5
Nutrigenomics in Livestock Production

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5.1 Introduction

The current global population is approximately 8 billion and is estimated to reach 9–11 billion by 2050, with a 50% growth in the demand for food products by 2030 and doubling by 2050 (Ma et al. 2021; Haq et al. 2022). Products of animal origin are particularly subject to high demand, especially meat and dairy products.

This rapid increase has greatly challenged livestock farming in order to meet the demand for food and high-quality animal protein and to adapt to environmental obstacles such as heat and nutritional stress (Benítez et al. 2017).

Livestock research has had to find new strategies to increase animal production, for example, the modulation and optimisation of animal diets.

Nutrigenomics and nutrigenetics study the effects of food/feed and nutrients at a genetic level. Nutrigenomics combines several disciplines such as nutrition, bioinformatics, molecular biology, genomics, functional genomics, epidemiology and epigenomics to study how selected nutrients and bioactive compounds in foods and feed, as well as additives, can affect animal metabolism by modulating/altering gene expression. In particular, nutrigenomics studies the impact of dietary components on genome functioning in terms of gene expression patterns and epigenetic modifications. These include DNA methylation and histone modifications, identifying the key molecular signature for the subsequent development of effective nutritional strategies aimed at improving feed efficiency, animal well-being and the sustainability of livestock production (Benítez et al. 2017).

A balanced diet for livestock animals not only has economic benefits but, above all, results in healthy and quality products (Nowacka-Woszuk 2020). Manipulating the diet can lead to changes in production traits such as growth, product quality and animal health. The response is also affected by the physiological state/age/period of life of the animal.

Recent advances in nutrigenomics have clearly highlighted the effects of different dietary treatments and exposure times during key life stages, from maternal diets (i.e. prenatal) to neonatal, weaning (when appropriate), growth, adults and ageing (Figure 5.1).

5.2 Nutrient–gene interactions require intermediates

Nutrigenomics research applied to livestock is useful to assess how nutrients or dietary regimens affect animal well-being, performance and productive yields. The effects of nutrients, additives and diets can be observed at epigenetic, transcriptional, translational or post-translational levels, which can be investigated through nutrigenomic-related approaches such as transcriptomics, proteomics and metabolomics. However, nutrigenomics is not related to direct effects on DNA but encompasses the

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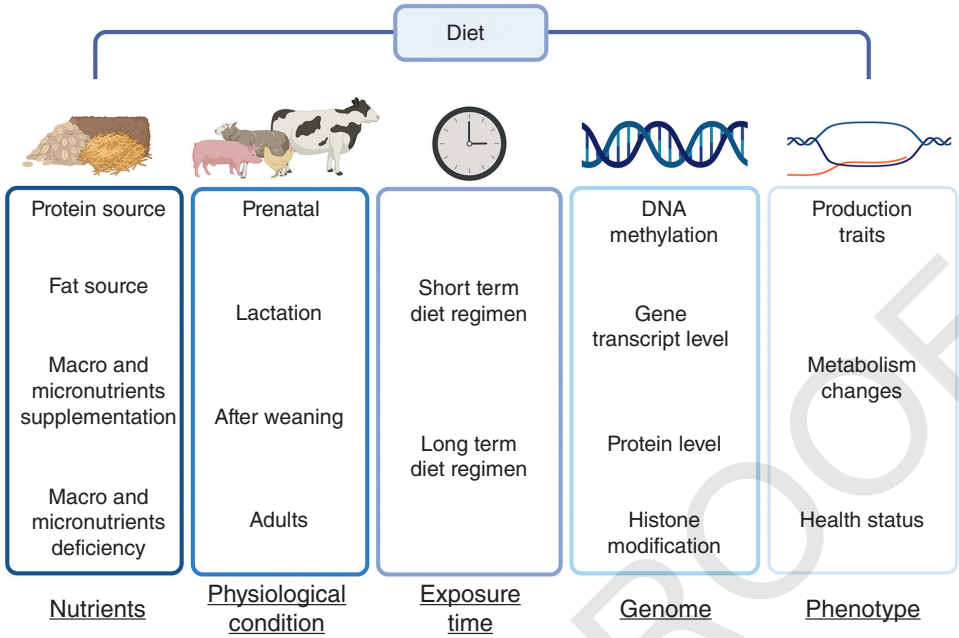

Figure 5.1 Overview of dietary aspects examined in nutrigenomic studies. Source: Modified by Nowacka-Woszuk (2020). The figure was partly generated using Servier Medical Art, provided by Servier, licensed under Creative Commons.

nutrient–gene interactions through the intermediate action of transcriptional regulatory factors (TFs) in the short to medium term and indirect epigenetic factors in the medium to long term.

Effect of nutrients on transcription factors

Bioactive nutrigenomic molecules directly or indirectly activate or repress TFs; thus, a nutrient–gene interaction is a TF-mediated interaction with the genome rather than the direct binding of nutrients to the genome. Some of the main TFs are reported in Table 5.1.

PPARs and SREBPs are the most common transcription factors studied. PPARs are a family of nuclear receptors that bind to fatty acids and regulate the transcription of genes involved in nutrient metabolism and energy homeostasis. PPAR isoforms work as heterodimers with the retinoid X receptor, and together, both bind to a specific DNA sequence in the promoter region of the target gene, thus inducing or repressing its transcription.

There are three PPAR isoforms, α, γ and β, which differ in terms of target tissue, physiological properties and developmental tissue stage (Ladeira et al. 2016). PPARγ is highly expressed in adipocytes and less in the muscle and plays a crucial role in controlling adipogenesis, lipogenesis and insulin sensitivity. PPARα is highly expressed in the liver, followed by the small intestine, adipose tissue and heart, while PPARβ is distributed throughout the body. In the liver, PPARα plays a key role in fatty acid oxidation by inducing the expression of long-chain fatty acid (LCFA) transporter proteins and other enzymes involved in peroxisomal β-oxidation (Ladeira et al. 2016).

PPARγ is the main regulator of fatty acid storage and adipogenesis, as it binds to the genes associated with lipid metabolism, including those that encode the fatty acid-binding protein (FABP), acyl-CoA synthetase long-chain family member 1 (*ACSL1*) and lipoprotein lipase (LPL). PPARγ thus plays a key role in ruminant research, especially because it is associated with candidate genes in regulating marbling. As with the other nuclear receptors, PPARγ also binds to and becomes activated by lipophilic molecules (fatty acids) causing transcription regulation. PPARγ can bind to two different fatty acids at the same time and is not specific for a single fatty acid, thus highlighting its ability to bind a

Table 5.1 Main transcriptional factors with nutrigenomic potential.

Protein	Agonist	Nutrigenomic activity
RARα	Retinoic acid	Development, differentiation, apoptosis
RARβ	Retinoic acid	Embryonic morphogenesis, cell growth, differentiation
RARγ	Retinoic acid	Limb bud development, skeletal growth, matrix homeostasis
PPARα	Fatty acids	Fatty acid metabolism, inflammation, tissue regeneration
PPARβ/δ	Fatty acids	Fatty acid metabolism, tissue regeneration, epidermal proliferation
PPARγ	Fatty acids	Adipogenesis, insulin sensitivity, lipogenesis
LXRα	Oxysterol/fatty acids	Cholesterol homeostasis, macrophage functions, inflammation
LXRβ	Oxysterol/fatty acids	
VDR	Vitamin D	Mineral metabolism, immune response
PXR	Vitamin E	Detoxification
HNF4α	Fatty acids	Liver, kidney and intestine development
RXRα	9-*cis*-retinoic acid	Leukocytes differentiation, formation of heterodimers with other IdNRs
RXRβ	9-*cis*-retinoic acid	Embryonic morphogenesis, cell growth, differentiation
SREBP1	Long-chain fatty acids	Role of milk fat and protein synthesis

Source: Adapted from Bionaz et al. (2015).

mixture of fatty acid molecules. In addition to lipogenesis, PPARγ may play an essential role in LCFAs oxidation by controlling the expression of carnitine palmitoyltransferase 2 (CPT2) and carnitine acetyltransferase (CRAT), which are involved in the entry of LCFAs into the mitochondria (Bionaz et al. 2015; Ladeira et al. 2016).

