the split within

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the EU28. In 2016 in the EU-28 480 million Mg was fed to animals. The largest part, 50% million Mg, consisted of on farm roughage production and about 10% originated from home grown cereals. Industrial compound feed accounted for about 30% (158 million Mg) and 10% are feeding stuff directly purchased by farmers (FEFAC, 2017).

In Figure 17.1, the feed nutritional pyramid is given. At its most basic level, the farmer feeds his animals with the feed ingredients directly available to him. This very rudimentary form has become scares worldwide. In many parts of the world the feed is at least supplemented with straights (i.e. protein rich sources) and a concentrate with the right enzymes, vitamins and trace minerals. When it is possible to include significantly more macro-ingredients of different origin (10-20) in the feed, a far more nutritional and optimal diet can be designed for an animal. At the top of the pyramid is the processing of the right mixture of nutritional ingredients in such a way that optimal animal performance is achieved. The current base line for feed processing is described in the next pages.

So, why are currently not all animals nutritional optimally fed? This has to do with economics. Edible biomass produced by the farmer are often the cheapest feed ingredients available due to the lack of transportation costs and margin loss involved with any trading transaction between parties. Transport costs are for example approximately 2-4 euro per Mg of feed for one 100 km distance using a truck (Western Europe example). Transportation of ingredient for feed by road is much more expensive than transportation by water. The proximity of a harbour has a positive effect on the availability of different types of raw materials for feed manufacturing due to lower costs. Animal feeds in countries having harbours and internal waterways like The Netherlands or South Korea, are based on different feedstuffs from all over the world. In contrast, diets in countries like for example Canada, USA or Brazil are based on more local available raw materials. A nutritional more optimal feed first has to compensate for transport and other necessary costs involved, before it becomes economically more attractive to the feed producer or farmer. Traditionally, the farmer is focused on the feed costs per unit of output. Recently, also impact costs such as the health status of a farm (pressure to reduce antibiotic usage) and the environmental

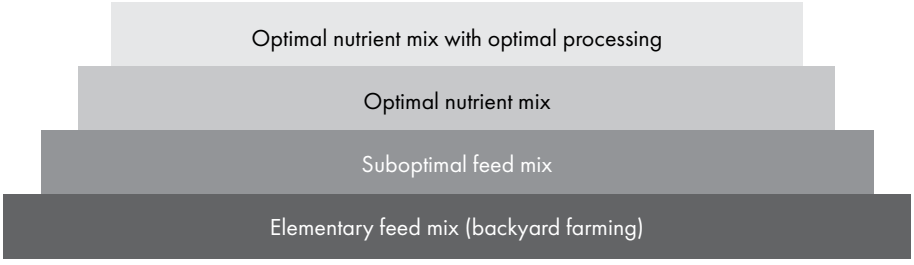

Figure 17.1. The feed nutrient pyramid.

impact (i.e. nitrogen and phosphorous emissions) have gained in importance. On the other hand, making the technological and nutritional most optimal diet also involve costs for equipment, energy, research and human labour. Overall, economic considerations dictate the feasibility to manufacture technologically more advanced diets. If the economic benefit is larger than the costs involved, there will be more room to involve technological treatments. In a general equation:

$$\%\ \text{improvement} \times (\text{feed price} + \text{impact costs}) > (\text{technology costs} + \text{transport costs}) \quad (1)$$

Most predictions are that feed prices will increase in the future. Since the improvement in the feeding value of diets can be expressed as a percentage of the total feed, the absolute economic gain increases with increasing feed price. If for example a diet is 2% more efficient, the economic impact doubles when feed prices double. Impact costs related to the environment and animal health are likely to increase in the future in many parts of the world.

Technology costs on the other hand are independent of raw material feed costs. For standard compound feed processes (generally consisting of grinding, mixing and pelleting), the direct costs for energy, capital and labour are approximately equal. For compound feed production, the processing costs without sales, transport and other indirect costs are currently for Western Europe in the range of 15-20 euro/Mg of feed. When energy prices increase, not only transport and processing costs are affected, also the raw material feed prices are affected by higher fertiliser, agrochemical and diesel costs. The influence of energy is, therefore, represented on both sides of Equation 1. The authors note, that prices differ greatly when considering other countries in the world. We conclude that the economic room for technologically and nutritionally more advanced diets will increase in the future.

Currently, the processing of animal feed has a number of beneficial effects including:
- homogenous mixture of feed ingredients (i.e. pellets versus meal);
- decrease in anti-nutritional factors (i.e. lectins, protease inhibitors, glucosinolates);
- increase in feed safety (i.e. deactivation of (micro)organisms);
- increase in nutrient absorption (i.e. increase feed efficiency);
- increase in feed intake (i.e. pellets versus meal, less spoilage);
- balance between efficiency and animal health (i.e. fine grinding versus coarse particles in relation to stomach ulcers for swine).

There is a large diversity of manufacturing flow diagrams, equipment and systems in feed mills. and this diversity is already present for each different unit operations: in the case of particle size reduction, there is a large choice in special equipment and the way the different steps are shaped into a grinding system. For agglomeration e.g. these may vary from having large flexibility in more basic production of different feed

forms, up to applying sophisticated equipment (expanders, compactors) to improve nutrient digestibility to have a strive towards high quality feeds.

In the next paragraphs, the current baseline of how to achieve these objectives is described in more detail. For a recent overview on the effects of processing on the different nutrient levels and its digestibility, the reader is referred to Van der Poel *et al.* (2017).

17.2 Base-line technologies

17.2.1 Particle size reduction

After a grinding process, diet ingredients have a certain fineness which is the result of the combination of mill type and ingredient stress. Motivations for a reduction in particle size may be different. Above all, it has the objective to improve feed efficiency. One general accepted fact is that a finer particle size increases the particle surface area to aid digestion by a better accessibility of enzymes. In addition, in compound feed manufacturing the downstream mixing process will show an improved uniformity of mixed products when using ground ingredients. Finer particles also facilitate the pelleting process by an easier uptake of moisture by the particles (Mania *et al.*, 2004). Particle size reduction can also cause problems with respect to the fineness of feeds where feed particles that are too fine are associated with higher energy costs, feed bridging in silos and respiratory diseases and gastric ulcers in animals (Alaviuhkola *et al.*, 1993). Currently similar general issues are experienced now as those reported 20 years ago (Behnke, 1996) when discussing cereals and mixed feed particle size and its effect on animal performance, both with respect to diets for poultry and pigs. In addition, health aspects still have to be taken into account to prevent diseases and gastric ulcers.

Nowadays, an increased focus is placed on more coarse diets for poultry. This may offer interesting perspectives to the poultry feed industry since coarse diets affect the development of the gastrointestinal tract and, as a consequence, the reflux phenomena, which has been indicated to have a large role in the nitrogen recycling (Kwakkel *et al.*, 2014). Coarser diets result in a higher efficiency of nitrogen utilisation and in a shift in the volatile fatty acid profiles in the gastrointestinal tract and are likely to improve the bird's gut health.

But optimising the particle size distribution with respect to nutrition means optimising the coarse and fines fractions and will impact on the energy input for milling operations. On one hand, a coarser grind is sometimes used as the pelleting process (agglomeration) which is often the next step in the feed processing process

also will decrease the size of particles. Furthermore, the fines fractions in laying hen diets are not desired and can be decreased by blending with liquid ingredients or the separate pelleting of this fraction. Therefore, the evaluation of feed particle size in view of its nutritional potency should take consideration of both grinding and other shear processes such as pelleting.

Inherent to these developments, modern measuring methods are replacing the well-known and often applied dry sieving procedures to evaluate the distribution of particles in a dry or wet medium. For example, if digestion processes are thought to have a better relationship with wet sieving procedures, wet sieving procedures may be applied. But in general, time is an important factor, where now advanced and rapid methods for particle size distribution are used such as NIR, laser diffraction, etc. Goelema (1999) discussed the wet sieving methodology as having perhaps a better resemblance with the animal's digestion processes. With wet sieving, mean particle sizes were shown to be lower where contrasts between different processing treatments were larger (Robertson *et al.*, 1984). Moreover, the distribution of nitrogen over the different fractions also differs substantial between the two sieving methods (Table 17.1).

Finally, it is the grinding equipment that has to decrease the particle size and is, together with up- and downstream equipment, to assure efficiency in feed conversion. Both hammer mills, roller mills and multicracker systems are available, all of which

Table 17.1. Effects of pressure toasting of faba beans on the nitrogen distribution over different particle size classes (Goelema, 1999).[1,2]

Sieve fraction (μm)	Dry sieving		Wet sieving	
	U (%)	T (%)	U (%)	T (%)
<71	30.0[a]	24.7[b]	66.3[a]	41.4[b]
71-160	5.3	8.8	0.5[a]	15.6[b]
160-315	5.4	5.0	0.7[a]	2.6[b]
315-630	11.3[a]	11.8[b]	3.1[a]	6.4[b]
630-1,250	33.9[a]	37.0[b]	12.5	16.4
1,250-2,500	14.1	12.7	16.6	17.4
>2,500	0.0	0.0	0.3	0.1
SEM	7.96	8.48	4.95	6.77
D_{50}	311	338	6[a]	123[b]

[1] Values within the type of sieve with different superscripts indicate significant differences ($P<0.05$).
[2] D_{50} = particle size median value; U = untreated; T = toasted under pressure.

have different modes of action by which the size reduction of ingredients is achieved. Hammer milling may result in finer particles and a smaller decrease in volume weight for wheat and barley compared to roller milling (Laurinen *et al.*, 2000). The multicracker system is a concept in which the particle size of diet ingredients is reduced by a cracking action of two rows of discs instead of being ground or rolled between two drums (Thomas *et al.*, 2012). These authors showed that in coarse grinding, maize, wheat and whole soybeans show a different grinding behaviour, with soybeans having the highest specific mechanical energy needed at the highest mean particle size. Overall decreases in particle size were small to modest (Thomas *et al.*, 2018).

Finally, it has to be remembered that there are differences in the way pigs and broilers are fed around the world, with large variations in ingredients that are used. For example in broiler diets, corn/soybean combinations are often used in USA/Canada where wheat is an important diet ingredient in European diets.

17.2.2 Mixing

Mixing is an important basic operation where uniformity has been described as one of the most important quality aspects of animal feed production. The relevance of the recommended uniformity standard, however, was shown by the experiments of McCoy and co-workers where it was shown that diet uniformity is an important factor but where coefficients of variation as large as 20% were shown adequate for maximum growth performance of broilers (Behnke and Beyer, 2002).

