Animal Science Papers and Reports vol. 39 (2021) no. 4, 321-338
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The use of nutrigenomics in animal improvement for product quality and health – a review

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(Accepted November 4, 2021)

Nutrigenomics it is novel and multidisciplinary research field in nutritional science that aims to elucidate how diet can influence animal health. The present review contains two chapters. The first chapter shows information about nutrigenomics and nutrigenetics, the comparison of these two research trends, describes applications of nutrigenomics in animal science, the connection of nutrigenomics and the "omic" technologies and future scopes of nutrigenomics. The second chapter is entitled: "Nutrigenomics in animal feeding". In individual subsections influences of protein, carbohydrates, fat, mineral substances and other substances in porcine diet on level expression of genes and proteins have been demonstrated. A better understanding of these mechanisms will allow for acquisition of better quality food, higher production and other benefits.

KEYWORDS: carbohydrates / diet / fat / nutrigenomics / pig / protein

In recent years, new research directions in the so called „smart specialisations" or „intelligent specialisations" have been developed worldwide. They strictly refer to global trends in the field of large scale analysis of the genome, transcriptome, proteome or epigenome. A part of these trends is research in the field of nutrigenomics and nutrigenetics, which elaborate relationships: "diet-gene" and "genotype-diet". These studies characterize the molecular mechanisms of genetic influence on the quality, nutritional value and health-oriented food of animal origin that meets the criteria of

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functional foods and is used in the prevention of diet related diseases. The purpose of the article is to show the role of nutrigenomics research in improving the quality of raw materials of animal origin. It is very important because the consumers lifestyle in Western Europe, also observed in Poland in recent years, has led to intense efforts related to improving the health and welfare of animals in order to obtain food of the best quality. Type of food consumed is a crucial factor for human health in term of specific nutrition related gene expression.

Nutrigenomics *vs* nutrigenetics

Studies conducted within the nutrigenomics research field verify the effect of the diet enriched with bioactive components on animal genome and its effect on the changes of genes expression profile. There are many different bioactive ingredients used in the nutrigenomics research, one of them are fatty acids, especially polyunsaturated fatty acids (PUFA), microelements, vitamins, polyphenols, flavonoids and other compounds etc. [Attia *et al*. 2019ab, Huminiecki *et al*. 2017, 2020, Tewari *et al.* 2017, Huminiecki, Horbańczuk 2018, Mozos *et al.* 2018, Pogorzelska-Nowicka *et al*. 2018, Wang *et al.* 2018, 2020, Yeung *et al*. 2019ab, 2020, Pieczyńska *et al*. 2020, Li *et al*. 2021]. The nutrigenomics research explains influence of the diet compound on transcriptomics and proteomics profiles, pointing out changes associated with the physiological state of the organism, and defining disturbances caused by unhealthy diet.

Nutrigenetics aims to define requirements of the diet compounds depending on the genotype polymorphism or variability of reactions of the organisms of various genotypes to the bioactive dietary components. Such research allows to elaborate criteria of selection of animals in terms of specific genotypes for various individual nutritional systems. Both nutrigenomics and nutrigenetics studies implemented on livestock animals allow improve quality of animal origin products with a possible maximum nutritional value and oriented for consumers health [Fenech *et al*. 2011, Marcum *et al*. 2020, Nasir *et al*. 2020, Peña-Romero *et al*. 2017].

Nutrigenomics concept

For the first time the term of "nutritional genomics" has been used by [DellePenna 1999]. According to this definition it was the general approach to gene discovery that is currently most applicable to compounds of nutritional importance that are synthesized or accumulated by plants and other organisms [Ordovas and Corella 2004].

Kore *et al*. 2008 defined nutrigenomics as the study of molecular relationships between nutritional stimuli, and the response of the genes. In turn, according to Müller and Kersten 2003 nutrigenomics is specified as the application of high throughput genomic tools in nutrition research.

The diet has long been regarded as a complex mixture of natural substances that

supply both the energy and building blocks to develop and sustain the organism. Nutrients have a variety of biological activities. Some nutrients have been found to act as radical scavengers known as antioxidants, and as such are involved in protection against diseases. Other nutrients have shown to be potent signaling molecules and act as nutritional hormones [Müller and Kersten 2003]. Some of the plant secondary metabolites, phytochemicals, serve as a modulator of animal health and production.

Genomes evolve in response to many types of environmental stimuli, including nutrition. Therefore, the expression of genetic information can be highly regulated by nutrients, micronutrients and phytochemicals found in food [Van Ommen 2004].