Another important TF is SREBP, which plays a crucial role in energy homeostasis, promoting glycolysis, lipogenesis and adipogenesis. The SREBP family has three members: 1a, 1c and 2. SREBP-1c is encoded by the sterol regulatory element-binding transcription factor 1 (SREBF1) gene and seems to act more specifically on the genes involved in fatty acid synthesis, while SREBP-2 has a greater influence on the regulation of the expression of cholesterogenic genes. SREBP-1c was originally identified in white adipose tissue and was initially named adipocyte determination and differentiation factor (ADD-1). It is also expressed in the liver and in the mammary gland (Bionaz et al. 2015; Ladeira et al. 2016).

Epigenetic effects of nutrients

Indirect methods concern epigenetic mechanisms. The term 'epigenetics' is used to refer to gene expression that occurs without changes in the DNA sequence. Although several studies have shown that there are numerous nutrients and bioactive compounds that can influence different pathways through which epigenetics affects gene expression, there is still relatively little information on the precise mechanisms through which nutrients modulate epigenetics. These multiple mechanisms are mutually compatible and may operate together over time, adding to the complexity of this regulatory pathway (Bordoni and Gabbianelli 2019).

The epigenetic mechanisms studied in the literature are many (ubiquitination, phosphorylation, transposons, miRNAs), but the most important are DNA methylation and histone acetylation. In eukaryotic cells, DNA is enveloped in chromatin in the nuclei. The nucleosome, the basic unit of chromatin, consists of 146–147 base pairs of DNA and an octamer of histones with one H2A-H2B tetramer and two HE-H4 dimers (Figure 5.2).

The N- and C-terminal tails of these histone proteins can be modified, for example, by methylation and acetylation. These modifications can change the electronic charge and structures of these histone tails, which bind to DNA, to alter chromatin status and subsequent gene expression (Zhang et al. 2021).

- *DNA methylation/histones*. Most of the knowledge on the ability of nutritional factors to modulate gene expression through epigenetic mechanisms refers to one-carbon metabolism, a complex network of interrelated biochemical reactions in which methyl donor nutrients

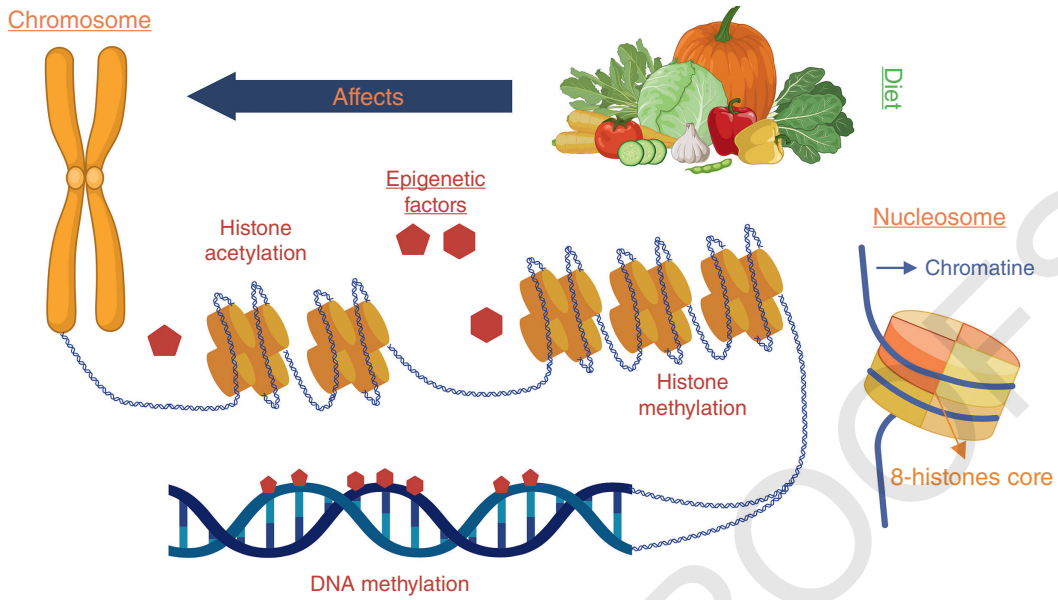

Figure 5.2 Histone/DNA methylation and histone acetylation. The figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported licence.

provide carbon units to various biochemical and molecular reactions (Bordoni and Gabbianelli 2019). Nutrients are processed through the folate cycle and the methionine cycle, which serve as methyl sources for the universal methyl donor, *S*-adenosylmethionine (SAM). The methyl group of SAM can be enzymatically transferred to other molecules (cytosine in DNA), generating *S*-adenosylhomocysteine (SAH) as an end product. At the level of the histone, on the other hand, the methyl group is added to the amino acids of the histone protein. As shown in Figure 5.2, the methyl group, which binds at the cytosine level of the DNA or histone, creates a clutter that prevents transcription factors from initiating gene transcription. The main nutrients and bioactive molecules involved in this mechanism are shown in Table 5.2 (Choi and Friso 2009).

- *Histone acetylation*. This process involves the addition of an acetyl group to the amino acid lysine in the N-terminal of histones H3-H4 (Turner 2000). This reaction is catalysed by histone acetyl transferases and results in the removal of the positive charge of the lysine side chain, causing a decreased binding to the histone. In this way, chromatin takes on a more relaxed conformation, allowing the binding and action of transcription factors, as shown in Figure 5.2. The reverse process, deacetylation, removes acetyl groups causing chromatin to thicken, thus blocking gene transcription.

Table 5.2 Main compounds involved in epigenomic methylation processes.

Nutrients	Role
Dietary methyl donor nutrients (methionine, choline, betaine, serine)	Dietary methyl donor nutrients
B vitamins (B12, B6, B2, B9)	Act as acceptor or donor of methyl groups
Micronutrients (zinc, retinoic acid, selenium)	Affect one-carbon metabolism
Bioactive food compounds	Modulate DNA methyltransferases' activity

Nutrigenomics and productive traits

To date, the nutrigenomic approach has been used in farm animals to study the effects of nutrients on well-being and animal performance, the modulation of microbiota and aspects related to environmental sustainability and, to a lesser extent, production traits.

However, the productive aspect is gaining importance in research on livestock production. In fact, the study of the nutrient-genome interaction using transcriptomics approaches (i.e. microarrays and next-generation sequencing) can detect key molecular markers related to animal production traits and thus the subsequent application of specific dietary guidelines required to achieve particular production goals.

Most transcriptomics and nutrigenomics studies are mainly related to milk production in dairy cows, beef cattle and meat production in pigs. In terms of milk, studies tend to focus on milk yield, fat composition, fat and protein content and fatty acid modifications in milk following specific dietary regimens. Studies on meat deal mostly with several features related to quality, fat composition, texture and marbling (Ladeira et al. 2016). With regard to the transition period in dairy cows (Elolimy et al. 2019), the influence of nutritional interventions on metabolic health and specifically on hepatic metabolism through altered gene expression has been widely addressed.