As for the mixing equipment, ribbon mixers and paddle mixers have a different way of moving particles where the paddle mixer fluidises the mixture in a very short time enabling also the proper addition of liquids into this mixer type, both as main mixer and as mixer of pellets and liquids. Especially nowadays, more than one liquid feed material has to be distributed over pellets and kibbles, especially the heat-sensitive additives (i.e. lysine, enzymes) by post-agglomeration coating (Engelen and Van der Poel, 1999) or by vacuum coating principles (Lamichhane *et al.*, 2015).

The mixing process is vital for the production of a homogeneous particle distribution of uniform mixtures, pre-mixtures or animal feeds. Motivations for mixing are the production of a uniform, consistent dry feed mixture where distinct differences can be observed between the manufacturing of compound feed, where the focus is on time of mixing and the manufacture of pre-mixtures, where the focus is more on homogeneity. The right mixer type and concomitant mixing time are important determinants for mixing quality. Again, much variation still exists in the standards used in the pre-mixture and feed industry to evaluate the uniformity of nutrients and feed additives. For example, when considering the evaluation of mixing uniformity based on the analysed levels of a marker and its coefficient of variation after mixing, the rating according to different references differs substantially (Table 17.2).

Table 17.2. Homogeneity rating according to different standards (L. Clasadonte, personal communication).

Standard	Product dosage	Coefficient of variation (%)
Mixer manufacturer	100 pm	<5
EU Regulation	1 to 500 mg/kg	<20
American Feed Association	not defined	<10
Feed company	all feed additives	<15
Behnke and Beyer (2002)	not defined	<10

Where a mash mixture is subject to segregation e.g. during transportation, this is the primary cause of reduced feed uniformity. This segregation is often avoided by pelleting the diet but in some cases, still mash diets are being fed to animals. Axe (1995) published an overview about mixing practices, mixer equipment, handling excesses and ingredient characteristics, all affecting segregation of mixtures and all required to control mixing uniformity.

In practice, this control in the feed industry includes especially feed additives/premixtures in addition to compound feeds, since it also has to control the problem of carry-over of critical additives. In Table 17.3 data are shown on the carry-over of critical additives in Dutch feed mills (Van der Poel, 2008). The mean carry-over of critical additives at the grinding/mixing line in 15 production locations was 3.1% (min. 0.8% and max. 6.4%) and of the pelleting line 2.5% (min. 0% and max. 4.9%). For the combined lines, the carry-over is thus 5.6%. For the separate process of post-pelleting mixing, the mean value of the carry-over during bulk blending was 2.0%. Except for the carry-over of drugs, the uniformity of mixing is also important for the effectiveness of the product. The uniformity of mixing was measured by using either the manganese/crude protein method or by using microtracers as markers; these were measured in a number of samples drawn after the mixing process (Van der Poel, 2008; Table 17.4).

Table 17.3. Carry-over percentages (mean ± SD) in the grinding/mixing line, the pelleting line and during bulk blending in Dutch feed mills in 2008.

Number of locations	Grinding/mixing	Pelleting line	Bulk blending
n	15	20	4
Carry-over (%)	3.07±1.76	2.48±1.56	2.00±1.24

Table 17.4. Mean (± SD) coefficient of variation as a parameter of uniformity of mixing of compound feed in Dutch feed mills using 2 different markers.

Number of locations	Marker		All locations
	Manganese & crude protein	Micro-tracers	
n	11	5	16
Uniformity (%)	3.45±2.33	5.04±1.83	3.95±2.41

In Germany, the standard for a good mixing uniformity was set to <7% (Kirchner, 2007).

Therefore, relative to the known measures to avoid segregation and its contamination, the order of addition of some additives in batches of mixed feed ingredients is critical.

17.2.3 Pre-conditioning/pelleting

For the manufacture of diets for poultry and pigs, pelleting has been the major process in agglomeration. Motivations for pelleting compared to feeding mash diets can be of technological origin such as efficient transportation, better flow properties and having less dust. Furthermore, animal related reasons are the fixation of the mixing ratio that is preventing segregation of particles. Compared to mash diets, pellets have a better flowability and under the practical situation of feeding, feed wastes are lower and no selection can occur. Early works on pellet agglomeration roughly cover the same issues (Behnke, 1996).

As for the effects on digestibility and performance of pigs, numerous studies have revealed the positive effects of pelleting. Early studies of Vanschoubroeck *et al.* (1971) and Andela (1986) showed positive effects on feed intake, growth of animals and showed remarkable data for improved feed conversion. These effects may have different causes such the quantity of spilled feed, segregation or effects on nutrient digestibility. Moreover, pellet quality and pellet size have to be mentioned (Hanrahan, 1984; Van den Brand *et al.*, 2014). Vanschoubroek *et al.* (1971) concluded from a thorough literature overview positive effects of pelleting for the digestion of crude protein, crude fat and organic matter. The absolute improvement of the latter dietary fractions in terms of faecal digestibility was 1.8, 6.8 and 2.0%, respectively. The improvement in fat digestibility as a result of pelleting has also been found in other studies as well. The most striking effects in pigs with respect to performance are presented in Table 17.5. It should be noted, that these trials have been performed under the so-called 'antibiotics base-line' (antimicrobial agents present in feed) and

that the improvements by pelleting were confirmed by other authors e.g. Chae and Han (1998). More recently, the pelleting pig feeds gave rise to a higher gain-to-feed ratio in weanling pigs when cold pelleting equipment was used to make pellets of 6 mm in diameter (Ulens *et al.*, 2015). Feeding the pelleted diets improved growth performance as well as a reduction of dust in the barn was observed., reason to think that the pellet coating may be advantageous for pig health.

Lundblad *et al.* (2012) examined the conditioning process prior to agglomeration and concluded that hydro-thermal processing improved gain/feed in piglets compared to when a mash diet was fed. It showed that specific nutrients were affected differently depending on the conditioning: protein by conditioning/pelleting and starch by extrusion/expander-conditioning. The latter processes can be classified as high-shear conditioning and its effects make sense with respect to starch and its gelatinisation (Table 17.6). This type of high-shear conditioning was earlier described by Behnke (1996) who already the interrelationship between ingredient properties, fineness of grind and the actual conditioning/pelleting process was justified. Accordingly, the ingredient properties that contribute to the pellet quality in terms of durability and hardness have been the subject of study (Wood, 1987; Thomas *et al.*, 1998).

Simple processing systems have been recognised such as a concept known as 'cafetaria-type' diets (Ammann, 1989). In this concept, separate ingredients or parts of the compound feed are processed into pellets where the final pig diet is established by mixing the pellets of the various 'ingredients' in the right proportions together. This type of processing attunes the core processes of grinding and pelleting to individual feed materials and was shown to have similar performance of 30 kg pigs for growth and feed efficiency (Van der Poel *et al.*, 1997) as compared to the same feed where the same ingredients were ground, mixed and steam-pelleted, respectively. However,

Table 17.5. Effect of pelleting on feed intake, weight gain and feed utilisation in fattening pigs (Vanschoubroek *et al.*, 1971).

Feed provision	No. of trials	No. of pigs given pellets[1]	Performance with pellets compared to mash (%)[2]		
			Daily feed intake	Daily weight gain	Feed utilisation
Unrestricted	79	1,148 (75)	96.9±8.4	107.2±8.7	90.6±8.2
Restricted	26	1,064 (25)	99.0±3.7	104.4±4.0	94.9±3.9
Unknown	27	461 (17)	99.8±6.2	107.1±10.6	93.6±6.2
Total	132	2,673 (117)	97.9±7.3	106.6±8.5	92.1±7.4

[1] Values in parentheses are the number of trials in which the number of animals is known.
[2] Per cent of that of mash; SD, standard deviation.

Table 17.6. Effects of steam conditioning at low or high temperature, expander conditioning and extruder processing prior to pelleting on apparent ileal digestibility of main nutrients and total lysine in pigs (Lundblad *et al.*, 2012).

Nutrient	Control	Hydrothermal treatment[1]					Contrasts (*P*-value)[2]	
		C/P L	C/P H	EXP	EXT	SEM	1	2
Dry matter	0.792	0.795	0.792	0.789	0.795	0.005	0.780	0.401
Starch	0.978	0.986	0.986	0.985	0.984	0.001	0.001	0.142
Phosphorus	0.541	0.531	0.529	0.535	0.555	0.020	0.852	0.897
Crude protein	0.838	0.830	0.839	0.823	0.843	0.001	0.782	0.477
Total lysine	0.866	0.878	0.871	0.887	0.894	0.006	0.006	0.031

[1] C/P = conditioning/pelleting; EXP = expander processing; EXT = extrusion; H = high shear; L = low shear.
[2] Contrast 1 = control vs treatments; 2 = pelleting processes (C/P) vs high-shear processes.

the practical implication was hampered by the variation of the individual quality of pellets, use of liquid ingredients, inclusion of pre-mixtures, different pellet densities, segregation, etc.

It has also been investigated whether a pig diet composed of ingredients and subsequently pelleted, results in differences in nutrient digestibility. To explore this, different set-ups for processing were examined; the ingredients were: (1) just mixed; or (2) ground/steam pelleted; or (3) processed as whole pellets with an expander (high or low mechanical shear) and then pelleted. The nutrient composition of the diet was (g/kg as-is): crude protein 211, crude fat 75, crude fibre 85, moisture 110, ash 66 and starch 220. The ingredient treatments were evaluated in young piglets (6 piglets/treatment) for apparent ileal digestibility (Table 17.7 and 17.8). Both the ileal digestibility of dry matter and nitrogen was the highest for the pellets that were just mixed. In this type of studies it is difficult to explain the contribution of the separate ingredients to the results. It indicates, however, that the interaction between primary (ingredient pelleting) and secondary processing (agglomeration of ground pellets) may exist and should be taken into account when formulating diets. One constraint of course is knowledge of these interaction per feed material. Salazar Villanea *et al.* (2017) explored this interaction and demonstrated that the negative effects of toasting of rapeseed meal on the protein digestibility in pigs was improved by subsequent pellet of extrusion of the diet in which this rapeseed meal was included.

For poultry, pelleting effects are not always positive and several reports have been described with no or negative effects of the conditioning/pelleting process (Behnke and Beyer, 2002; Lundblad *et al.*, 2012; Abdollahi *et al.*, 2013). This is related to, amongst

Table 17.7. Ingredient composition of the experimental pig diets composed of 7 different pellets (pellet diameter in mm between brackets).