Applications of nutrigenomics in animal science

Over the past two decades, nutritional research has moved its focus from classical epidemiology and physiology to molecular biology and genetics. For this reason nutrigenomics should be understood as a novel and multidisciplinary research field in nutritional science that aims to elucidate how diet can influence animal health [Canas *et al*. 2009]. It is expected that nutritional genomics will be a key area in nutritional research over the next decades [Kaput *et al*.2005]

From a practical point of view, gene expression studies will allow for the identification of pathways and candidate genes responsible for crucial biological mechanisms, metabolic pathways and final phenotypes of farm animal. Nutritional manipulations and nutritional strategies are key tools for influencing ruminant production. The examples of applications of nutrigenomics in animal science are:

- Developing animal feed or food matching to its genotype. The development of the foods and feeds that can be matched to genotypes of animals to benefit health and enhance correct physiological processes
- Selecting nutrients fine-tuned with genes of animal. Through nutrigenomics we are carefully selecting nutrients for fine-tuning genes and DNA present in every cell and every tissue of an animal.
- Understanding role of nutritional management in performance of animal. Nutrigenomics and nutritional genomics are providing new tools that can be used to more clearly understand how nutritional management can be applied to address disease, performance and productivity in animals [Rao *et al*. 2001]
- Understanding the aging process in animals. A nutrigenomic approach can be applied to understanding the aging process in companion animals [Ghormada *et al*. 2011]
- Nutrition influence on immune system. The basis of nutrigenomics is that nutrition is the key element of health maintenance, particularly for the immune system. Also, an optimum level of nutrition will ensure optimum animal health. There is a defined relationship between production and immune status of animals [Ghormada *et al.* 2011]
- Nutrition and diseases development. Essential nutrients and other bioactive

food components as macronutrients, vitamins, minerals can modify gene transcription, and translation, which can after biological responses such as metabolism, cell growth and differentiation, all of which are important in the disease process [Mariman 2006, Van Ommen 2004]

- Nutrition and reproduction. Preliminary studies have shown the value of such techniques and suggest that it will be possible to use specific gene expression patterns to evaluate the effects of nutrition on key metabolic processes relating to reproductive performance [Dawson 2006].

Nutrigenomics and the "omic" technologies

The deep knowledge of the genomes structure has become possible thanks to the development of a modern scientific methodology. These new areas of scientific studies usually include the "omics " suffix. The technical developments have given us novel tools enabling high throughput genome wide approaches. These are tools of the "biomics" era: genomics, transcriptomics, proteomics, metabolomics, and systems biology, integrating all "omics" techniques [Mutch *et al*. 2005].

Role of transcriptomics in nutrigenomics

The transcriptomics is the study of gene expression at the level of the transcriptome, taking into account all mRNA transcripts. The aim of transcriptomics is to characterise the level of all or the selected subsets of genes based on the variability of quantity of specificity mRNA transcripts - present in particular tissue samples. Using NGS (RNA-seq) or expression microarray technology, it describes profile of genes expression in analysed biological samples. Nutrigenomics is aimed on regulation of the rate of the genes transcription by food components, and it explains the impact of nutrition on individuals phenotype [Trujillo *et al*. 2006]. A number of essential nutrients can serve as regulators of gene expression patterns by modification gene transcription and translation processes, influencing biological processes metabolics pathways, important for cell growth and differentiation. On the level nutritional research, the aim of transcriptomic technology is to find relations between nutrient disease and predispositions, individualized food, functional food and diagnosis.

Role of proteomics in nutrigenomics

Proteomics is the study of the proteins in a particular cell, tissue or compartment [Banks *et al*. 2000]. The major tools used in proteomics are two-dimensional gel electrophoresis (2D), liquid chromatography and mass spectrometry. The proteomics explains the post translational modification, important for protein-protein interaction. It allows to point out biomarkers – for verification of bioprocess cultivation conditions, food quality control, and diagnosis of influence of functional food on organism. The proteomic analysis was quite effective to evaluate the effect of dietary methionine on

breast-meat accretion and protein expression in skeletal muscle [Corzo *et al*. 2006].

Role of metabolomics in nutrigenomics

Metabolomics is the final step in understanding the function of genes and their products. The main aim of metabolomics is to determine the sum of all metabolites in a biological system: organism, organ, tissue or cell [Müller and Kersten 2003]. The techniques employed in metabolomics to investigate the metabolome are high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance spectroscopy (NMR). These modern technologies combine the following common features: miniaturization, automation, high throughput and computerization [Corthesy-Theulaz *et al*. 2005]. As in the case of transcriptomics and proteomics, the scope of metabolomics analysis is mainly to assess the influence of dietary components on the metabolome of selected organs or tissues in animal nutrition studies [Bertram *et al*. 2006].