Table 5.3 lists examples of how nutrients and dietary interventions can alter the genome or epigenome of livestock animals, mainly regarding genetic production traits. Most genetic traits for meat and milk production depend on a multifactorial background. For an extensive knowledge of the manipulation of dietary regimens, both the genetic background and the effects of the environment need to be considered. For instance, the response to diet is dependent on both inherent genetics as well as environmental features and variables that are reflected in the quality of animal products, such as meat and milk (Benítez et al. 2017).

5.3 Dietary interventions modulating gene expression related to production traits in livestock animals

Ruminants

Ruminants such as dairy cows and beef cattle play a key role in livestock production since they can convert undigestible plant biomass into the energy and animal proteins found in ruminant-derived food, i.e. meat and milk (Pinotti et al. 2021). Nutrigenomics can help researchers and farmers in understanding the biological processes related to milk synthesis, meat quality and productive yields, thus leading to specific dietary interventions focused on improved performance and production (Baldi and Pinotti 2008).

However, it is clear that nutrients can affect gene expression directly or indirectly. Nutrients are thus bioactive molecules because not only do they provide energy and building blocks for cells, but they also act as signals detected by cellular sensors, which provoke a change in the physiological state of the cells. However, other components in the diet can also have nutrigenomic effects without being specifically bioactive, for example, the restriction of dietary energy (Bionaz et al. 2015; McFadden et al. 2020). As mentioned previously, the nutrient and energy levels can affect the genome, particularly through transcriptional regulators. The biology of the organism can thus be fine-tuned by modifying the diet (Bionaz et al. 2015).

Dietary interventions with different lipid sources can alter the gene expression in the adipose tissue. Two different lipid sources in steer diets have been found to alter the gene expression related to lipid synthesis and adipogenesis. A palm oil-supplemented diet has been shown to decrease the expression of AMP-activated protein kinase alpha (AMPKα) and CCAAT enhancer binding protein β (CEBPβ), whereas a soybean-supplemented diet reduced the expression of SCD gene (Choi et al. 2016). The modulation of diet through different lipid sources was also found to alter several genes related to lipid metabolism in German Holstein bulls. A diet rich in *n*-3 fatty acids was found to decrease the *n*-6/*n*-3 ratio and downregulate the gene expression of sterol regulatory element-binding protein-1 (SREBP1), acetyl-CoA carboxylase alpha (ACACA), Fatty acid sinthase (FAS) and SCD compared to a control diet rich in *n*-6 fatty acids, thus altering lipogenesis in adipose tissue (Hiller et al. 2011). In another study (Vogel et al. 2021), dairy cows fed with a diet supplemented with linseed oil rich in *n*-3 fatty acids increased the growth hormone receptor 1A (GHR1A), insulin receptor (INSR) and insulin-like growth factor 1 (IGF1) gene expression in the liver. Therefore, *n*-3 fatty acids affect both lipogenesis and somatotropic axis-related genes (Vogel et al. 2021).

Table 5.3 Nutrients with epigenetic and genetic modulation potential in different farm animals.

Nutrients	Species	Dietary treatment	Effects
Methionine	Bovine	Rumen-protected methionine (0.09% DM) in peripartal period (21–130 d in milk)	↑ Methylation of PPARα, ↓ global DNA methylation (liver)
	Swine	30% methionine > basal diet methionine content in weaning piglets (21 d)	↑ Methylation of MSTN (skeletal muscle)
	Poultry	L-Methionine, DL-methionine and methionine analogue, DL-2-hydroxy-(4-methylthio)-butanoic acid to provide 0.22% methionine equivalent	↔ Global DNA methylation in male cobb-500 broilers at 3 d of age (liver)
Methyl donors (choline, betaine, folic acid)	Swine	Maternal supply with betaine (3 g/kg diet), choline (400 mg/kg diet), folic acid (15 mg/kg diet), vit B12 (150 mg/kg diet)	↑ Methylation of IGF1 in offspring pigs at 110 kg (offspring liver) ↑ Methylation of SLC15A1 in offspring pigs at birth (offspring small intestine)
Methionine	Bovine	Rumen-protected Met (RPM 1.05 g/kg of DMI) during a heat stress (HS) challenge	RPM may help cows maintain homeostasis in mTOR, insulin signalling and 1-carbon metabolism
Choline	Ruminants	Dairy cows exposed to different levels of dietary energy (DE) or supplementing rumen-protected choline (RPC) or both	The three-way interaction of energy × choline and days in milk affected expression of carnitine palmitoyltransferase 1A, glucose-6-phosphatase and peroxisome proliferator-activated receptor α and tended to affect cytosolic phosphoenolpyruvate carboxykinase
Betaine	Wine Poultry	Maternal supply with betaine (3 g/kg diet) Breeders 0.5% betaine-supplemented diet	↑ Methylation of GALK1 in male piglets at birth (offspring in liver) ↓ Methylation of SREBF1, SREBP-2 and APOA1 (Hypothalamus) in offspring broilers at 56 d of age, ↑ Methylation of DIO1 (liver)
Vit. B12	Sheep	Oocytes matured *in vitro* in a medium supplemented with 200 pmol/l vitamin B12	↑ Global DNA methylation (embryonic cells)
SFA and PUFA	Swine	Pigs fed SFA-enriched diet (5% hydrogenated lard) and pigs fed with PUFA (5% sunflower oil)	Upregulation of SCD, ACACA and ME1 genes in SFA dietary group (adipose tissue, liver, muscle)
Selenium	Swine	Diet with added organic Se (0.3 mg/kg)	Modulatory effect on multiple physiological pathways (leukocytes)
	Bovine	Se supplements that contained none (control) or 3 mg Se/d in ISe (sodium selenite) or OSe	Ise and OSe supplementation upregulated mitochondrial gene expression capacity. Improvements reported in the ADG of OSe-supplemented feedlot steers and calves born from OSe-supplemented cows (liver)
Vit. A	Swine	Three groups: Vit A-enriched diet (10,000 IU vitamin A/kg) (CTR), without supplementation early restriction group (ER), late restriction group (LR)	Small Vit A effect observed on fatty acid composition (backfat, liver) ↑ ACSL4, CEBPB and IGF1 genes in the ER group (hepatic tissue) ↑ CRABPII and SCD genes in ER group (adipose tissue) ↓ RXRG gene in the ER group (adipose tissue) Long-term restriction of dietary vitamin A →positive effect on nutritional/sensorial parameters of ham meat. Gene expression results →consistent with the vitamin A transcriptional regulation of adipogenesis and lipogenesis (meat and fat composition)

Table 5.3 (Continued)