Diet ingredient[1]	Inclusion level	
	% in feed	% in complementary feed[2]
Beet pulp pellet (11)	10	
Maize gluten feed pellet (5)	15	
Sunflower seed meal pellet (11)	15	
Animal meal pellet (5)	8	
Wheat middlings pellet (9)	15	
Common beans pellet (7)	15	
Rest pellet (5)	22	
Maize	16.05	73.0
Soybean oil	4.0	18.2
Limestone	0.6	2.7
$NaHCO_3$	0.15	0.68
L-lysine-HCl	0.2	0.91
Pre-mixture vit./min.	1.0	4.5

[1] All pellets were purchased from commercial suppliers except the rest pellet.
[2] Ingredients of the complementary feed ingredients brought together in one pellet.

Table 17.8. Effect of processing of pelleted ingredients (agglomeration or just mixing) on its nutrient digestibility in pigs.[1]

	Ground, mixed, steam- pelleted	Pellets, just mixed together[2]
Pellet quality		
Diameter (mm)	5	5, 7, 9 and 11
Hardness	7.5 ± 1.5^{a}	18.1^{b*}
Durability	80.0^{a}	75.7^{b*}
Ileal digestibility (%)		
Dry matter	54.7±4.9	60.0±3.9
Nitrogen	59.0±5.2	64.3±5.2
Crude fat	78.4 ± 2.3^{a}	85.1 ± 2.6^{b}
Neutral detergent fibre	17.2 ± 7.7^{a}	32.9 ± 6.9^{b}

[1] ^{a,b} means with different superscript letters are statistically significant ($P<0.05$).
[2] * = calculated average of hardness (range 5.1 up to >50 kg) and durability (range 41-98%).

others, the particle size distribution of the feed with positive effects of larger particles in the overall development of the digestive tract, especially the gizzard. Pelleting decreases particle size by its shearing action of the rollers/die. The larger particles associated with the feeding of whole seeds improve the performance and feed/gain of broilers (Svihus, 2011). Other methods of conditioning are pre-conditioning with a BOA compactor (Erwann *et al.*, 2003) or double pelleting. In principle, these are all methods that try to balance heat and moisture with the objective to bind and modify particles, eventually to eliminate pathogens with the ultimate goal of improved pellet quality.

Abdollahi *et al.* (2013) evaluated the pelleting process for poultry and described the process as being important in the manufacturing of animal feeds for its primary effects on nutrients, the interaction with the intestinal tract and, therefore, animal performances. It was shown that pelleting exerted beneficial effects for nutrients such as starch and proteins, but those effects were relatively small (Abdollahi *et al.*, 2013).

Larger effects have been observed for extra toasting of pig feed ingredients and the effect of pelleting on a whole diet. In both cases, these heat treatments gave rise to detrimental effects when the reactive lysine content as a result of the Maillard reaction was analysed. These data are presented in Table 17.9 (Van Rooijen *et al.*, 2014; Hulshof *et al.*, 2016). The same treatments also gave rise to a substantial increase in Maillard reaction products where, for example, in pelleting, both steam conditioning temperature and the used pellet die affected Maillard reaction products originating from amino acids; these Maillard reaction products being fructoselysine and carboxymethyllysine and furthermore the cross-linked amino acid lysino-alanine.

Table 17.9. Total and O-methylisourea (OMIU) reactive lysine contents in (un)processed ingredients for pig feed and dog food.

Species	Total lysine	OMIU-reactive lysine
Pigs (n=1)[1]		
Soybean meal	6.3	6.0
HT-soybean meal[3]	4.6	3.7
Rapeseed meal	5.6	5.0
HT-rapeseed meal	4.3	3.1
Dogs (n=3)[2]		
Pelleted food	10.7±0.1	9.7±0.1

[1] Values in g/100 g crude protein (Hulshof *et al.*, 2016).
[2] Values in g/kg DM (Van Rooijen *et al.*, 2014).
[3] HT = re-toasted in presence of lignosulfonate.

Much more complicated is the effect on non-starch polysaccharides (NSP) as influenced by feed manufacturing. One objective in the future is the development of technologies to enhance the degradability and digestive utilisation of fibre-rich feed ingredients. Current feed processing methods may affect the degradability of soluble NSP, but hardly affect the recalcitrant fibre fractions as found in maize and oilseed/oilseed by-products. The use of specific enzymes, targeting these recalcitrant NSP may be of interest especially when saving amino acids is accompanying a positive enzyme effect (De Vries, 2012; Pustjens *et al.*, 2014).

Raising the subject of pellet quality, it was Briggs *et al.* (1999), who reminded us that producing good quality pellets is sometimes thought as an art rather than science by many mill operators. As indicated above, ingredients strongly affect pellet quality (Wood, 1987; Thomas *et al.*, 1998; Briggs *et al.*, 1999). Ultimately, it is a balance between nutrient availability and physical quality of pellets that is critical in the more practical guarantee to deliver good quality pellets to the farmer and for an optimal animal performance. As such, pellet control technology, therefore, has to start with the very important procedure for conditioning since conditioning is vital for the final agglomeration of particles (Váhl, 1994; Thomas, 1998) and to obtain a durable pellet.

17.2.4 Extrusion/universal pellet cooker

Extruder technology for the manufacture of feeds for pets and livestock animals is based on the idea of forcing mixed, heated diets/diet ingredients through a die with a design specific to the feed and cutting the feed to a specified size by a rotating knife. Animal feed products manufactured using extrusion usually include dry and semi-moist pet foods, fish diets as well as piglet feeds.

The technique of extrusion is important to be able to produce diets that improve the performance and/or health of livestock and pet animals. For the extrusion process, raw materials are first ground to a correct particle size. The dry or additionally moistened mixture of ingredients is then passed through a pre-conditioner, in which other ingredients are added depending on the target product: liquid sugar, fats, meat products or water. Steam is injected to start the cooking process, and the preconditioned mixture (dough) is then passed into the extruder. The extruder consists of a large rotating screw, tightly fitted within a stationary barrel, at the end of which is the die. The extruder's rotating screw forces the dough towards the die, through which it then passes. For the required residence time, a length/diameter ratio is desired of about 12-16.

The extruder enables the production of animal feeds via a continuous, efficient system, that should ensure uniformity of the final product. This is achieved by controlling various aspects of the extrusion process such as screw speed, specific mechanical energy, product temperature and pressure at the die. It has also to enable the further processing of the extruded kibble such as liquid infusion of certain ingredients. The

extrusion process results in 'chemical reactions that occur within the extruder barrel and at the die'. The extrusion process and its resulting nutritional quality has been well described by Camire (2000). The motivations for extrusion can be various and include:

- use of higher moisture and fat contents compared to pelleting; use of liquid ingredients;
- improvement of nutritional value due to pasteurisation/inactivation of undesired components;
- cooked products with increased porosity, suitable to infuse liquid additives/fats.

Table 17.10 shows the results of a study into extrusion processing on the ileal digestibility of different nutrients. For the diet ingredients, barley, sorghum, corn, wheat, potato, faba beans, beans (*Phaseolus*), peas, soybeans and rapeseed, extrusion processing especially improved the protein and starch digestibility. Somewhat comparable with the extrusion process is the universal pellet cooker. In its barrel, the rotor moves faster but capacity of the total system is higher compared to extrusion. This system can be applied to complete pig diets to produce diets somewhat more expanded at the end of the die.

17.3 Shifts in technologies and mechanisms

Modern feed technology has been built on the essence of manufacturing technologies: dosing, grinding, mixing and agglomeration, but there is a shift from the more generic technology towards other technologies, where various stages can be used and flexibility in processes is more common practice.

We have to deal with the routinely used diet ingredients and the high capital technological infrastructure (available equipment/existing systems) that we have: infrastructure and local feed ingredients often dictate the ways diets are produced.

Table 17.10. Average differences in ileal apparent nutrient digestibility coefficients for pigs as a result of extrusion processing.

Parameter	DM	OM	CP	Fat	Cfibre	NFE	Starch
Number of experiments	21	11	42	5	3	3	17
Average difference to untreated (%)[2]	4.9	8.6	4.5	0.4	-0.7	1.7	11.4
Number of trials positive	11	8	34	3	2	2	13
% trial positive	52	73	81	60	67	67	76

[1] Cfibre = crude fibre; CP = crude protein; DM = dry matter; NFE = N-free extract; OM = organic matter.
[2] Unit percentages.

Where necessary, we need to produce mash diets or we have to use the specific ingredients from the own region instead of purchasing/transportation of ingredients from all parts of the world. Moreover, when farmers have their own ingredients on the farm, concentrates will be used as supplementary diets, so they will purchase feeds with a higher nutrient density.

In the process diagrams of the animal feed industry, this will ask for sophisticated factory designs with sufficient possibilities for flexibility in production: it is aimed at the variation in the diet ingredients to be used and sometimes the relationship with the food industry as a supplier. The use of molasses and vinasses in the feed industry for example, is now completed by the use of other liquid ingredients, other than just water or steam. The food industry itself is also changing as related to their processing objectives and ingredient use (Van der Goot *et al.*, 2016) such as less use of water, less drying and the use of enriched fractions instead of relatively pure fractions.

Further trends are observed, that comprise possibilities for the primary processing i.e. the treatments of ingredients such as separate ingredient grinding to attune the grinding conditions to this ingredient. To understand the grinding processes, the breaking principles of raw materials should be studied and apply its law's to generate equations to be used in the future. We can ask ourselves whether just the size of particles is sufficient to explain effects on nutrient digestibility or should other particle characteristics based on volume, on surface, on its combinations, should be used.

Pelleting but especially its variation in conditioning methods using pre-compaction methods has not been well examined for its potential positive effects on nutrient digestibility. Expander processing has been more a subject of study but does not always show similar directions for its result on animal performance. Since pelleting or extrusion as secondary processing in the feed mill may improve the nutritional value of rapeseed meal for pigs after a demonstrated decrease of its protein nutritional value due to desolventising/toasting (Salazar Villanea, 2017), incorporating technology as a factor in feed formulation should be considered.

Finally, and especially with respect to temperature sensitive feed additives such as enzymes and probiotics, the use of post-pelleting applications (Engelen and Van der Poel, 1999; Pierce *et al.*, 2003) of this kind of liquid products either coated or vacuum infused is well established nowadays. In the end, all standards should be met for the feeds: safe, hygienic, environmentally friendly and having a high nutritional value. It is, however, recognised that fines from pellets after transporting stress, will contain all the expensive and valuable products which is not the objective of coating. There has been only limited research into well-designed studies to optimise conditions for application of post-pelleting applications in the feed mill.

To meet the world wide increasing demand for feed raw materials, there are basically three strategies (Choct, 2014): increase the cultivation/supply of the basic feed raw materials, a better use of the existing raw materials or to examine new alternative raw materials.