Future scopes of nutrigenomics

The development of this research is possible thanks to modern technology and new tools bioinformatic analyses. The new biomarker in nutrigenomics is a revolutionary new way to view the food and the pharmaceutical capabilities of the food to reverse disease and slow down the process of ageing [Bhatt and Sharma 2011]. Nutrigenomics is a recent subject and there is scarcity of reports regarding the nutrition related gene expression. Exploration of the genetic polymorphism and nutrition related gene expression allows better understanding of the individual requirements and preparation personalized diet.

Nutrigenomics in animal feeding

Influence of proteins level in diet

The group of Yunnan Agricultural University researched impact of dietary protein on lipid metabolism and related gene expression in porcine adipose tissue. Their analyses were conducted on Sixty Wujin pigs of about 15 kg weight, which were fed either high protein (HP-18%) and low protein (LP-14%). HP significantly reduced gene expression of acetyl CoA carboxylase (ACC), fatty acid synthase (FAS) and sterol regulatory element binding protein 1c (SREBP-1c) at 60 kg and 100 kg. The mRNA level and enzyme activity of FAS were increased at 30 kg. HP promoted gene and protein expression and enzyme activities of lipoprotein lipase (LPL), carnitine palmtoyltransferase-1B (CPT-1B), peroxisome proliferator-activated receptor ɣ (PPARɣ) and adipocyter-fatty acid binding proteins (A-FABP) at 60 kg, but reduced their expression at 100 kg. Gene expression and enzyme activity of hormone sensitive lipase (HSL) was reduced markedly at 60 kg, but increased at 100 kg by the high dietary protein. Levels of mRNA, enzyme activities and protein expression of ACC,

FAS, SREBP-1c and PPARɣ in both LP and HP groups with increasing body weight. However, gene and protein expression levels/enzyme activities of LPL, CPT-1B, A-FABP and HSL in both groups were higher at 60 kg than at 30 and 100 kg [Zhao *et al*. 2010].

HP significantly reduced adipocyte size, fat meat percentage and backfat thickness, but significantly increased gain, lean meat percentage and loin eye area at 60 and 100 kg. Serum free fatty acid and triglyceride concentrations in the HP group were significantly higher than in the LP group [Zhao *et al*. 2010].

The researchers from Teagesc Food Research Centre Ashtown in Ireland compared gene expression profiles of *Musculus semimembranosus* of animals divergent for IMF as a consequence of protein dietary restriction in an isocaloric diet. A total of 542 annotated genes were differentially expressed (DE) between animals on low and high protein diets, with 351 down-regulated and 191 up regulated on the low protein diet. Many of identified transcriptomic responses have also been observed in genetic and fetal programming models of differential IMF accumulation, indicating they may be robust biological indicators of IMF content [Hamill *et al*. 2013]. Factors affecting IMF are very important because they define pork quality from a sensory perspective, since IMF is positively correlated with flavour, juiciness, tenderness/firmness and overall acceptability of pork [Fortin *et al*. 2005]. In this studies IMF content of SM muscle increased on the low protein diet (3.60 vs. 19.2).

In a study from Mexico the effect *Leu* : *Lys* ratios on expression of the: transporters b^{0+}, cationic amino acid transporters (CAT-1) and myosin were analyzed. The studies were conducted on 96 pigs, which were feeding basal diet with added 5 SID *Leu*: *Lys* ratios (88, 100, 120, 140 and 160% in diets 1-5). b^{0+} expression in the jejunum was higher, but lower in the liver of pigs with the 120% ratio compared to those with the 88 or 160% ratio; myosin expression in *longissimus dorsi* was also higher in pigs with the 120% ratio. The level expression of CAT-1 was lower in the jejunum and *longissimus dorsi* of pigs with 120% or 160% ratios, than in pigs with 88%. It was reported that dietary *Leu* and *myosin*: *Lys* ratio affected the expression of genes coding for amino acid transporters and myosin, the availability of *Lys*, and the growth rate and efficiency in pigs [Garcia *et al*. 2015].