Nutrients	Species	Dietary treatment	Effects
Omega-6	Bovine	Steers fed increasing levels of dietary corn oil, three groups: 0 (NONE), 0.31 kg/d (MED) and 0.62 kg/d (HI)	MED level of oil supplementation ↑ gene expression of key lipogenic enzymes, with HI level mRNA encoding lipogenic enzymes responsible for *de novo* synthesis and desaturation are ↓ regulated. Changes in specific lipogenic mRNA → ↓ of *de novo* and desaturated FAs with HI of oil supplementation)
Oleic acid	Swine	ST diet with carbohydrates as energy source (CH) or diet enriched with high oleic sunflower oil (HO)	RXRG, LEP and FABP5 genes ↑regulated in HO group ME1, FASN, ACACA and ELOVL6 ↑regulated in CH Higher ME1 gene expression in CH than HO groups ↑ *de novo* endogenous synthesis of FA in the CH group and a ↑ FA transport in the HO group PPARγ showed higher expression in finishing pigs (adipose tissue)
Linoleic acid	Swine	Pigs fed with CLA-supplemented diet	Proteome changes in LM contributed to greater intramuscular lipid content in CLA-supplemented pigs (muscle)
Vit. E	Poultry	Chickens received three treatments: control corn-soybean diet, control supplemented with red grape pomace (8%) and control supplemented with VE (200 IU) Control diet Control diet + 50 IU of Vit E/kg, Control diet + 100 IU of Vit E/kg, Control diet + 200 g of EcoE/ton	Higher expression of CAT gene in both Vit E- and GP-supplemented diets compared to control HMOX2 gene showed higher expression in GP than Vit E groups SCP2 gene (associated with increasing PUFA contents) has higher expression in supplemented animals (muscle) Altered expression of 542 genes in breast muscle, of which a significant amount was regulated by EcoE and Vit E (especially the control diet + 50 IU of Vit E/kg). EcoE and Vit E involved in cell morphology, skeletal and muscular system development and function, immune response and multiple metabolic processes, including lipid and carbohydrate metabolism (muscle)
Inulin	Poultry	Two dietary treatments: control diet without inulin, control diet + 5 g of inulin/kg of diet	Modification of the lipid metabolism in chickens fed with inulin (liver) Modulation of gene expression of three main classes: (i) basal processes, including tissue development and maintenance (muscle, nervous system processes, cell organelle processes, protein metabolism, gene transcription and response to hormones); (ii) immune system processes; and (iii) fatty acid metabolism

Source: Adapted from Benítez et al. (2017) and Elolimy et al. (2019).

Nutritional status and energy balance during the transition period (before and after calving) are two nutrigenomic targets in dairy cows. An insufficient feed intake during early lactation leads to increased body fat mobilisation in order to meet the energy demands for milk production. In this scenario, the hepatic energy metabolism plays a key role through increased endogenous glucose production, hepatic glucose output for milk synthesis and adaptation of post-calving fuel oxidation.

Cows differ both in their degree of fat mobilisation around parturition and total liver fat concentration, thus affecting hepatic gene expression involved in gluconeogenesis, fatty acid oxidation, ketogenesis and cholesterol synthesis

as well as transcriptional factors related to energy metabolism. In addition, the mRNA abundance of most enzymes and transcription factors changes over time during the transition period (Weber et al. 2013). Pyruvate carboxylase (PC) mRNA was found to increase at parturition to a greater extent in cows with high and medium liver fat accumulation than in the low liver fat group. Significant liver fat content for carnitine palmitoyl-transferase 1A (CPT1A) and acyl-CoA synthetase, long chain 1 (ASCL1) during the transition period indicated variable gene expression caused by liver fat accumulation. Hepatic gene expression, performance and plasma concentrations of metabolites and hormones were found to correlate positively with time-specific relations during the transition period. Elevated body fat mobilisation during early lactation affects gene expression involved in gluconeogenesis to a greater extent than gene expression involved in lipid metabolism, indicating the dependence of hepatic glucose metabolism on hepatic lipid status and fat mobilisation during early lactation.

Rumen-protected methionine (RPM) supplemented in the diet of Holstein cows was found to alter the expression of genes related to methionine metabolism, such as betaine-homocysteine S methyltransferase 2 (BHMT2), cystathionine-β-synthase (CBS) and adenosylhomocysteinase (AHCY). This alteration was related to the level of SAM and thus the methylation cell potential (Jacometo et al. 2017). Rumen-protected methionine has also been investigated for its potential effects in mitigating heat stress (HS). Exposing cows to an HS challenge increased AKT activation and abundance of INSR and SLC2A4 in liver tissue, thus dysregulating hepatic insulin signalling, while at the same time the dry matter intake decreased and gluconeogenesis upregulated. HS has also been found to upregulate hepatic 1-carbon metabolism and downregulate innate immune function. However, feeding RPM during an HS challenge attenuated increases in liver insulin signalling, maintained homeostasis in hepatic 1-carbon metabolism, whole-blood *trans*-sulphuration and antioxidant gene expression, and upregulated mTOR signalling. RPM was therefore shown to promote liver function and antioxidant mechanisms in whole blood. In the same direction, the peripartum supplementation of rumen-protected choline and the controlled prepartum energy supply were two factors involved in the alteration of gluconeogenesis (glucose-6-phosphatase [G6PC], [PC], cytosolic phosphoenolpyruvate carboxykinase [PCK1]) or lipid metabolism (carnitine palmitoyltransferase 1A [CPT1A], microsomal TG transfer protein [MTTP] and peroxisome proliferator-activated receptor α) (McFadden et al. 2020). The support of hepatic glucose and lipid metabolism is part of the mechanism for production and health responses associated with these peripartum interventions, mainly focused on the supply of methyl group sources (such as choline and methionine). Several studies (see McFadden et al. 2020) have indicated that the dietary supplementation with rumen by-pass methyl group sources (i.e. rumen-protected methionine, choline as well as folic acid, vitamin B12 and betaine) is a potential nutritional approach to target one-carbon pools and improve methyl donor balance, especially in transition cows. Such nutritional intervention has been investigated in several researches, demonstrating the ability of cows to improve milk production efficiency, milk protein synthesis, hepatic health and immune response (see McFadden et al. 2020).

The role of nutrigenomics has also been investigated in milk synthesis in the mammary gland. Extensive research on lipid uptake, trafficking and secretion at the cellular level has been carried out over the last 30 years. Much of the knowledge has been obtained by studying milk fat depression. The effect of t10–c12 CLA (C18:2 *cis*-9, *trans*-11) on depressing milk fat synthesis via the inhibition of SREBP1 is among the major nutrigenomic examples in dairy cows. Regarding the transcription factor SREBP-1c, grain-rich diets appear to decrease the expression of the SREBF1 gene in the mammary gland due to a decrease in ruminal pH, which might alter the biohydrogenation pathways and increase C18:2 *trans*-10, *cis*-12 synthesis. The increase in this metabolite (fatty acid) decreases SREBF1 mRNA levels, consequently decreasing the activity of the enzymes involved in *de novo* synthesis.

The biohydrogenation theory of milk fat depression proposed by Bauman and Griinari (2001) involves the concept that rumen biohydrogenation can produce *trans*-10, *cis*-12 CLA,

which seems to be a powerful inhibitor of milk fat synthesis. In fact, post-ruminal infusion of *trans*-10, *cis*-12 CLA reduced the mRNA abundance of genes involved in fatty acid uptake (LPL), fatty acid transport (fatty acid binding protein), *de novo* fatty acid synthesis (acetyl-CoA carboxylase, ACC and fatty acid synthase, FAS), desaturation (stearoyl-CoA desaturase, SCD) and triglyceride synthesis (acylglycerol phosphate acyltransferase and glycerol phosphate acyl transferase) in the mammary gland.

Levels of *trans*-10 and *cis*-12 CLA in milk fat have also been correlated with decreased ACC mRNA and with lower levels of the enzymes FAS, LPL and glycerol-3-phosphate acyltransferase (GPAT) (Griinari and Bauman 2006). Unsaturated fatty acids are also regulators of the fatty acid profile of milk. Desaturase activity in the mammary gland cells not only converts stearic acid arising from ruminal biohydrogenation to oleic acid, which is secreted in milk, but is also involved in the synthesis of CLA isomers in the mammary gland (Bauman and Griinari 2001). However, the availability of substrates in the diet is the main factor influencing the content and profile of milk fatty acids and affecting the expression of various lipogenic genes in the mammary gland.