In the next paragraphs the different technologies available to improve feed nutrient utilisation and the use of alternative raw materials, both to be used at the level of the animal feed producer or directly at farm level are discussed.

17.3.1 Enzymes

The use of exogenous enzymes in poultry and swine diets has seen an enormous increase since the first introduction of phytase in 1991 (Joshi, 2014). At present, phytase represents some 60% of the total feed enzymes used and has been well established as in terms of efficacy (Adeola and Cowieson, 2011). The other enzymes used are mainly applied to hydrolyse NSP. At first, these enzymes were used primarily in countries that use wheat and barley in diets, whereas today their application has been extended to other cereals in corn, soybean, and sorghum diets (Moura *et al.*, 2014). With the NSP enzymes, however, there is less consensus on the mode of action, particular in corn based diets. There are two long standing mechanisms by which NSP-degrading enzymes are thought to improve performance: (1) eliminating the nutrient encapsulation effect of the cell wall; and/or (2) reducing viscosity problems associated with certain NSPs, particularly arabinoxylans and β-glucans (Masey O'Neill *et al.*, 2014).

There is a synergistic effect of processing and the impact of enzymes (Amerah *et al.*, 2011). Modification in cell wall architecture obtained by processing technologies can improve the accessibility of NSP to enzymes (De Vries *et al.*, 2012). In their review, these authors observed a 1.5-6 times larger effect of the addition of enzymes on the digestibility of fibre when applied to heat processed diets compared to unprocessed diets.

A more consistent use of NSP enzymes has been shown in poultry compared to swine diets (Kerr and Shurson, 2013) at least for non-corn-soy diets (Aftab, 2012). Generally more effects are seen in young, fast growing animals rather than in older animals (Cowieson *et al.*, 2006). Recently, more attention is paid to the influence of these enzymes on the type of microflora which is promoted in the gut. Given the large difference in the range in microbiota composition likely to exist between studies, the responses to feed enzymes are a continuum or a population of responses (Bredford and Cowieson, 2012; Kiarie *et al.*, 2013) rather than being an absolute response. The future will most likely show developments of enzymes, capable of modulating the gastrointestinal tract microbiota to the benefit of the host's health under specific production conditions.

The feed form has an influence on the effects observed with different enzymes. In piglets for NSP, amylase, proteases alone and in combination it was shown that particle size, nutrient density of the diet and feed form interacted with result of the individual enzymes (Torres-Pitarch *et al.*, 2017).

With the growing demand for less antibiotic use and even total antibiotic free production, the market for specific non-antibiotic solutions against pathogens increases. Enzymes can and will play a role here in the future, either by attacking the pathogen directly, neutralising the toxin or blocking the adhesion possibilities.

17.3.2 Fermentation

One of the areas which has regained interest in recent times is the fermentation of diets for pigs and poultry. Both fermentation of the whole diets and single feed ingredients are studied.

At present, two distinct research areas can be distinguished:
- anaerobic lactic acid fermentation;
- fermentation by fungi.

In both cases the aim is to increase nutrient digestibility and possibly health status of the animal.

Lactic acid fermentation has been long used to stabilise grass and corn silages for ruminants. Besides ease of storage, the protein digestibility increases, the starch is more rapidly degraded and the amount of lactic acid increases. Also reduction of anti-nutritional compounds such as tannins and trypsin inhibitory activity and oxalic acid have been reported for forage legumes (Martens *et al.*, 2014).

Liquid feeding of swine has long been used to valorise by-products from the food industry. In these processes, starting already at the supplier, always some spontaneous lactic acid fermentation took place. In the last two decades, increased research efforts into the effect of controlled fermentation inoculated with selected lactic acid bacteria (Scholten *et al.*, 1999) have been made. Reported results have varied depending on hygienic status and strategies used. When performed properly, a better feed conversion, phosphate and nitrogen efficiency, reduced pathogen prevalence and improved health status have been reported (Plumed-Ferrer and Von Wright, 2009; Missotten *et al.*, 2010; Canibe and Jensen, 2012). Selective hygienic lactic fermentation of (part of) grain fractions using a liquid starter culture has evolved as the most promising strategy in praxis. In this process, each batch is pasteurised by soaking the grains in water of 65 °C and by adding cold water brought to 37-38 °C before the start of fermentation. A fresh liquid starter culture (with no lag phase) is used to quickly lower the pH and

results in a fermentation with low amounts of acetic acid, ethanol and high amounts of lactic acid (Figure 17.2; Scholten *et al.*, 2016).

Research of the use of fermentation technology for poultry diets has been much more limited (Skrede *et al.*, 2003). Providing a liquid feed would prove challenging in the current feeding systems for layers and broilers which are fully geared to accommodate dry feeds. Having said that, advantages especially in the first days and weeks of a chick life are reported which validates further research (Niba *et al.*, 2009).

Research in the use of fungal microorganisms can be divided in two main categories. First the fermentation of mainly soybeans with fungi like *Aspergillus* (Matsiu *et al.*, 1996; Liu *et al.*, 2007) or *Rhizopus* (Nout and Kiers, 2005) which show positive effects on nutrient digestibility and fecal microflora for piglets. Secondly research focusing on lignin degradation by fungi such as *Ceriporipsis subvermispora*, *Lentinula edodes* or *Pleurotus eryngii* (Van Kuijk, 2014). Although most research focuses on fungi, also some bacteria are able to degrade lignin (Brown and Chang, 2014). Since the latter fungi only degrade lignin when little amounts of nutrients are available, the research focusses on upgrading high lignin, low energy feedstocks like wheat or rice straw, or maize cops. Since these raw materials are classically ruminant feedstocks, the focus is still on ruminant applications (Mahesh and Mohini, 2013). Fungi treatment which takes several weeks and needs to start from sanitised feedstock, leads a higher digestibility and increased feeding value. To be able to implement this in practice at

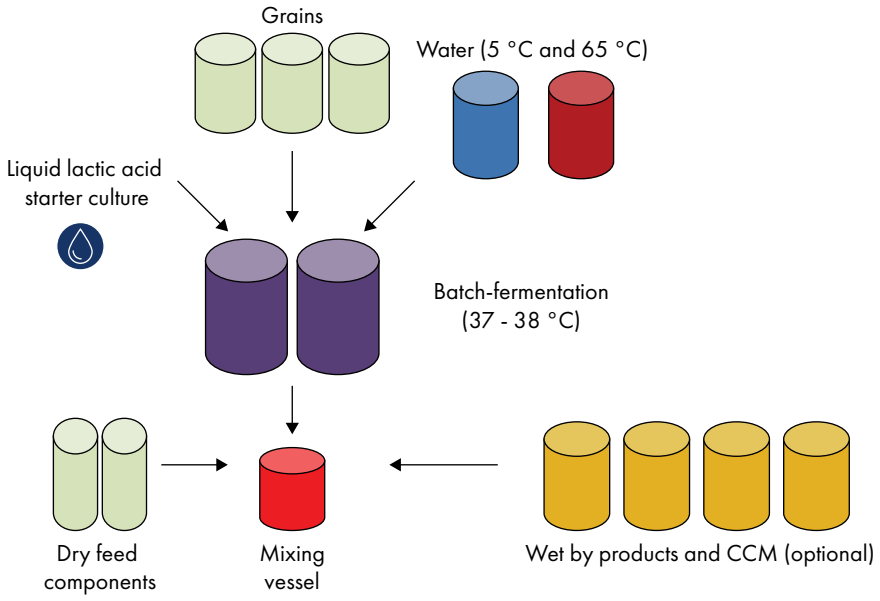

Figure 17.2. Lactic acid fermentation strategy (Scholten *et al.*, 2016).

a farm level, robust fast growing fungal strains have to be selected that are able to effectively colonise the feedstock and degrade the lignin (Van Kuijk, 2016). Although this technology can be seen as still in its infancies, it is the opinion of the authors that much is still to be expected from this low cost, mild energy and sustainable treatment method.

17.3.3 Physical treatment

The bioethanol industry and the aim for second generation biofuels is boosting research into the physical (pre)treatment of biomass like steam explosion or high temperature and prolonged residence time to increase its digestibility (Chang *et al.*, 2012; Oliva *et al.*, 2017). In general large scale installations are needed to achieve a sufficient economy of scale per Mg of treated material to make the technology cost effective. The relatively harsh treatment has a negative impact on protein digestibility. The combination of high-capital investment and the negative impact on protein digestibility currently prohibits its application in feed production.

17.4 Future perspectives

Farms are becoming larger and in certain countries farmers are using on-farm processing of feed ingredients in addition to a purchased concentrate. There is a small but growing trend in organic farming to include the use of other ingredients for animal diets such as grass for sows, fermented ingredients, etc. The potential use of more co-products from industry also includes the use of wet products. And each group of ingredients has its specific challenges; anti-nutritional factors in especially pulses/legume and oil seeds, mycotoxins, agglomerating gluten in calf milk replacers, etc. We have to consider more carefully the role of the impact on society, since we can try to optimise feed costs, including environmental costs but have to also consider regulatory consequences, investment risks, and consumer acceptance. Den Hartog refers to a new business model in agriculture where the goal has to be a balance between economy, ecology and society, and calls it 'sustainable precision husbandry' (Den Hartog, 2015). In the case that ingredient processing plants, feed mills, farms, retailers and consumers are connected, it also has consequences for the feed manufacturing industry such as the alignment on the use of certain feed ingredients. But as soon as this direction is known, it does not mean that there is just one general process of making animal feeds: yes, to increasing efficiency of feed production and no, to large diversity of current manufacturing diagrams and systems of the feed manufacturer; the different manufacturing set-ups, however, largely will remain because of the investments done and available feed plants.

For the manufacture of animal feed, several combinations of processes are possible. Compound feed manufacturing is a relative 'dry' technology that includes moisture

levels of feeds to be guaranteed below 130 g/kg. This standard was based in earlier times on cereals as the main ingredients. Some years ago, however, the type of ingredients that are included into animal feeds has changed. The inclusion level of cereals in diets was only 20% in the past but nowadays in some animal feeds, the level of cereals has increased again to 50-60%. Some feed ingredients enter the market as a more moisture-rich or 'wet' product. For these products it is worthwhile for the feed manufacturer to evaluate possibilities to process larger volumes of these 'wet' ingredients. As such, one should also have a technology that is in line with the use of these 'wet' ingredients. Apart from just feeding wet diets to more animals, the extrusion or extrusion-like process may also offer opportunities to process these 'wet' ingredients. By extrusion, the feeding value of starch/fibrous nutrients of the mixed diet and its hygienic quality will be improved, while the permissive moisture level during extrusion is higher compared to that during the pelleting process.