Studies by Liu *et al*. 2011 aimed on characterisation of the effects of maternal dietary protein on transcriptional regulation of Myostatin (MSTN) in skeletal muscle of pig offspring. MSTN, a member of the transforming growth factor type β (TGFβ) super-family, is a negative regulator of skeletal muscle mass [McPherron *et al*. 1997]. Myostatin is responsive to the nutritional status. MSTN is suggested to mediate the effect of maternal nutrition on offspring phenotype, yet the mechanisms underlying such adaptive gene regulation is elusive. Animals were fed either low-protein (LP) or standard-protein (SP) diets throughout gestation and lactation. MSTN mRNA abundance was down-regulated at weaning, but upregulated at finishing stages. At weaning, CCAAT/enhancer-binding protein β (C/EBPβ) in muscle nuclear lysate decreased in LP piglets, associated with diminished binding of C/EBPβ to all three putative binding

sites in MSTN promoter. Among 12 microRNAs predicted to target MSTN, none was expressed differently. Expression of ssc-miR-136 and ssc-miR-500 was significantly reduced. These results indicate that maternal dietary protein affects MSTN expression through distinct regulatory mechanisms at different stages [Liu *et al*. 2011].

High-protein diet led also to higher MSTN level in rat gastrocnemius muscle [Nakazato *et al*. 2006].

The group of researchers from China Agricultural University tested the hypothesis that weaning or glutamine may modulate expression of genes that are crucial for intestinal metabolism and function [Wang *et al*. 2008].

Glutamine, as one of the important aminoacids, is a major substrate for the endogenous synthesis of arginine in most mammals, including humans and pigs, via intestinal-renal axis. Glutamine, is also required for the synthesis of N-acetylglucosamine-6-phosphate, a common substrate for the synthesis of glycoproteins that are particularly rich in intestinal mucosa [Wang *et al*. 2007]. Early weaning resulted in increased (from 50 to over 350%) expression of genes related to oxidative stress and immune activation but decreased (35-about 80%), expression of genes related to macronutrient metabolism and cell proliferation in the gut. Dietary glutamine supplementation increased intestinal expression (120-125%) of genes that are necessary for cell growth and removal of oxidants, while reducing (35-75%) expression of genes that promote oxidative stress and immune activation. These findings reveal coordinated alterations of gene expression in response to weaning and aid in providing molecular mechanisms for the beneficial effect of dietary glutamine supplementation to improve nutrition status in young mammals [Wang *et al*. 2008].

The researchers from Germany showed that the dietary L-carnitine alters gene expression in skeletal muscle of piglets [Keller *et al*. 2011].

Carnitine is a water soluble quaternary amine, which is essential for normal function of all tissues. On the basis of the literature its role is known in energy homeostasis [Kerner and Hoppel 2000]. Supplementation of L-carnitine was reported to cause several pleiotropic and often beneficial effects, such as protecting against neurodegeneration and mitochondrial decay resulting from aging [Calabrese *et al*. 2005]. Transcript profiling revealed 211 genes to be differentially expressed in muscle by carnitine supplementation. The identified genes were mainly involved in molecular processes such as cytoskeletal protein binding, insulin-like growth factor (IGF) binding, transcription factor activity, and insulin receptor binding. Identified genes encoded primarily transcription factors, most of which were down-regulated by carnitine, including pro-apoptotic transcription factors such as: proto-oncogene *c-Fos*, proto-oncogene *c-jun* and activating transcription factor 3. In conclusion, carnitine may have beneficial effects on skeletal muscle mass through stimulating the anabolic IGF-1 pathway and suppressing pro-apoptotic and atrophy-related genes, which are involved in apoptosis of muscle fibers and proteolysis of muscle proteins [Keller *et al*. 2011].

Influence of carbohydrates level in diet

In recent years mannan-oligosaccharides (MOS) often are used as alternatives to

antibiotic growth promoters (AGP). MOS are mannose-rich carbohydrates found in the yeast cell wall [Young *et al*. 1998].

MOS products are used as natural feed additives in livestock and poultry because of documented benefits in performance and gastrointestinal health [Baurhoo *et al*. 2009]. These are often referred to as one of the potential alternatives for antimicrobial growth promoters. The groups of researchers showed the effect of dietary MOS on the different biological processes. Effect of dietary MOS supplementation on non-specific and specific cellular immune response of weaned pigs was also investigated [Nochta *et al*. 2009].

Other researchers showed that dietary MOS supplementation can efficiently reduce the number of pathogens post-infection [Leroy *et al*. 2003].

The review of literature data showed also the possible effect of dietary MOS on the growth performance of weaned pigs. Other authors reported conflicting results. White et al. 2001 reported no benefits, while others found an improved rate of daily gain and feed efficiency in weaned pigs [Castillo *et al*. 2008, Le Mieux *et al*. 2010].