Transcriptomic studies in cows have shown that vegetable oil supplementation often affects the expression of genes involved in the remodelling of the mammary gland, which might be accompanied by modifications in the milk FA profiles. As previously reported (see Baldi and Pinotti 2008) in several studies, supplementation with unsaturated FAs (using either unprotected rapeseed, soybean, linseed oils or a proportional mix of them) induced a reduction in short-chain FAs, reflecting a decrease in *de novo* FA synthesis. These kinds of studies not only showed an altered expression of genes involved in molecular transport, lipid and protein metabolism and nutrient metabolism but also some side effects on a set of genes involved in cell development and remodelling, apoptosis and immune response. These latter pathways were predominantly downregulated and negatively correlated with milk *trans*-FA concentrations.

The type of fat source is also important. In fact, studies on different plant oil supplementations have shown different effects on gene expression. In particular, linseed oil affected the expression of more than 1,000 bovine mammary gland genes, whereas 5% of safflower oil affected less than 200 genes. However, both diets led to a decreased milk fat percentage, with a decrease in saturated FAs, including short-chain FAs, which was due to the decrease in *de novo* synthesis in the mammary gland. The interaction between the nature of the lipid and the basal diet also had different effects on gene expression. Supplementation with sunflower oil (4%) in a low-forage-based diet thus showed a higher amplitude of milk composition and mammary transcriptome responses than supplementation with whole intact rapeseed (14%) in a high-forage diet.

Similar supplementation diets have been studied in caprine mammary transcriptomes, which showed lower responses in goats than the responses observed in cows. The same scenario has also been reported for marine oils and marine material (algae), which are able to decrease milk fat synthesis in the mammary gland. These findings indicate that not only different dairy ruminants but also the individual ingredients and their specific combination in the diets, can change the transcriptome and thus the productive response of the mammary gland (Hue-Beauvais et al. 2021).

In terms of beef cattle, the scenario is similar. To date, the results in the literature indicate that the expression of genes involved in lipid metabolism is influenced by beef cattle nutrition and that diet manipulation might change muscle marbling and the molecular composition of fat in beef. Among the options for dietary manipulation, PUFA sources, starch concentration, forage proportions and vitamins stand out (Ladeira et al. 2016).

In terms of the transcription factors involved, those associated with lipid metabolism, such as PPARs and SREBPs, are the most important. PUFA supplementation inhibited the expression of the gene encoding for SCD in beef cattle. SCD1 expression was also significantly reduced in the subcutaneous adipose tissue of cattle-fed diets with a high *n*-3 PUFA content, which reduced CLA and oleic acid (C18:1 *cis*-9) contents (Herdmann et al. 2010). These findings are consistent with the data in the literature on the inhibitory effects of PUFAs on SCD1 in other

species (Flowers and Ntambi 2008). Different expression levels of this gene in response to dietary manipulation suggest the existence of a tissue-specific mechanism and possibly different actions of the transcription factors related to their regulation in ruminants.

Regarding different dietary starch sources, beef cattle fed a diet composed of corn silage and ground corn showed an increased muscle expression of the fatty acid binding protein 4 (FABP4), ACACA and SCD1 genes. In the same study, beef cattle fed with a diet containing whole-shelled corn without forage showed an increased muscle gene expression of peroxisome PPAR-α and a reduced expression of SREBF1. The diet containing whole-shelled corn therefore did not increase fat marbling due to a reduced SREBF1 expression in muscles and thus a reduced lipid synthesis (Ladeira et al. 2016). Similarly, Graugnard et al. (2009) found higher FABP expression in longissimus lumborum of animals fed a high-starch diet, and Peng et al. (2012) found higher LPL expression in the muscle of animals fed a diet with a higher energy density. When the forage content was considered, animals fed a diet with a high forage percentage tended to exhibit a higher LPL expression in the muscle.

In contrast, Zhang et al. (2015) found greater LPL and FABP expression in subcutaneous adipose tissue of animals fed high-energy diets compared to low-energy diets. According to these authors, an increased dietary energy content might improve nutrient digestion and absorption, and thus stimulate the expression of the genes responsible for fatty acid transport and traffic. The cellular uptake of the circulating triglycerides in lipoproteins, such as chylomicrons, is mediated by the action of the enzyme LPL and then carried by FABP4, which is responsible for the transport of fatty acids into cells. LPL and FABP4 expression levels therefore depend on the energy content of the diet. In addition, changes in the composition of the dietary fatty acids that are absorbed into the small intestine might alter the expression of PPARα in the *longissimus dorsii* muscle, due to the positive correlation between those genes. When the expression of one gene increases, the others therefore exhibit a similar behaviour (Ladeira et al. 2016).

Although LCFA are clearly the most powerful, short-chain fatty acids have been suggested to play a nutrigenomic role. Saturated compared with unsaturated LCFAs have a greater nutrigenomic effect *in vitro*, likely through PPAR (Bionaz et al. 2015). *In vivo*, the effect of saturated LCFA is more modest, with contrasting effects among tissues.

The nutrigenomic effects of amino acids are also being investigated, particularly for the regulation of milk protein synthesis-associated genes. Molecular studies have examined the nutrigenomic effect of single amino acids on milk protein synthesis *in vitro* with primary bovine mammary cells. Some of the focus has been on lysine (Lys) and methionine (Met), which are thought to be the most limiting aminoacids for milk synthesis. Nan et al. (2014) provided evidence that peak synthesis of casein at a Lys to Met ratio of 3:1 was driven partly by an increase in mTOR phosphorylation but also the upregulation of mRNA expression of MTOR itself, as well as casein and lactalbumin genes, and the transcription regulator E74-like factor 5. Another study provided evidence that Arg, a conditionally essential aminoacids, is also capable of increasing the expression of casein genes along with MTOR, RPS6KB1 and STAT5 and decreasing the expression of the translation inhibitor 4EBP1 when supplemented at a level equivalent to 2× the concentration found in casein.

Pigs

The diet of pigs can be enriched in fatty acids to manipulate the fatty acid metabolism and the lipid deposition in tissues in order to improve the meat quality and ensure the nutritional value of meat (Nowacka-Woszuk 2020). The nutrients, in this case fatty acids, influence the genetic traits associated with lipid metabolism (Benítez et al. 2017; Malgwi et al. 2022). For example, a pig diet enriched in PUFAs leads to a decreased SCD gene expression. SCD is a desaturase enzyme catalysing the synthesis of MUFA, thus being a regulator of lipid metabolism. A pig diet enriched in saturated fatty acids led to an upregulation of lipogenic genes in the liver such as SCD, ACACA and ME1. ACACA and ME1 are both involved differently in fatty acid

biosynthesis (Benítez et al. 2017). ACACA gene expression was also shown to be affected by the supplementation of palm oil in a low-protein pig diet, whereas soybean oil did not exert the same effect, also suggesting that the specific fatty acid composition of the oil needs to be considered.

A transcriptomics-based investigation on pigs fed a diet supplemented with PUFA (linoleic acid and α-linolenic acid rich in *n*-6 and *n*-3) showed an altered expression of 3,500 genes in adipose and muscle tissues related to fatty acid biosynthesis and activation, lipid transport and binding, thus showing that this dietary intervention significantly affected the lipid metabolism (Malgwi et al. 2022). Regarding proteins, weaned piglets fed a glutamine-rich diet showed a positive correlation with genes responsible for small intestine growth, body weight gain and intestinal antioxidant capacity (Wang et al. 2008).