17.4.1 Ingredients and formulation

In Europe there is an increased need for vegetable proteins in order to replace fish meal (price and sustainability), soybean meal (sustainability), meat meal and swill (currently not approved under EU legislation) and there is an increased focus on the development and validation of alternative protein sources for pig and poultry diets. The cultivation, processing and nutritional aspects of different alternative protein sources (insects, seaweed, duckweed) have been well described (Van Krimpen *et al.,* 2013; Makkar *et al*., 2016; Van Huis and Tomberlin, 2017).

However, the prices of these alternative proteins are currently much higher than traditional sources like soy, sunflower or rapeseed. To bridge this gap either the consumer should be willing to pay more for the concept (i.e. based on CO_2-foot print) and/or the costs of production should be decreased substantially by upscaling.

The use of controlled lactic acid fermentation is gaining momentum in especially the German swine market with about 50-100 farms using this technology as of the beginning of 2017. Benefits on animal health, increased feed, N and P efficiency and the possibilities to better use alternative proteins are main drivers (Van den Over, 2017; pers. communications).

In diet formulation, a mixture of ingredients has to be provided with respect to the nutrient requirements for animal performance. So, in addition to meeting nutritional requirements, further considerations such as palatability, the physical quality of feeds, no undesired constituents, etc. have to be taken into account when formulating feeds. Developments in feed formulation include multiblend formulation and risk formulation. There is a development trying to switch from 'ingredient'-thinking to 'nutrient'-thinking, since constraints for ingredients are prone to emotions rather than proven facts. Moreover, since pelleting or extrusion may improve the feeding

value of heat-processed ingredients (Salazar Villanea, 2017), ideas to implement this extra value to ingredients in the linear programming – technology matrix values in formulation – are welcome.

17.4.2 Manufacturing technologies

In animal feed manufacturing, it is the equipment that has to be refined in the manufacturing phases together with a reduction of energy use for sustainability reasons. Over the past years, only innovations in equipment were observed: dosing equipment, the multicracker device for grinding and the coating/vacuum coating systems being examples.

Some feed manufacturers have invested in improvements for the rapid production of different feed forms: mash, pellets, crumbles, expandate in order for them to be flexible in the feed forms they are able to produce. Part of this strive should be the optimisation in conditioning process for feeds prior to pre-compaction/pelleting. Yet, there is still a need to optimise this important process in terms of moisture absorption and particle characteristics. We have noted that factories invest equipment which allows maximum levels of liquids to be incorporated in animal feeds; these liquid ingredients require pumps, network of pipes, etc. to be implemented into the existing production line. Liquid coating and infusion is a way to increase the energy content and/or palatability of pet foods, it is also a way to decrease the contamination problem in feed production lines where critical feed additives are involved. Using these additives in a liquid form at the end of the production line clearly diminishes its carry-over level and these techniques have also been applied to pellets meant for pigs and poultry. Grinding is still a process being researched due to its large contribution to processing costs. New systems and further processing of the fine and coarse fractions is a current topic of discussion in feed milling operations. In research, we can ask ourselves whether the particle size as a two-dimensional diameter expression is the correct parameter to be the link of grinding, mixing and pelleting results to the digestive processes in the animal.

We also note that the type of thermal/shear processing (expander processing; extrusion) requires further research in terms of flexibility of use and this may provide possibilities to produce more or less 'functional' feeds. In addition, from a nutrient supply point of view, the emphasis in research should be to find also suitable conditions for the production of nutritious and health promoting (use of antioxidants, vitamin supplements, controlling fibre types), including immunity enhancing (use of prebiotics, yeast products) feeds. It is about the integration of nutrition and feed manufacturing, with economy, ecology and society as the essential pre-conditions (see above) to guarantee the successful development of new, modern, functional feeds.

17.4.3 Sensors and automation

In the feed industry, information technology, greater quantities of information, and digitalisation is already wide-spread. Factories can be fully automated where currently process data are used in optimising the feed manufacturing technology. In the whole society, including the feed manufacturers, the number of available data increases dramatically.

Feed moisture level, for example will affect both feed quality and shelf life. It will also directly affect the profitability of the feed mill. It is, therefore, evident that this parameter should be monitored and controlled and maintained at an optimum level at each stage in the process. The use of proper sensors contributes to an accurate management of product characteristics at the feed mill. Older methods used include, for example oven drying, a destructive and time consuming method that requires taking representative feed samples. New on-line/in-line methods are widely accepted and outweigh the investment/onward costs. For example, the monitoring of the moisture level in diet ingredients, controlling water addition in the mixing/drying processes and controlling the moisture in the finished feeds is profitable. Both NIR and measuring the dielectric properties of ingredients (with microwaves or electrical capacitance) can be used as methods to monitor feed moisture level.

Feed manufacturer's advice to farmers is also provided especially nowadays with the easy access to internet. Decision support systems based on historical data are used to model dairy farms, to determine risk factors and to even provide controlling tasks. Big data in general are still challenging as is the modelling dynamics of nutrient digestion for ruminants and non-ruminants to contribute to precision livestock feeding. Digital innovations on-farm are present: the use of app's and of google glass, use of drones to detect deviations in animal behaviour, all contribute to a better farm security.

17.4.4 Rapid methods for analysis

A number of reasons can be mentioned why rapid tests have been/must be developed. In addition to the manufacturer compliance to legislative rules (e.g. traceability), manufacturing practices such as ingredient quality monitoring, production of feed via the concept of 'just-in-time', ingredient and feed safety including HACCP, are relevant reasons for such a development. Moreover, the analytical variation of diet ingredients has to be controlled. These different reasons are also a reason for the fact that more analyses are carried out at the manufacturing lines, where time is nowadays an important factor in feed production. The availability of rapid analyses systems has to do with the analytical information accuracy that has to be in time.

As for safety, especially ingredient and supplier judgement on risks are important. All kinds of threshold levels must be monitored for specific components such as

mycotoxins, heavy metals, pesticides, etc., all contributing to company alertness. The carry-over of certain feed additives to non-target animals forms an additional issue and safety is the first reason for increasing the number of analyses. In addition, matrix values of certain additives in feed formulation will depend on the physical form (like for example the coating) of the final formulation, all reasons to control its processing.

NIR developments are on-going where the number and accuracy of nutrients measured in both ingredients and final product are steadily increasing. This rapid analytical tool is also used for in-line ingredient analysis to optimise moisture levels and to reduce nutrient variability. Moreover, real-time data can be obtained for process control and feed production optimisation to meet requirements for nutritional as well as feed safety analysis.

Relevant reference analysis to estimate the nutritional values exists for parameters such as dry matter, NDF, starch, etc. Additional calibrations should be developed of course, where present examples on the availability of calibration lines for phytate should encourage further examples. We might think of (anti)nutrients such as trypsin inhibitors, mycotoxins but also ingredient digestibility values and reactive lysine as an unwanted result of ingredient or diet processing.

Both at-line or in-line measurements are used, but currently, the feed company also can use mobile NIR to farms to optimise its feeding strategies.

17.4.5 Economy

For the feed mill, the key issue to be implemented in the future will certainly be the manufacturing of animal feeds being part of a balance between economy, ecology and society. In the chain of ingredient supply, feed mill, farm, retailer and consumer, it means taking responsibility for feed quality and safety while applying recent feed manufacturing quality systems. In a changing world with increasing attention for the role of society, transparency in the way of producing feeds as well as traceability for feed constituents, are major issues.

With the increasing scale of farms more and more farmers will start home mixing in which they produce or buy part of the overall feed mix themselves. Together with a supplementary feed or concentrate, the total ration is fed to the animals. The feed producer has to compete with better advise on the overall nutrient composition and advised feeding strategy compared to his competitors. Although the overall performance of this feed approach is mostly lower than compound feed (with all its efforts put into optimal balancing and processing of the nutrients), the overall economics will dictate in which direction this development will go. It is the challenge of the compound feed industry to innovate in the coming years to keep their competitive advantage.

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18

Precision livestock feeding, principle and practice

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Summary points

- Precision livestock farming (PLF) is proposed to the livestock industry as an essential tool to enhance sustainability and competitiveness.
- Precision livestock feeding is part of PLF and can have a great impact in livestock profitability due the ability of feeding pigs with diets tailored daily to their nutrient requirements.
- Precision livestock feeding can decrease livestock environmental impacts by optimising the use of dietary nutrients and animal nutrient utilisation efficiency which results in less nutrient excretion.
- Mathematical models developed for precision livestock feeding must be designed to operate in real-time using system measurements. These models are structurally different from traditional nutrition models.
- The success of PLF is dependent on the precision livestock feeding integration into the system, as well, the adaptability and training of the farmers to use PLF systems.

Keywords: precision livestock farming, farm management, automatisation, modern livestock production, nutrition

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18.1 Introduction

Precision livestock farming (PLF) is an innovative production system approach that can be defined as the management of livestock using the principles and technologies of process engineering (Wathes *et al.*, 2008). The intensive and integrated use of advances in animal science and in the technology of information and communication are the basis for the development of PLF. One of the objectives for developing PLF systems is the on-line continuous and automatic monitoring of animals to support farmers in the management of animal production such as feeding strategies, control of the growth rate, and health management (Berckmans, 2004). The main purpose of PLF is, however, to enhance farm profitability, efficiency, and sustainability (Banhazi *et al.*, 2012a). Precision animal nutrition or precision livestock feeding is considered in this document as part of the PLF approach and involves the use of feeding techniques that allow the proper amount of feed with the suitable composition to be supplied in a timely manner to a group of animals (Parsons *et al.*, 2007; Cangar *et al.*, 2008; Niemi *et al.*, 2010) or to individual animals in a group (Pomar *et al.*, 2009; Andretta *et al.*, 2014). The on-farm application of precision livestock feeding requires the design and development of measuring devices (e.g. to determine the animal's feed intake and weight), computational methods (e.g. estimating in a timely manner nutrient requirements based on the actual animal's growth), and feeding systems capable of providing the required amount and composition of feeds that will generate the desired production trajectory.