The goal of the study conducted in the research center in Nicholasville, Kentucky was to use a transcrtiptomics approach to investigate the effects of MOS on the intestinal gene expression profile of young broilers and characterise biological gene pathways responsible for the actions of MOS. These animals were randomly divided into 2 groups and were fed either a standard wheat-soybean meal-based (control) diet, or the same diet supplemented with 2.2 g/kg of MOS. Results indicated that a total of 672 genes were differentially expressed in the jejunum by MOS supplementation. Association analysis indicated that differentially expressed genes are involved in diverse biological functions including energy production, cell death and protein translation. Expression of 77 protein synthesis-related genes was differentially regulated by MOS in the jejunum. Pathway analysis indicated that 15 genes related to oxidative phosphorylation were upregulated in the jejunum, and expression of genes important in cellular stress response. Differential expression of genes associated with other biological processes were also observed in MOS-fed broilers [Xiao *et al*. 2012].

The use of plant-derived products to improve performance and health of animals and humans is center of interest in recent years. One of the products is silymarin. It is a mixture of flavonolignans derived from the plant *Silybum marianum*, and possess hepatoprotective, anti-inflammatory and antioxidant properties [Giese 2001, Wu *et al*.2011]. The active component of silymarin is silybin, which concentration may be different in particular silymarin extracts. These extracts increase milk yield in lactating cows [Tedesco *et al*. 2004], women [Di Pierro *et al*. 2008] and enhance bovine and murine mammary cell proliferation as well as β-casein gene expression [Starvaggi-Cucuzza *et al*. 2010]. Silymarin was used in alternative medicine to increase milk yield in women with hypogalactia [Di Pierro *et al*. 2008].

The group of Canadian researchers from College St. Sherbrooke showed the effects of supplementing the diet of gestating gilts with the plant extract silymarin on circulating hormonal concentrations, oxidative status, mammary development and mammary gene

expression at the end of gestation. In this experiment gilts were fed conventional diets during gestation and on day 90 they were assigned as controls (CTL) or treated (TRT) animals. Treatment consisted of providing 4 g of silymarin twice daily until day 110 [Farmer *et al*. 2014]. Silymarin increased circulating concentrations of prolactin in all samples in the repeated in time analysis. In separate analysis for each sampling time, prolactin concentrations in TRT gilts tended to be greater than in CLT gilts on day 94 of gestation. Silymarin also reduced plasmatic accumulation of biomarkers of oxidative damage to protein between day 89 and 109 d, while no effect of treatment on progesterone, estradiol, leptin concentrations were observed. Percent of fat in mammary parenchyma was greater, percent of protein was lesser and concentrations of both RNA and DNA were lesser in TRT than CTL gilts. Silymarin reduced concentrations of protein carbonyls in liver of TRT gilts. Whereas, no effect of treatment was observed on antioxidant gene expression and enzymatic activities in liver samples. These results suggest that in female pigs silymarin can increase concentrations of prolactin. But, this increase of prolactin was not enough to have beneficial effects on mammary gland development in late gestation [Farmer *et al*. 2014].

Three years later the same team showed effects of addition extract silymarin on litter performance and oxidative stress in lactating sows [Farmer *et al*. 2017]. In their study authors provided either 1 g/day or 8 g/day of this extract to lactating sows. The experiments were carried on all 99 Yorkshire x Landrace sows. These animals received no silymarin (the control group, n=33), or received 1g/day of silymarin (SYL1, n=33), or 8 g/day (SYL8, n=33). The silymarin was provided in two equal amounts per day, and was fed throughout a 20-day lactation. In this experiment there was no effect of silymarin on circulating prolactin or urea, or on oxidative damage to proteins or antioxidant potential in sows. Results demonstrated that providing up to 8g/day of the plant extract silymarin to lactating sows had no beneficial effects in terms of circulating prolactin concentrations or oxidative status of sows, or in terms of performances of sows and their litters [Farmer *et al*. 2017].