Supplementation of L-carnitine in the diet of growing piglets altered the muscle transcriptome, in particular the expression of 211 genes, which were mainly involved in the upregulation of insulin-like growth factor (IGF) binding and insulin receptor binding pathways. Overall, this supplementation exerted a beneficial effect on skeletal muscle mass (Keller et al. 2011)

However, in pig production, one of the main nutrigenomic focuses is the intra muscular fat (IMF) content (Malgwi et al. 2022). It is known that the IMF content in pigs is indirectly influenced by the majority of fat metabolism-related genes. The effects of these genes vary in terms of the lipogenesis and adipogenesis mechanisms. For example, local pig breeds (especially Italian Landrace, local Basque, local Wujin, Mangalitsa, Meishan, etc) showed a higher IMF content and better meat quality traits compared to modern breeds. The higher presence of IMF is justified by the higher expression of genes and enzymes involved in fatty acid synthesis and lipid metabolism. Specifically, in addition to the formation of mature adipocytes, fatty acid and triglyceride synthesis and storage (lipogenesis) are crucial in determining IMF in pigs. Both mechanisms occur under the regulation of adipogenic (e.g. PPARγ) and lipogenic genes (Malgwi et al. 2022).

The main genes involved in adipogenesis, lipogenesis and IMF deposition are reported in Table 5.4.

Poultry

Nutrigenomics in poultry research is useful in terms of improving performance, feed efficiency and the quality of meat and eggs. As with pigs, diet can influence the transcriptome of poultry.

Few studies have reported the effects of supplementation or deprivation of specific amino acids. Some authors have investigated the supplementation of arginine (Arg) and glutamine (Gln), either alone or in combination, as well as amino acid-based solution (MIX) containing Arg, Gln, threonine (Thr) and grape extract in broiler chickens challenged with dexamethasone (DEX) as a gut barrier dysfunction model (Barekatain et al. 2021). Growth performance was not affected by the treatments, but the feed conversion ratio was higher with the supplementation of Arg, Arg and Gln or MIX. The amino acids restored the ileal levels of Nrf2 and IL1β, which were impaired by DEX, thus restoring/repairing the gut inflammation and permeability (Barekatain et al. 2021). The *in ovo* injection of methionine upregulated several genes responsible for growth and metabolism (somatostatin, R5 and thyroid stimulating hormones) and antioxidant defence (superoxide dismutase, glutathione *S*-transferase and glutathione peroxidase) in the liver of newly hatched broiler chicks (Alagawany et al. 2022).

A methionine-deficient diet decreased the transcription of methionine adenosyl transferase 1 gene at the hepatic level, thus reducing the conversion rate of methionine to *S*-adenosyl methionine (SAM). SAM is an important methyl donor, and a low level was found to be positively correlated with the reduced growth performance of poultry fed the methionine-deficient diet (Aggrey et al. 2018). The deficiency of other dietary amino acids, such as cysteine, led to an increased expression level of cystathionine beta synthase (CBS) and cystathionine-lyase (CTL), thus altering the *trans*-sulphuration pathway. The disruption of the *trans*-sulphuration pathway affected thermoregulation and subsequently the growth rate, feed efficiency and well-being of poultry (Vilar da Silva et al. 2020).

As previously mentioned, the level of dietary amino acids is essential to ensure animal welfare, growth performance and feed efficiency. These features are strictly related to productive poultry

Table 5.4 Genes directly or indirectly involved in fat metabolism and IMF content in pigs.

Genes involved	Pig breed	Tissue	Trait
FABP4 and FASN	Chinese local, Large White	*Longissimus dorsii*, liver	IMF
ADIPOQ, PPARG, LIPE, CIDEC, PLIN1, CIDEA and FABP4	Purebred Duroc	*Longissimus dorsii*	IMF
ATGL, FAS, HSL, CPT-1B, SREBP-1c, SCD, A-FABP and H-FABP	Wujin, Landrace	*Longissimus dorsii*	IMF
RAD9A, IGF2R, SCAP, TCAP, SMYD1, PFKM, DGAT1, GPS2, IGF1, MAPK8, FABP, FABP5, LEPR, UCP3, APOF and FASN	Landrace, Sonigliao, Black sows	Subcutaneous fat, *Longissimus dorsii*, liver	Fat deposition
H-FABP and LEPR	Duroc, Pietrain, Puławska, Polish Large White, Polish Landrace	*Longissimus dorsii*, semimembranosus muscle, liver	Fat deposition and IMF
FABP3 and LEPR	Duroc, Pietrain, Puławska, Polish Large White, Polish Landrace	*Longissimus dorsii*	Fatty acid metabolism and IMF levels
FABP3 and LEPR	Korean native pig, Yorkshire crossed animals	*Longissimus dorsii*	IMF
H-FABP and MASTR	Large White	Blood	IMF
PRKAG3	Large White × Duroc × Pietrain	Skeletal muscle	IMF
EEF1A2, FABP3, LDLR, OBSCN, PDHB, TRDN and RYR1	Landrace × Large White × Pietrain	*Longissimus dorsii*	IMF
IGF2	Large White, Polish Landrace, Puławska pigs	Blood	IMF
PPARG and ADRP	Laiwu, Lulai Black, Large Whites	*Longissimus dorsii*	Fat deposition and IMF
PPARA, PPARG, SCD and PCK2	Shanzhu × Duroc commercial crossbreds	*Longissimus dorsii*	Lipid deposition and IMF
BMPER promoter	Duroc × Large White × Yorkshire	*Longissimus dorsii*	IMF
FABP3 promoter	Large White × Landrace background × Pietrain	*Longissimus thoracis* and lumborum, semimembranosus muscle, blood	IMF
SCD and LEPR	Duroc	Gluteus medius, *Longissimus dorsii*	IMF and fatty acid composition
FASN and LIPE	Jinhua and Landrace	Subcutaneous adipose	IMF
CAV2, MYOZ2, FRZB, FASN, SCD, ESR1 and ADORA1	Chinese Diannan Small-ear pig, Tibetan, Landrace and Yorkshire	*Longissimus dorsii*	Lipid deposition and muscle growth
SCD, ACACA and FASN	Puławska, Polish Large White and Polish Landrace	*Longissimus dorsii*, blood	IMF and lipid metabolism
MSTN	MSTN-knockout cloned Meishan	Subcutaneous fat, blood	Fatty acid metabolism
FGF2	Italian Large White	Semimembranosus muscle	IMF
FABP3, LIPE, IGF1, IGF2, LEP, LEPR, MC4R, PHKG1, RETN, RYR1, SCD and UBE3C	Chinese Shuai pigs	*Longissimus dorsii*	IMF
FASN, SCD, ELOVL6, DGAT2, PLIN1, CIDEC and ADIPOQ	Iberian	*Longissimus dorsii*	Lipid metabolism and higher content of IMF

Source: Adapted from Malgwi et al. (2022).

yields, and therefore it is crucial to supply them through the diet.