The practical application of precision livestock feeding can have great impact in livestock profitability. Feed is the most important cost component in commercial growing-finishing pig production systems and represents between 60 and 70% of the overall production costs. Similar figures hold for broilers and other livestock. Given that nutrients that are not retained by the animal or in animal products are excreted via the urine and faeces or as heat, and that the efficiency by which domestic animals transform dietary nutrients into animal products are generally low, improving the nutrient efficiency can largely contribute to reducing production costs and improve the sustainability of livestock production systems. In fact, nitrogen and phosphorous, which are among the most costly nutrients in livestock feeds, are retained with efficiency rarely greater than 35% (Dourmad *et al.*, 1999; Poulsen *et al.*, 1999). The inefficiency of nitrogen and phosphorous use has different causes. First, part of these ingested nutrients are used for basal metabolic processes involving degradation (catabolism) and synthesis (anabolism), or are lost in the digestive tract through desquamation and endogenous secretions. These losses are generally referred to as maintenance losses. Nutrients are also lost during the production of animal products (e.g. body protein and lipid, milk, and eggs). In growing animals, the losses associated with the utilisation of the first-limiting amino acid for body protein deposition can largely be attributed to the inevitable catabolism (Heger and Frydrych, 1985; Mohn *et al.*, 2000). These inevitable amino acid losses should be differentiated from other metabolic losses

related to the preferential amino acid catabolism, which results from the catabolism of amino acids given in excess, from the excretion of chemically unavailable absorbed amino acids (e.g. heat damaged proteins) (Batterham *et al.*, 1990), and from the use of amino acids for the synthesis of non-protein body compounds (Moughan, 1989). In growing animals fed cereal-based diets, the sum of the undigested nitrogen and the losses associated with digestion, maintenance functions, and body protein deposition may represent more than 40% of the total ingested nitrogen.

Pigs, broilers, and other livestock animals are typically raised and fed in groups, usually with the same feed that is given to all animals in the group during a given period of time. However, nutrient requirements vary largely among animals in a population (Pomar *et al.*, 2003; Brossard *et al.*, 2009) and these requirements evolve over time following individual patterns (Hauschild *et al.*, 2012; Andretta *et al.*, 2014). When growth maximisation is the objective of a commercial production system, nutrients have to be provided at a level that will allow the most nutrient demanding animals in the group to express their growth potential (Hauschild *et al.*, 2010). In this situation, almost all animals receive more nutrients than they need. Providing animals with high levels of nutrients to maximise herd performance is common practice in commercial livestock operations even though maximum growth does not ensure maximum economic efficiency (Hauschild *et al.*, 2010; Niemi *et al.*, 2010). Besides the estimated 40% nitrogen loss associated with digestion, maintenance, and production inefficiencies, an additional 30% loss results from protein given in excess to optimise the production response of the group. To account for the variability among animals but also among feed ingredients and other uncontrolled factors (e.g. environment, health) nutritionists include safety margins when formulating diets to ensure the maximum population responses. The need of these safety margins can be seen as an admission of our inability to precisely estimate the nutrient requirements of groups of animals (Patience, 1996). Precision nutrition will play an important role in future animal production systems because innovative monitoring approaches simplify the determination of nutrient requirements which, when estimated in real-time, allow for the possibility of feeding animals, individually or as a group, according to specific production objectives. These objectives include the maximisation or the controlling of growth rate, or to minimise the excess supply of nutrients and reducing environmental impacts. Safety margins are not required in precision livestock feeding. Compared to a 3-phase feeding program for growing pigs, precision livestock feeding can reduce protein intake by 25% and reduce nitrogen excretion by almost 40% while feed cost can be reduced more than 10% (Pomar *et al.*, 2010). Because animals and feed distribution are monitored and controlled automatically, precision livestock feeding will reduce the time that nutritionists and farm staff will spend on animal observation, decision-making, and applying production strategies, enabling them to work on other aspects of farm management. The objective of this chapter is to describe the basic concepts of precision livestock feeding, its essential elements and illustrate practical applications of precision livestock feeding for growing and finishing pigs.

18.2 The basic concepts of precision livestock feeding

Precision animal nutrition or precision feeding concerns the use of feeding techniques that provide animals with diets tailored according to the production objectives (i.e. maximum or controlled production rates), including environmental and animal welfare issues. Precision livestock feeding is presented in this document as the practice of feeding individual animals or groups of animals while accounting for the changes in nutrient requirements that occur over time and for the variation in nutrient requirements that exists among animals. As defined in this document, the accurate determination of available nutrients in feeds and feed ingredients, precise diet formulation, and the determination of the nutrient requirements of individual animals or groups of animals should be included in the development of precision livestock feeding (Sifri, 1997; Van Kempen and Simmins, 1997; Pomar *et al.*, 2009). The operation of precision livestock feeding in commercial farms requires the integration of three types of activities: (1) automatic collection of data; (2) data processing; and (3) actions concerning the control of the system (Aerts *et al.*, 2003; Berckmans, 2004; Banhazi *et al.*, 2012b). Application of precision livestock feeding at the individual level is only possible where measurements, data processing, and control actions can be applied to the individual animal (Wathes *et al.*, 2008).

18.2.1 Automatic data collection

Measurements on the animal, the feeds and the environment are essential in precision livestock feeding and these have to be measured directly and frequently (if possible, continuously). Measurements that can be made at the animal level include feed intake (e.g. quantity eaten, feed intake behaviour), its physical state (e.g. body weight, body composition), and indicators of its behavioural and health status (e.g. physical activity, interactions among animals). The availability and the rapid development of new devices and emerging sensor technologies to PLF and precision livestock feeding, offer a great potential for animal monitoring. Available technologies and sensors have been described by Wathes *et al.* (2008) and include low-cost cameras which, in combination with image analysis, can be used to quantify animal behaviour and estimate body weight. Real-time sound analysis and audio-visual observations have been proposed to monitor health status and welfare in pigs (Vranken and Berckmans, 2017) and behaviour in laying hens (Berckmans, 2004; Vranken and Berckmans, 2017).

Besides the availability of technologies allowing the measurement of animal traits, some guiding principles have to be used for choosing the appropriate and relevant devices and sensors to be used in precision livestock feeding. Black and Scott (2002) used the hazard analysis critical control point (HACCP) in the Australian 'More Beef

from Pastures' program. The HACCP was proposed to ensure that the most important processes determining productivity and profitability in an animal enterprise were identified and could be controlled and manipulated with the least chance of failure (Black, 2007) including the development of PLF applications (Banhazi *et al.*, 2012b). In the context of automatic data collection for precision livestock feeding, the HACCP principles are: (1) to identify the factors that have quantitatively a major impact in the determination of the response of the animal or of the population to the nutrient supply; and (2) for each one of these factors, determine the measurements that have to be taken at the farm or animal level to ensure the application of precision livestock feeding. At this point, precision livestock feeding developers have to avoid the temptation of looking for practical applications of currently available sensors but rather concentrate on identifying the most important physiological factors and measurements needed to establish optimal feeding strategies. These measurements have to be related to the precise evaluation of the nutritional value of the diet, the real-time determination of nutrient requirements (Pomar *et al.*, 2009), and the responses of the animal to the nutrient supply. The application of HACCP principles to identify production hazards is not addressed further in this paper and the reader is referred to Black (2007) for more information on this issue.

18.2.2 Data processing

Collected data has to be processed to allow for control activity in precision livestock feeding. Mathematical modelling is a methodology used to understand and to quantify complex biological phenomena involved in animal production and can be the basis for data processing in precision livestock feeding control systems. A mathematical model is an equation or a set of equations representing the behaviour of the system (Thornley and France, 2006). Computer simulation, in its broadest sense, is described as the process of defining a mathematical-logical model for the real system and experimenting with this model on a computer (Pritsker, 1986; Thornley and France, 2006). By definition, models are a simplification of the system they represent, but the most relevant factors implicated in the animal responses that are to be controlled in precision livestock feeding need to be represented into the model. Mathematical models developed for precision livestock feeding, however, have to be designed to operate in real-time using real-time system measurements and, therefore, they are structurally different from traditional nutrition models, which are developed to work in a retrospective manner to simulate and understand known production situations. The basic principles for model development have to be reviewed because not all the models are adequate for precision livestock feeding and a model structure has to be chosen according to the available information and the desired control design of the system.

Mathematical models can take many different forms depending on model objectives and structure. Indeed, models can be empirical or mechanistic, deterministic or

stochastic, static or dynamic, and real-time or prospective. Information about the development of mathematical models in animal science can be found elsewhere (Thornley and France, 2006). Different approaches have been used to predict animal growth (Van Milgen *et al.*, 2012). The earliest and still very common approach is empirical in which growth is described by a single or few mathematical equations. Empirical models use a black-box approach and are developed to describe the responses of a system without a description of the system itself and unconstrained by biological principles (Thornley and France, 2006). The empirical approach can provide effective prediction in a narrow range of situations related to experimental conditions under which the data were collected. However, the empirical approach fails to extrapolate results in situations beyond those used in the original experimental conditions. Because model parameters and structure do not have a biological meaning, these models need to be fitted with appropriate data to simulate each situation. Therefore, to ensure flexibility and to allow effective prediction in a wide range of situations, models with mechanistic (deductive) components are preferred (Baldwin, 1976; Whittemore, 1986). Mechanistic models provide some degree of understanding of the biological phenomena implicated in the response of the system (Thornley and France, 2006). Mechanistic mathematical models have been the preferred approach in animal sciences since the 70s when protein and lipid deposition (and the resulting body weight gain) was modelled from the nutrient supply (Whittemore and Fawcett, 1976). This and other early models have inspired the development of other nutritional models simulating growth in pigs (Black *et al.*, 1986; Moughan *et al.*, 1987; Pomar *et al.*, 1991; Birkett and de Lange, 2001; Green and Whittemore, 2003; Halas *et al.*, 2004; Van Milgen *et al.*, 2008), poultry (Emmans, 1981, 1988; Hancock *et al.*, 1995; Gous *et al.*, 1999), turkeys (Hurwitz *et al.*, 1991; Rivera-Torres *et al.*, 2011), conceptus growth and milk production in sows (Dourmad *et al.*, 2008; NRC, 2012) and egg production in hens (Fisher *et al.*, 1973).

Mathematical models can be deterministic or stochastic. Deterministic models make a unique prediction for each specific set of input variables without any associated probability distribution. Stochastic models contain random elements in the model, so that, in addition to predicting the expected value of a performance trait, they also predict its dispersion (Thornley and France, 2006). Variation is essential and inherent to living systems and variation among the animals significantly contributes to the efficiency with which nutrients can be used (Curnow, 1973) independently of genetic variation (Knap, 2000; Knap and Jorgensen, 2000; Pomar *et al.*, 2003; Brossard *et al.*, 2009; Vautier *et al.*, 2013), and environmental or animal management aspects (Wellock *et al.*, 2004). This variation is essential for the understanding of the biological mechanisms implicated in the response of populations to the nutrient intake, given that the response of a population to treatments differs in magnitude and shape from that of an individual animal (Pomar *et al.*, 2003). Mathematical models designed to estimate nutrient requirements and responses in a population of animals need to account for individual variation.