Researchers from Germany investigated effect of a mixed diet on other biological parameters. High levels of undigested fermentable protein reaching the colon of pigs are associated with the formation of potentially harmful metabolites and impairment of intestinal barrier function. In turn, balancing the ratio of fermentable protein to carbohydrates in the pig diet can reduce the level of potentially harmful colonic metabolites [Bikker *et al*. 2006]. Mass spectrometry was used to profile changes in metabolite composition in colon and urine associated with variation in dietary fermentable carbohydrates (fCHO) and fermentable protein (fCP) composition and mucosal physiology. Thirty two of weaned piglets were fed 4 diets with other quantity fCHO and fCP (high and low). Analysis of mass spectra by partial least squares approach indicated a clustering of both colonic and urinary profiles for each pig by feeding group. KEGG metabolic pathways revealed increased abundance of metabolites associated with arachidonic acid metabolism in colon of pigs fed a high concentration of fCP irrespective of dietary fCHO. Mass spectrometry can effectively differentiate metabolite

profiles in colon contents and urine associated with changes in dietary composition [Pieper *et al*. 2012].

Influence of fat composition in diet

The composition fatty acid (FA) of muscle and in adipocytes tissue determines sensorial, technological and nutritional aspects of meat influencing its perception by the consumers [Wood *et al*. 2008]. From a nutritional point of view, medical recommendations are now shifting from the reduction of fat intake towards increasing fat quality in order to maintain cardiovascular health. High consumption of saturated fatty acids (SFA) has been associated with obesity, high plasma cholesterol and cardiovascular diseases [Chizzolini *et al*. 1999]. The effects of two diets, respectively enriched with SFA (S) and polyunsaturaed fatty acids (PUFA, P) on FA tissue composition and gene expression was studied in fattened Iberian pigs by spanish researchers. The FA composition of adipose, muscular and liver tissues was affected by dietary treatment. S group showed higher monounsaturated fatty acids (MUFA) and MUFA/SFA ration and lower PUFA and n-6/n-3 ratio than P group in all analyzed tissues. The expression of six candidate genes related to lipogenesis and FA oxidation was analyzed by qPCR. In liver, stearoyl CoA desaturase (SCD), acetyl CoA carboxylase alpha (ACACA) and malic enzyme 1 (*ME1*) genes showed higher expression in S group. *SCD*, *ACACA*, *ME1* and fatty acid synthase (*FASN*) gene expression levels showed a wide variation across the tested tissues, with much higher expression levels observed in adipose tissue than other tissues. Tissue FA profile and gene expression resulted in the deposition of dietary FA, the lipogenic effect of dietary saturated fat in liver and the employment of saturated dietary fat for endogenous synthesis of MUFA in all the analyzed tissues [Benítez *et al*. 2015].

Polish researchers from Institute of Genetics and Animal Breeding of Polish Academy of Sciences in Jastrzębiec (IGAB PAS) analysed dietary effects of omega-6 and omega-3 fatty acids on biological mechanisms. Omega-6 and omega-3 fatty acids are polyunsaturated fatty acids (PUFA), which belong to the essential fatty acid family and must be obtained from food sources. Omega-6 and omega-3 fatty acids modulate gene expression and provide substrates for the production of signaling molecules of functioning mediators. A correct balance of these fatty acids is important for the proper function and development of various cell types. Additions of omega-6 and omega-3 fatty acids in the diet is important to prevent the development of metabolic diseases such as heart attacks, atherosclerosis, thrombosis, arrhytmia, stroke, immune-inflammatory disorders, asthma, arthritis, cancer proliferation, obesity and psychiatric disorders [Lands 2012, Kang and Liu 2013]. The aim of first part of the study conducted in IGAB PAS was to investigate changes in the muscle transcriptome and the biological functions regulated by increased consumption of omega-3 and omega-6 fatty acids in the pig *gluteus medius* muscle. In these analyses one of the most modern techniques such as next-generation sequencing (NGS) was used. Comparative expression analyses identified 749 genes significantly differing at least

in the two-fold of change between two groups of animals fed with divergent level of omega-3 and omega-6 fatty acids. The expression of 219 genes was upregulated, and the expression of 530 genes was down-regulated in the group of pigs supplemented with omega-3 and omega-6 fatty acids in relation to control group pigs. These results indicated the role of fatty acid in the regulation of the expression of genes which are essential for muscle tissue development and functioning [Ogłuszka *et al*. 2017].

The objective of the second part of these studies was to identify changes in the pig liver transcriptome induced by a diet enriched with omega-6 and omega-3 fatty acids and to characterize the biological mechanisms related to PUFA metabolism. Here the next – generation sequencing (NGS) was used as well to identify differentially expressed genes (DEG) between transcriptomes between dietary groups. Analysis of fatty acid profile indicated a higher contribution of PUFAs in the liver of LA- and ALA-enriched diet group. Next-generation sequencing identified 3565 DEG, 1484 of which were induced and 2081 were suppressed by PUFA supplementation. A low ratio of omega-6/omega-3 fatty acids resulted in the modulation of fatty acid metabolism pathways and over-representation of genes involved in energy metabolism, signal transduction, and immune response pathways [Szostak *et al*. 2016]. Summarizing the obtained results provided by Polish researchers, omega -6 and omega 3 fatty acids regulated fundamental metabolic processes in muscle tissue development and functioning and would influence animal health status.