Other dietary interventions, such as the modulation of fat in poultry diets, also impact the gene expression and physiological response of poultry. In particular, specific fatty acids, fatty acyl-coenzyme A or eicosanoids in the diet impact expression of PPARs and the intracellular calcium level, which is linked to the activation or inhibition of signalling cascades (Alagawany et al. 2022). Apart from the main components of feed, there are also other constituents or supplements that can exert a nutrigenomic effect on poultry. Minerals such as zinc, iron and

cadmium modulate the mRNA expression of genes related to iron homeostasis (transferrin and ferritin) and inflammation (COX-2) in the intestine. In addition, phytochemicals and plant extracts used as feed additives impact the gene expression of the intestinal mucosa of broiler chickens. Thymol nanoemulsion was shown to upregulate IgA, IL-10, FABP2 and mucin 2 and downregulate IL-6 and IL-2 gene expression in the spleen and upregulate tight junction protein genes in cecal samples (Alagawany et al. 2022). Integrating resveratrol into the poultry diet was shown to both preserve gut integrity and permeability by upregulating jejunal occluding, claudin-1 and E-cadherin gene expression and upregulating the antioxidant-related gene expression, such as Nrf2, SOD1, GPX and GST. The same effect on antioxidant-related genes was also obtained by dietary supplementation with quercetin (Alagawany et al. 2022).

5.4 Intestine as a target tissue of nutrigenomics in monogastric animals

Nutrigenomics often focuses on the interaction between different bioactive molecules and targeted cells through the investigation of the global gene expression and proteomics of the systemic metabolites and tissues. Nutrients are crucial in regulating gut health because they interact directly with the intestinal epithelial barrier, gut microbiota and intestinal immune system.

Nutrigenomics considers nutrients as dietary signals that can influence genes, proteins and metabolites, and thus can be used to assess how nutrients can modulate gut health. Several genetic clusters responsible for productive traits can be influenced by nutrients, which can act directly as transcription factors or indirectly on gene products (Asmare and Negewo 2019).

Until recently, the gut microbiota was not considered in nutrigenomics-related studies despite being a key part in the host metabolism. The gut microbiota is affected both quantitatively and qualitatively by several factors, such as age and anatomical sites of the intestine. However, solid feed has a greater impact on bacterial community structure than age, solid feed type and environment.

The introduction of solid feed during weaning is probably the most stressful step in the pig's life, which can easily lead to post-weaning syndrome, characterised by high morbidity (e.g. diarrhoea), a high mortality rate, but also impaired gut barrier function and increased pathogen infection. This syndrome is mainly associated with gut microbiota dysbiosis, which leads to significant economic losses in the swine industry. The best strategies are thus being investigated to modulate gut microbiota composition in monogastrics via nutritional approaches such as probiotics, prebiotics, symbiotics or polyphenols in order to prevent gut microbiota dysbiosis and consequently, to prevent pathogen infections.

Fanalli et al. (2022) evaluated the effects of soybean oil in pigs' diets on gene expression and metabolic pathways, considering both skeletal muscle and liver. Initially, a higher level of soybean oil increased the level of MUFA deposition in muscle. Several differentially expressed genes (DEGs) were then identified between the two experimental diets containing different levels of soybean oil, in particular 45 DEG for skeletal muscle and 281 DEG for liver tissue. The authors observed that higher levels of soybean oil caused an increased level of genes related to lipid oxidation, metabolic diseases and inflammation. For example, muscle AL3A2 was upregulated, which can lead to a higher rate of lipid oxidation and cell damage caused by free radicals and inflammation, since AL3A2 also regulates the biosynthesis of leukotriene 4, a mediator of inflammation. The authors concluded that soybean oil can affect the transcriptome profile of skeletal muscle and liver tissue of pigs (Fanalli et al. 2022).

The effect of soybean meal, cottonseed meal (individually or in combination) and fishmeal on the gut microbiota has also been tested in pig diets. Fishmeal correlated positively with an increased abundance of Escherichia and Shigella, two bacterial taxa usually related to post-weaning diarrhoea in pigs (Cao et al. 2016).

Dietary fibre is another popular field of research because the differences among the fibre sources can lead to different outputs in the modulation of gut epithelial barrier and gut microbiota. Several fibres derived from different grain products have been tested in pig diets. Wheat bran fibre was found to increase villus height and the villus: crypt ratio in pig ileum compared to maize and soybean fibre. Wheat bran fibre also enhanced the integrity of the gut

barrier by increasing the transcription of the tight junction proteins, zonula occludens 1 and occluding (Chen et al. 2013).

Wheat bran fibre has a high dietary fibre content, ranging from 33.4% to 63.0%. The soluble fibre is usually <5% of total dietary fibre; therefore, the main component is non-soluble fibre. Non-soluble fibre has been related to increased growth in commensal gut bacterial taxa, leading to a higher production of volatile fatty acids (VFAs) and a subsequent lower pH in the large intestine. In an acidic environment, VFAs are able to mitigate the growth of bacterial pathogens such as *Salmonella*, *E. coli* and *Clostridia*. The overall effect of wheat bran fibre is increased gut health with higher epithelial integrity and a gut microbiota rich in commensal bacteria (Lindberg 2014; Yang and Zhao 2021).

In addition to lipid and fibre, several amino acids such as methionine, lysine, leucine, phenylananine, thr and tryptophan are essential in various metabolic pathways and gene expression. Research on the nutritional and physiological functions of essential amino acids has been very in-depth, and there are suitable recommended supplementations for pigs. Le Floc'h et al. (2018) reviewed these aspects with special emphasis on stress and local or systemic inflammation. Inflammation states alter animal metabolism in such a way that nutrients (particularly amino acids) are diverted from their use for growth towards the production of defence-related proteins and low-molecular-weight compounds (e.g. nitric oxide and glutathione) for supporting the activity of rapidly dividing cells such as immune cells and enterocytes. Furthermore, amino acids may act specifically as signalling molecules to regulate metabolic pathways during inflammation. For instance, the perfusion of a solution containing enterotoxigenic *Escherichia coli K88* (ETEC) into the jejunal loops from young pigs reduced the expression of genes encoding for apical and basolateral amino acid transporters, in association with the general upregulation of genes related to the induction of inflammation (see Le Floc'h et al. 2018). This finding could indicate that the acute inflammation induced by ETEC may reduce the intestinal absorption of some AAs as well as their concentrations in the blood. In addition, amino acids, such as lysine, are essential in defining several quality metabolic pathways and have a huge nutrigenetic impact on pigs. A low supply of lysine in the diet of heavy-finishing pigs promotes intramuscular fat deposition and better marbling. Studies showed that a 0.78% lysine supply results in higher intramuscular fat content in growing pigs.

Other authors have focused on the nutrigenomic evaluation of polyphenols, phytochemicals and plant-secondary metabolites as feed supplements in pig diets. In a study by Fiesel et al. (2014), a natural polyphenol-rich source (a grape marc meal extract) was used as a feed supplement for pigs to observe the effects on gut health. First, the grape extract downregulated the expression of several pro-inflammatory cytokines (such as ICAM1, IL-1β, IL-8 and TNF-α) in the intestinal mucosa. The authors also found lower VFA production and a subsequent increase in pH, confirming that polyphenols and plant extracts likely mitigate gut microbial fermentation (Fiesel et al. 2014). In another study (Zou et al. 2016), oregano essential oil was used as a feed supplement to assess its role in the gut health of pigs. It significantly increased villus height in the gut epithelial barrier and also increased the mRNA and protein levels of tight junction proteins, such as occluding and zonulin. This experimental diet also led to a decrease in the abundance of *E. coli* in the jejunum, ileum and colon compared to the control diet. The supplementation decreased mRNA levels of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, MCP-1 and INF-γ in the jejunum, compared to the control group (Zou et al. 2016). This latter result is similar to the one above (Fiesel et al. 2014).

Table 5.5 reports on how different nutritional strategies improve the gut health of pigs through the modulation of the gut microbiota.