Static models do not contain time as a driving variable and do not make time-dependent predictions. Dynamic models are developed to quantify and to study the evolution of a system over time (Thornley and France, 2006). Essential elements of dynamic models are the differential equations in which time is an independent variable driving the rate of change of the state variables of the system. Most models in animal science and specifically in swine nutrition are dynamic because of the animal responses and requirements change over time.

The utilisation of mechanistic models in precision livestock feeding systems has been criticised because these models are overly complex and the information required by the model to simulate practical conditions is not always available (Aerts *et al.*, 2003; Wathes *et al.*, 2008). On the other hand, the simplicity of empirical models is counteracted by the difficulty to represent interactions between nutrients and animals. Despite the fundamental structural differences between empirical and mechanistic models in the way they predict the response of the animal to the nutrient supply, both types of models have to be calibrated *a priori* using data collected from reference populations (Pomar *et al.*, 2015) in which the phenotypic performance potential of the animal is quantified. Indeed, mechanistic growth models for pigs use intrinsic characteristics of a reference population either to describe the potential (phenotypic) protein deposition and feed intake patterns (Dourmad *et al.*, 2008; Van Milgen *et al.*, 2008; NRC, 2012) or potential body protein and lipid deposition (Emmans, 1981; Black *et al.*, 1986) while empirical models have the animal responses embedded into the model. To be used in precision livestock feeding, empirical and mechanistic models are, therefore, challenged by the difficulty of identifying the right reference population for its calibration, the fact that actual populations and individual animals may follow feed intake and growth patterns different than the ones observed in the reference population (Pomar *et al.*, 2015).

The computational power and reliability of modern information technologies empower the utilisation of advanced recursive technologies in the development of PLF and precision livestock feeding applications (Wathes *et al.*, 2008). These modelling techniques (e.g. artificial neural networks) estimate unknown model parameters of an abstract mathematical model, based on on-line input and output measurements. Model parameters are estimated on-line during the process, resulting in a model that continuously adapts its response to on-line process inputs and outputs. There are few examples in which these models have been used in PLF or precision livestock feeding applications (Korthals *et al.*, 1994; Bridges *et al.*, 1995; Aerts *et al.*, 2000; Thomson and Smith, 2000). The limitation of using the recursive approach in precision livestock feeding is related to the fact that model parameters and model structure do not provide biological insight in the causal mechanisms implicated in animal responses, that animal response and input parameters may have unsymmetrical variation, and that the animal responses to input variation does not evolve in the same timeframe. For example, when animal processes are modelled for which there is a significant time

lag between the effects of varying input parameters (e.g. dietary lysine intake) and the response (e.g. body weight gain and composition), the autocalibration capability of these recursive models is limited and they will generate irregular control signals (Cangar *et al.*, 2008). Rapid animal responses such as a behavioural response to inputs such as temperature and light intensity may be easily controlled by recursive models in PLF applications (Aerts *et al.*, 2000).

The disadvantages associated with black-box models can be overcome by using an intermediate approach of grey-box models in which recursive technologies and mechanistic models are combined. This approach was suggested by Bridges *et al.* (1995) who used a mechanistic swine growth model to generate physiological response data and this response data were then used to train and validate three backward propagation neural network models describing the effect of the environment on average daily gain, feed intake, heat production, and physiological status of the animal. The authors concluded that neural network models can be used to simplify data extraction from complex models and be used in instances where the use of the full model is difficult or impossible. Another grey-box model application was proposed by Hauschild *et al.* (2012), who combined black-box (i.e. empirical) and 'knowledge-based' (i.e. mechanistic) model components to estimate daily amino acid requirements in individual growing-finishing pigs. The empirical component of this model estimated daily feed intake, body weight, and daily gain based on individual information collected in real time. Based on these daily estimations, the mechanistic model component predicted the concentration of amino acids required to meet the daily growth needs. The principles behind this model approach have been described (Hauschild *et al.*, 2010; Pomar *et al.*, 2015) and validated (Andretta *et al.*, 2014, 2016b).

18.2.3 Control of the system

The main objective of precision livestock feeding is to monitor, manage, and control animal feeding and nutrition continuously and automatically. Data collection and monitoring devices provide the farmer with detailed information about the animal's actual conditions and performance, the utilisation of farm resources, while data processing helps with system surveillance (e.g. disease detection) and the estimation of optimal production strategies (e.g. optimal slaughter and production strategies). This information can also be used by an automatic controller to make decisions, which in the context of precision livestock feeding, will typically be the amount and the composition of the feed to be given to an individual or to a group of animals. Depending on the production objectives, the controller can be programmed to maximise growth rate, to minimise feed cost, to minimise nutrient excretion, or to meet another objective.

The determination of nutrient requirements and the control of the nutrient intake through feed composition and intake are two essential elements of precision

livestock feeding. For a given animal and at a given time during his life, daily nutrient requirements can be estimated by the sum of the requirements for maintenance and production. These requirements are estimated for each nutrient taking into account the efficiency with which each nutrient is used. For a given animal, maintenance and production requirements change over time and so do nutrient requirements (NRC, 2012). Farm animals are often raised and fed in groups although, within a group, animals differ in feed intake and growth potential. Consequently, nutrient requirements vary among animals (Pomar *et al.*, 2003; Wellock *et al.*, 2004; Brossard *et al.*, 2009). The dynamic and the between-animal variation are the two main sources of variation in nutrient requirements that can be controlled in precision livestock feeding systems. Production systems in which animals are fed individually can be used to control both sources of variation while in group-fed systems only the time-dependent variation can be controlled.

Therefore, several potential control strategies are available for the application of precision livestock feeding in commercial conditions. In feeding systems where animals are offered with feed *ad libitum*, the only way to control the nutrient intake is by varying the composition of the distributed feed. In *ad libitum* group-fed systems, animals can be fed for maximal production by providing nutrients following the time-dependent nutrient requirements of the group or for a given production strategy (i.e. body composition, population uniformity), by controlling the composition of the served feed. When animals are individually fed and offered feed *ad libitum*, both the between animal and the time-dependent nutrient requirements variation can be controlled. In feeding systems where animals are offered feed restrictively, the amount and the composition of the feed can be controlled. Maximum growth rate will not be attained in this situation although feeding strategies can be established to account for between animal and the time-dependent variation in nutrient requirements. For example, feed restriction in pregnant sows allows controlling body weight gain and fatness while maintaining conceptus growth. Examples of these control approaches for growing animals will be given in the following section.

18.3 The implementation of precision livestock feeding principles in growing and finishing pig production systems

Conventional growing and finishing pig feeding programs are designed to maximise population body weight gain, optimise carcass fatness, etc., and they provide a single feed to all the pigs in the pen or herd within each feeding phase. One to four-phase feeding systems are nowadays popular in commercial growing-finishing pig operations (Niemi *et al.*, 2010; NRC, 2012) but it is acknowledged that increasing the number of feeding phases reduces feed costs, improves feed efficiency, and decreases

nutrient excretion (Letourneau Montminy *et al.*, 2005; Brossard *et al.*, 2010). Multi-phase group-feeding systems allow the adjustment of the feed composition over time to better match the population nutrient requirements. Moving from conventional feeding systems to precision livestock feeding systems requires not only to increase the number of feeding phases, but also using the information concerning the actual status and evolution of the animal (e.g. feed intake, body weight, body composition) to control feed supply.

Accurate and automatic measurement of the amount of feed consumed daily by individuals or groups of pigs is an essential information element required for the implementation of precision livestock feeding in growing and finishing pig operations. Although liquid feeding systems provide predetermined amounts of feeds to pens, they are of limited use to provide information on the feed intake because feed is provided at restricted levels with these systems. The availability of commercial devices for measuring dry feed intake is still limited and seldom used for the implementation of precision livestock feeding in commercial piggeries. An individual feed intake recording system has been developed in the UK for the real-time control of growth (Parsons *et al.*, 2007). The system is able to weigh the feed delivered to each pig at each visit. Similar precision livestock feeding system has been developed by Pomar *et al.* (2014) using an automated recording system (IVOG system, Insentec B.V., Marknesse, the Netherlands). Another example of dry feeders measuring the consumed feed has been developed in Australia (Banhazi *et al.*, 2009, 2012a). This device can accurately measure the amount of feed supplied through the feed line (by an innovative feed sensor), estimating the amount of feed delivered to each feeder. Finally, an automatic and intelligent precision feeder developed for precision feeding of growing-finishing pigs has been developed (Pomar *et al.*, 2011), which is able to provide a specific quantity and composition of feed to individual pigs at each feeder visit. The functioning of these automatic and intelligent precision feeders has been described elsewhere (Pomar *et al.*, 2011, 2015) and the feeders have been used in several research projects (Andretta *et al.*, 2014; Cloutier *et al.*, 2015; Andretta *et al.*, 2016a).

Accurate and regular body weight measurements performed without causing stress and requiring labour input is a great asset for the implementation of precision livestock feeding in growing-finishing pig facilities. Available technologies for automatic animal weighing include conventional load cell platforms (Turner *et al.*, 1985) and the combination of video cameras and image analysis. The possibility of estimating the weight of a pig from specific areas and dimensions through digital image analysis has been developed (Schofield, 1990; Brandl and Jørgensen, 1996; Whittemore and Schofield, 2000; Doeschl-Wilson *et al.*, 2004; White *et al.*, 2004a) and used in several experiments.

Lean growth is the major determinant of amino acid requirements in growing animals. Modern pigs are capable of maintaining high levels of lean deposition at heavier live weights. Measuring backfat and muscle thickness can be precious information to estimate body fat and protein. Although different technologies are available, ultrasound is without doubt the most widely used because of its cost, reliability, and portability (Moeller, 2002). However, it is still a manual operation and although automatic measurement methods of backfat thickness have been proposed (Tillett *et al.*, 2002; Frost *et al.*, 2004), these technologies have not been developed further since then.