The researchers from Chinese Medical University in Zhejiang performed studies on pigs which were divided into three groups: intact male (IM) pigs fed a high fat and high cholesterol (HFC) diet (IM); castrated male (CM) pigs fed and HFC diet (CM); and castrated male pigs fed and HFC diet and given testosterone replacament therapy (CMT). The effects of testosterone on the development of hepatic steatosis in pigs and hepatic gene expression by RNA-Seq in three groups of pigs was investigated. Low levels of testosterone were associated with metabolic disorders, including obesity, dyslipidemia, hypertension, and insulin resistance. To determine the expression RNA-Seq analyses of the liver transcriptomes were used. In total, 18093, 18481 and 17740 expressed genes were detected in the livers of IM, CM and CMT pigs, respectively. Of these genes, a total of 16981 genes were identified commonly between each pair of groups, while 337, 538 and 253 genes were discovered exclusively for IM, CM and CMT, respectively. This analysis revealed that upregulated genes in the livers of CM pigs were mainly enriched for genes mediating immune and inflammatory responses, oxidative stress, and apoptosis. Surprisingly, the down-regulated genes mainly included those that regulate metabolism-related processes, including fatty acid oxidation, steroid biosynthesis, cholesterol and bile acid metabolism, and glucose metabolism. KEGG analysis showed that metabolic pathways, fatty acid degradation, pyruvate metabolism, the tricarboxylic acid cycle, and the nuclear factor-Kappa β signaling pathway were the major pathways altered in CM pigs [Cai *et al*. 2015].

Influence of mineral substances level in diet

Selenium (Se) is one of the critical mineral elements required for normal physiological processes, such as: homeostasis, apoptosis, immune cell functions. Dietary supplementation of Se improved growth and reproductive performances of domestic animals by antagonizing reactive oxygen [Choct *et al*. 2004, Beckett and Arthur 2005]. In turn, the anti-oxidative activity of Se may also help to improve water-holding capacity of meat products by preventing membrane oxidation [Mateo *et al*. 2007]. The team from Carolina University studied how long-term dietary supplementation of selenium modulates gene expression profiles in porcine leukocytes [Song *et al*. 2013]. In pig oligonucleotide microarray, they identified a total of 52 genes which changed in Se-fed pig leukocytes compared to control leukocytes. In Se-fed pig leukocytes, gene expression of 28 genes were up-regulated, whereas 24 genes were down-regulated in these animals compared to control pig leukocytes. The greatest increase was with MHCI, ferroxidase, SLA 3/2, arginase I, RAMP2, GlxII, steroid receptor coactivator 1e, inulin β-1 subunit, TLR2, and protein kinase Cβ1. The genes were down-regulated in the Se-fed pigs compared with control pigs. The greatest decrease was of TF, opticin, SAA2, SREBP-2, p27Kip1 and β-catenin. These results showed that many of gene activities found in Se-fed pigs indicate that dietary Se may modulate multiple physiological pathways, for example, immune responses, inflammatory response, oxidative stress status and cholesterol metabolism [Song *et al*. 2013].

Influence of other substances level in diet

Polish researchers provided valuable contribution to the development of the field of nutrigenomics. The team from West Pomeranian University of Technology in Szczecin characterized the systemic immune and metabolic alterations in the blood serum of growing pigs in response to a dietary supplementation with 4% of dried chicory roots. Chicory root is a natural source of inulin-type fructans such as inulin and oligofructose. Inulin is composed of a set of sucrose molecules of which the fructose moiety is substituted with a linear chain of β fructans. Fructans such as oligofructose (OF) and inulin are defined as components of the diet that are not digested in the upper gastrointestinal tract of monogastric animals and are able to selectively stimulate the growth and activity of beneficial intestinal microflora, mainly *Bifidobacteria* and *Lactobacilli* [Robefroid 2007]. Their study was performed on 12 castrated male piglets (PIC x Penarlan P76), which were divided into 2 groups: control group (C) unsupplemented cereal-based diet, and group supplemented with 4% of dried chicory root (T group). They found that experimental diet triggered significant changes in 37 protein spots. 14 of these were up-regulated, and 23 showed down-regulation. Of 37 significantly altered protein spots, 24 were successfully identified, representing 14 distinct gene products. Implementation of the dried chicory roots into the diet of growing pigs caused a significant down-regulation of apolipoprotein C-II complement component C6, C-reactive protein, CD14 antigen, C4b binding protein α and β chains and fibrinogen. Also, pigs fed experimental diet had similar IgA, IgG and IgM concentrations, although the level of IgM tended to be lower compared to

the control group. They showed that diet supplemented with 4% of dried chicory root may exert anti-inflammatory properties and affect lipid metabolism in growing pigs [Lepczyński *et al*. 2015].