Alterations in the microbiota composition have been correlated with metabolic disorders and unhealthy status, with increasing attention on the bacteria potentially shared across healthy individuals. The abundance of bacterial species inhabiting the intestinal ecosystem fluctuates since they are significantly influenced by the diet and other environmental variables. However, some bacteria shared by all individuals have been found to be more persistent and probably co-evolved with their host. This set of shared

Table 5.5 The effects of probiotics, prebiotics, symbiotics and polyphenols on gut microbiota in pigs.

Dietary strategy	Aim
Postbiotic heat-killed lactobacilli	Effects of heat-killed *Ligilactobacillus salivarius* strain 189 (HK LS 189) supplementation on anti-obesity and gut microbiota
Multi-strain probiotics	Effects of probiotics on microbiome composition, resistome, digestive function and oxidative stress responses
Multi-strain probiotics	Effects of maternal probiotic supplementation on gut microbiota and metabolites of sows and their suckling piglets
Two probiotics	Effects of two probiotics and one intramuscularly administered antibiotic treatment on the developing gut microbiome of post-weaning piglets between their third and ninth week of life
Multi-strain probiotics	Effects of supplementation with multispecies probiotics (MSPs) containing *Bacillus amyloliquefaciens*, *Limosilactobacillus reuteri* and *Levilactobacillus brevis* on the gut microbiota and faecal SCFAs and lactate levels of weaned pigs.
Seaweed polysaccharide	Dietary seaweed polysaccharide supplementation in pig used as prebiotics to positively modulate gut health and microbiota composition
Fermented wheat bran	Fermented wheat bran and yeast culture on growth performance, immune levels and intestinal microbiota in growing-finishing pigs
Pectin	Effects of pectin on the microbiome and mucosal immunity in pigs
Xylanase	Impact of insoluble corn-based fibre and xylanase on ileal digesta and mucosa microbiome of pigs
Xylooligosaccharide	Effects of xylooligosaccharide on gut barrier and gut immunity mediated by gut microbiota modifications
Synbiotic preparations	Effect of recently developed synbiotic preparations on dominant faecal microbiota and organic acid concentrations in faces of piglets from nursing to fattening
Clostridium butyricum plus corn bran	Synbiotics in weaned pig model to investigate their regulation of intestinal health and microbial fermentation
Synbiotics	Influence of synbiotics on intestinal microbiota and its metabolism in sows
Synbiotics	Effect of dietary probiotics or synbiotic supplementation on colonic microbiota, antioxidant capacity and immune function in weaned piglets
Single dose of synbiotics and vitamins	Whether a single-dose supplement given only at birth improves piglet performance and modifies their faecal microbiota during the suckling and post-weaning periods
Hydrolysable tannins	Impact of hydrolysable tannins on growth performance, board taint compounds and gut microbiota in entire males
Holly polyphenols	Effects of holly polyphenols on intestinal inflammation and microbiota composition in lipopolysaccharide-induced intestinal injury in piglets
Red-osier dogwood extract	Impact of ROD polyphenol extract on the ileal microbiota
A mix of functional amino acids and grade polyphenols	Combination of amino acids and polyphenol to facilitate the weaning transition
Barley and cocoa polyphenols	Combined effects of barley and cocoa polyphenols supplementation on faecal microbiota

and probably health-associated symbionts represents the core microbiota. Essentially, modulating gut microbiota through dietary interventions in order to increase gut health entails focusing on bacteria that are not part of the core microbiota (Salonen et al. 2012). It is possible, in fact, that bacteria belonging to the core microbiota are less influenced by environmental variations, such as nutrition, and are perhaps less susceptible to modulation.

On the other hand, when ascertaining the health status of the gut ecosystem, it is important to consider that a smaller core has been found in unhealthy individuals, compared with healthy subjects, suggesting the loss of some health-associated core bacteria. Unfortunately, results on the gut microbiota composition are significantly affected by the techniques or hypervariable regions used for the bacterial characterisation. Despite these variations, one meta-analysis found that by analysing 20 public datasets from high-throughput 16S rRNA gene sequencing studies of swine gut microbiota, numerous commonalities were found. Although no operational taxonomic units (OTUs)/amplicon sequence variants (ASVs) or genera were shared among all the samples

analysed, Clostridium, Blautia, Lactobacillus, Prevotella, Ruminococcus, Roseburia, the RC9 gut group and Subdoligranulum genera were shared by >90% of all stool samples, suggesting that these taxa could represent the core microbiota for commercial swine worldwide. Interestingly, these taxa were shared among pigs irrespective of the country of origin, diet, age or breed, thus providing important insights to help identify potential targets for dietary interventions in the swine production system.

Clostridium, Blautia and Ruminococcus belong to the order of Clostridiales and are widely found in the mammalian gut (Holman et al. 2017). Clostridia members can produce SCFAs, but they are also involved in the last step of the pathway in which skatole is produced via the fermentation of L-tryptophan in entire male pigs, contributing to the typical boar taint in the meat. Lactobacilli contribute to intestinal physiology and immune system regulation and are often used as probiotics in swine husbandry to improve growth performance and productivity. Prevotella spp. are likely associated with dietary carbohydrates in humans and part of the core microbiota in post-weaning piglets fed diets containing a high simple sugar content. In addition, Prevotella made up the core microbiota of entire male finishing pigs fed diets including different amounts of PUFAs and/or hydrolysable tannins from chestnut extracts. These findings confirm that the presence of Prevotella is not related to either the age or the breed, and that this genus is not an optimal target to be modulated by dietary interventions.

All this information demonstrates that even strong perturbations are not able to modify easily the structure and the population of the intestinal core microbiota. Strong perturbations such as the use of antibiotics, for example, mainly have short-term effects on the dominant microbiota. Microbially-integrated nutrigenomics still presents several open questions that need exploring. However, nutrigenomics research needs to take into account the resident microbiota and constantly consider intestinal bacteria as part of the animal holobiont.

There are few examples in the literature of the nutrigenomic approach to studying how nutrients modulate gut health in poultry, despite the intestinal permeability being a key condition for healthy and high-yielding animals. The few examples report the inclusion of prebiotics and amino acids or proteins in the poultry diet to assess their role in the modulation of gut barrier integrity and permeability. However, further research is still needed.

5.5 Conclusions

Nutrigenomics has many benefits for animal nutrition, including higher animal yield, feed efficiency, reproductive efficiency and health and well-being. In production animals, nutrient–gene interactions occur through the intermediate action of transcriptional regulatory factors and/or epigenetic factors, which determine the modulation of key gene expression. The genes that have been widely studied to date are those related to animal health and production traits in livestock species.

While the traditional target tissues of nutrigenomic studies are the liver, mammary gland and adipose tissue, the intestine is an emerging target tissue, particularly in monogastric animals. The liver and mammary gland have been extensively investigated in dairy ruminants (cows > goat > ewe), while the adipose tissue and fat deposition have been studied in meat-producing animals (pigs, beef).

In the case of poultry health, production traits have also been addressed, with the gut having increased relevance as a target for nutrigenomics studies. Besides being the primary tissue for nutrient digestion and absorption, the intestine can orchestrate the health homeostasis of the entire organism and modulate the production outputs of livestock animals through the action of gut microbiota.

The nutrigenomic effects of bioactive compounds could help fine-tune dietary requirements to optimise the production and health of livestock. The inclusion of nutrients and nutraceuticals in the diets of livestock can thus enhance the expression of various genes related to health, metabolism, growth, yield, immunity and antioxidant status. In particular, fatty acids, methyl group sources, amino acids, trace nutrients as well as feed and energy intake have shown the strongest nutrigenomics potential.

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