After measuring the essential information concerning the feeds and animals, precision livestock feeding requires to determine optimal nutrient concentration of feeds to automatically provide animals with the amount and composition of the feed according to the established production objectives. Precision nutrition can be used in pig growing facilities to either allow pigs perform at their maximal growth potential or drive growth rate and body composition by restricting feed or nutrient intake. Actual pig growth models (e.g. Van Milgen *et al.*, 2008; NRC, 2012) have been developed to operate in a retrospective manner and are calibrated after all growth data have been collected to simulate the production situation. These models are designed to predict, under specific situations, the consequences of feed and nutrient intake in terms of animal responses (e.g. protein and lipid deposition, body weight growth). These models are used to evaluate the nutrient utilisation by the animal and to test nutritional strategies. Mathematical models developed to be used in precision livestock feeding systems need to operate in real-time using appropriate real-time animal information (e.g. body weight and composition), behaviour (e.g. feed intake), environment (e.g. ambient temperature), health (e.g. body temperature, sounds to detect health status), and other parameters. When these models are conceived to achieve the animals' full growth potential, they can be devoid of feedback control elements and provide predictions based on actual and recent animal information. The objective of this real-time model-control approach is not to manipulate the animal response (i.e. body weight gain or composition) but to deliver the controlled production factors (e.g. feed composition) at the levels required for maximum growth.

Automatic blenders, feeders and feed, and animal management devices are required to apply the controller decisions. The development of feeding systems that allow blend-feeding and the automatic distribution of two or more feeds that, when combined in variable ratios, can meet the requirements of pigs throughout their growing period (Feddes *et al.*, 2000; Pomar *et al.*, 2014) makes the phase-feeding technique cost-effective. The feeds can be complete diets formulated to satisfy the requirements of pigs at the beginning and at the end of their growing period or to contain complementary amounts of nutrients in such a way that when blended, the feeds become complete diets (Joannopoulos *et al.*, 2015). Blending two feeds may be seen as a promising option for feed companies, since it means that there are just two feeds to prepare, with

only the proportions changing between the feeding phases and between farms. These group and individual feeding precision livestock feeding systems will benefit from using accurate and individual feed intake and body weight measurements to drive the amount and composition of the feeds to be served to the pigs.

To further develop precision livestock feeding systems, it is necessary to improve our actual understanding of several animal metabolic processes. Precision livestock feeding is still based on models and nutritional concepts of average population responses. When feeding individual pigs with daily tailored diets, these traditional nutritional concepts seem insufficient (Remus, 2015; Ghimire *et al.*, 2016; Remus *et al.*, 2017). It is necessary to distinguish the nutritional requirements of a population from those of an individual. Individual pigs are able to modulate growth and the composition of growth according to the level of available amino acids (Remus, 2018). Also, pigs can respond differently to the same amount of ingested amino acid, due to differences in the efficiency of amino acid utilisation. These aspects are not considered in current nutritional models, which assume that the efficiency by which animals use the available amino acids is constant. Similarly, the amino acid composition of whole body protein is assumed to be constant as well, while it has been shown that it can vary. Similar results have been found for the efficiency of calcium and phosphorus utilisation (Gonzalo *et al.*, 2018). Understanding the metabolic processes responsible for the observed variation between individual animals in their ability to use dietary nutrients is challenging nutritionists and modellers but is required to further improve the efficiency of livestock production. Advances in precision livestock feeding rely on the development of sound nutritional concepts and comprehensive biological models developed to more precisely estimate individual nutrient requirements in real-time.

18.4 PLF and precision livestock feeding systems used in practice

The real-time modelling-control approach was used by Pomar *et al.* (2014) to control the time-dependent variation of group-housed pigs offered feed *ad libitum*. Feed intake was measured daily with an automatic device and animals were weighed manually every two weeks. The desired diet composition was obtained by blending two feeds with a high and a low nutrient concentration. Nutrient requirements of the group were estimated each day based on body protein and growth rates observed in animals of similar genetic background. Comparing the traditional three-phase feeding system to the daily-phase feeding system, the authors concluded that protein intake could be reduced by 7% while nitrogen excretion was reduced by 12%.

Controlling the time-dependent and the among-animal variation can further help reducing nutrient intake and excretion. This modelling approach was used to estimate

nutrient requirements in real-time in individual pigs (Hauschild *et al.*, 2012; Pomar *et al.*, 2015) and applied to feed pigs individually with daily tailored diets (Andretta *et al.*, 2014). The latter authors showed that daily adjustment of the diet resulted in a 27% reduction in total lysine intake, without affecting growth. This additional 20% reduction in lysine intake in relation to group-fed pigs could be obtained by feeding the animals individually and thus controlling simultaneously the time-dependent and the between-animal variation. Although reducing feed cost depends to a great extent on feed prices, it is expected that feed cost can be reduced by 1-3% when only controlling the time-dependent variation while a 8-10% reduction can be obtained when controlling also the among-animal variation.

Restricting feed or nutrient intake has been proposed in several precision livestock feeding systems with the objective to minimise feed cost, ammonia emissions, or to maximise the return per pig space. Demmers *et al.* (2012) used an automated feeding system to provide the desired amount of feed of fixed composition to each pen. Daily body weight was estimated using a commercial visual image analysis system. The controller was based on a recursive neural network of growth and ammonia emission models, which were calibrated from previous experiments. The system was used to control the amount of feed delivered to pens and the ambient temperature to optimise growth and reduce ammonia emissions. A precision livestock feeding system was also used by Niemi *et al.* (2010) to study multi-phase and two phases feeding systems and growth patterns in terms of economic return per pig space. In this multi-phase feeding system, the amount of feed, the protein concentration in the diet, and the time to reach slaughter weight were optimised on a daily basis. The controller included a stochastic dynamic model that estimated nutrient requirements as a function of body weight and evaluated the different scenarios to maximise the return on capital investment. The authors concluded that producers would benefit from adjusting diet composition on a daily basis but that the optimal production strategy and the return on investment are affected by the variation among pigs and the variation in feed and carcass prices.

A real-time system for the integrated control of population pig growth and pollutant emissions was also proposed (Whittemore *et al.*, 2001; Parsons *et al.*, 2007) using an automatic daily feed intake recording device and a visual image analysis system to estimate daily body weight (Schofield *et al.*, 1999; White *et al.*, 2004b). Pigs were fed *ad libitum* in this precision livestock feeding system with diets varying in crude protein concentration. A high and a low-protein diets were manually blended to obtain the desired level of protein in the final mix to be served. The authors concluded that weight gain in pigs can be controlled through the proposed *ad libitum* feeding precision livestock feeding system and that some control of body fatness may also be possible.

18.5 Factors that can influence the successful application of precision livestock feeding systems on farms

Precision livestock feeding can be considered as a component of a PLF system and the successful on-farm application of precision livestock feeding systems will face similar challenges as other PLF systems. Wathes *et al.* (2008) considered PLF as an 'embryonic technology with great promise' but they also acknowledged that few PLF have been implemented successfully so far. In addition, there may be a long time path between development and application. For example, the milking robot was developed in the 80s and has been commercialised since the early 90s but, despite 25 years of availability, it has yet to revolutionise the dairy industry. In an article with the provocative title 'Is precision livestock farming an engineer's daydream or nightmare, an animal's friend or foe, and a farmer's panacea or pitfall?', Wathes *et al.* (2008) discussed the development and adoption of PLF systems. Others (Groot Koerkamp *et al.*, 2007; Banhazi *et al.*, 2012a,b) have expanded on these ideas and the main issues in the development and successful adoption of PLF (and thus precision livestock feeding) can be summarised as follows:

- There is a strong need for coordination and to involve different experts and stakeholders in the development and implementation of PLF (i.e. researchers, engineers, technology suppliers, economists, farmers, consumers, and citizens).
- With the rapid development and available of sensors, more focus should be paid to data interpretation and control mechanisms.
- Not all processes need to be automated; it is about assisting farmers, not about automatic farms. Groot Koerkamp *et al.* (2007) argued that there is not necessarily an intrinsic connection between (better) measurements and (better) control and that the allocation of controlling power is an important factor to consider. Who is in control: a machine, the farmer, the animal? Groot Koerkamp *et al.* (2007) suggested that recursive control by animals may be an alternative means to create order in complex biological systems, which, to some extent, can be interpreted as the consideration of agro-ecological principles in PLF.
- The benefits of PLF systems should be verified on the farm.
- Appropriate deployment of PLF systems and training, service and support for farmers should be assured. The latter may imply the development of a new service industry. As indicated by Banhazi *et al.* (2012b), farmers are biologists by nature and only technologists occasionally. Although they do invest in technology, it is typical machinery that they look forward to buying as opposed to software, sensors or services.
- Awareness and education for consumers and citizens. Citizens may perceive PLF as a further industrialisation of livestock production. Education should help appreciating controlled, animal-centric livestock production, while looking for attributes that make modern production better for the animal and more

sustainable. Food production should be made more transparent. For example, the EU-funded BrightAnimal project suggested using social networks, FarmCams, and a 'Be a farmer for a day' initiative to improve awareness.

Some of these issues addressed above have been considered in projects funded by EU-funded projects such as EU-PLF (www.eu-plf.eu), ALL-SMART-PIGS (www.all-smart-pigs.com), and Feed-a-Gene (www.feed-a-gene.eu).

An increasing concern is the adaptability and training required by farmers to use PLF systems. Some authors (Van Hertem *et al.*, 2017) believe that use of appropriate data visualisation tools can facilitate the farmer acceptance and adoption of PLF applications. These authors tested and evaluated PLF systems on ten fattening pig farms and five broiler farms. Data of production, climate and behaviour was continuously measured, analysed daily and made available on a web-based tool. Nearly 50% of the farmers took the training, but only 28% of the trained farmers actively used the tool. According to the authors, the success of the training seemed to be dependent on the complexity of the system installed on the farm (e.g. environmental sensors) and the training/education of the end user. They conclude that training is fundamental for the adoption of such systems.

18.6 Future perspectives

As indicated above, different technologies are now available for real-time and individual phenotyping and the availability of feeder equipment allows the distribution of specific diets to individual animals. An important issue that needs to be addressed further in the future is the control of the system, and how precision livestock feeding can and should interact with other components of PLF and with livestock production in general. For example, precision livestock feeding allows having large groups of pigs in a single pen, but this raises questions on how the group size affects animal behaviour and health, on pen design, and on management of animals in the pen. In recent years, some growing-finishing pig facilities are moving to larger groups of up to 1000 pigs/pen. These facilities are generally equipped with auto-sorting systems that weigh individual animals before entering the feed court, identifying pigs that reach market weight, and sort them into a loading pen (Street and Gonyou, 2008). The development of nutritional concepts and models specially designed for precision livestock feeding and the system integration, to provide early alerts about changes in the system (e.g. health status based on a reduction in the feed intake) seem to be of great importance. These issues need to be addressed in the future if precision livestock feeding is to go beyond being an alternative feeding technique based on the optimisation of the nutrient supply to the animal. Precision livestock feeding has the potential to be an important element of innovative livestock production systems,

which may involve changes in several processes and elements within the system (Groot Koerkamp *et al.*, 2007).

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