The same team carried experiment on a total 12 castrated male piglets, randomly divided into two group, but feeding diet supplemented with 2% water extract of inulin from chicory roots. The chemical composition of the inulin extract contained approximately 92% of inulin and 8% of other carbohydrates (glucose, fructose and sucrose). In the present study proteins which show altered expression as a result of the addition of 2% of water extract of inulin to the diet of growing piglets were identified. This analysis allowed us to detect an average of 240 spots per gel. Twenty protein spots were found to show statistically significant differences in their expression. Of these, 7 protein spots were up-regulated, whereas 13 showed down-regulation in response to the experimental diet. In total, 13 spots were identified representing 8 distinct gene products. The experimental diet caused a significant change in proteins directly or indirectly involved in hemostasis, and the innate immune response. They also found increased expression of vitronectin and the alpha subunit of the complement component C8 which may protect the host organism against excessive cytolitic activity of the activated complement [Herosimczyk *et al*. 2015].

The next studies by the same team were also performed on 24 castrated male piglets (PIC x Penarlan P76), but animals were assigned to three equal groups (n=8) and fed cereal-based isoenergetic diets: control, supplemented with 2% of inulin extract from chicory root or 4% of fried chicory root. Both experimental factors significantly modulated the expression of liver proteins associated with energetic metabolism, particularly those involved in cholesterol and triglyceride metabolism. Additionally, both dietary additives induced increased expression of proteins involved in hepatocyte protection against oxidative stress. These studies showed the first time that the diet supplementation with dried chicory root or inulin caused significant changes in the expression of liver cytoskeletal proteins [Lepczyński *et al*. 2016].

Curcuma longa possesses several phytochemical compounds exhibiting a wide variety of pharmacological properties, including those against tumor cells, hormonal disorders, inflammation, bacterial infection, oxidative stress and parasitosis. The major active ingredient of turmeric is curcumin (diferuloymethane), a lipophilic polyphenol [Bengmark *et al*. 2009].

Kim *et al*. studied the effects of dietary supplementation with an organic extract of *Curcuma longa* on systemic and local immune responses to experimental *Eimeria maxima* and *Eimeria tenella* infections in broiler chickens. The chickens fed *Curcuma longa*-supplemented diet showed enhanced systemic humoral immunity, as assessed by greater levels of serum antibodies. At the intestinal level, genome-wide gene expression profiling by microarray hybridization identified 601 differentially expressed transcripts (287 up-regulated and 314 down-regulated) in gut lymphocytes of *C. longa* – fed chickens compared with non- supplemented controls. The *C. longa* which induced intestinal transcriptome was mostly associated with genes mediating

anti-inflammatory effects [Kim *et al*. 2013].

One of the alternative strategies includes dietary immunomodulation [Chae *et al*. 2006]. Understanding the genetic regulation of protective functions by immunomodulators may lead to improvement of the safety and economics of poultry production and reduce the potential for development of resistance to antimicrobial treatments. Kumar *et al*. 2011 used vaccines, antibiotics, and other therapeutics to characterize the effects of dietary supplementation with immunomodulators on cytokine gene expression in the spleen of 3 distinct genetic lines of chickens. The mRNA expression did not differ significantly among diets or genetic lines for any studied genes. The only detected significant effect was sex effect on expression of IL-1β. Therefore, the results suggested the need for further investigations into the effects of dietary immunomodulators on cytokine or other gene expression in chickens [Kumar *et al*. 2011].

Summary

Nutrigenomics applies genomic technologies to study how nutrients affect expression of genes. With the advent of the post genomic era and with the use of functional genomic tools, the new strategies for evaluating the effects of nutrition on production efficiency and nutrient utilization are becoming available. Nutrigenomics plays an efficient role in various fields linked to animal health like nutrition, production, reproduction, disease process etc. Nutrigenomic approaches will enhance researchers' abilities to improve animal health and performance and the quality of animal products.

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