

# Application of nutrigenomics tools in animal feeding and nutritional research\*

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## ABSTRACT

The development of nutrigenomic studies has brought about a number of new research tools (transcriptomics, proteomics and metabolomics), which are important in animal nutrition and food research. This review presents an overview on the application of nutrigenomics tools in this scientific area. The use of microarray technologies - the main tools of transcriptomics, has allowed new information concerning the physiological effect of different dietary proteins, of omega-3 polyunsaturated fatty acids and dietary conditioning of colon cancer, to be obtained. The use of proteomics tools (mainly two-dimensional electrophoresis) revealed new information concerning the protein composition of egg and poultry meat proteins, the effect of dietary methionine on breast-meat accretion, the toxicity of dioxin and the safe use of transgenic crops in animal nutrition. Metabolomic analysis allowed the detection of changes in the biochemical profiles of plasma and urine from pigs fed different diets and the determination of metabolite profiles in the liver of rats used as an animal model to characterize the toxicity of triazol fungicides. In livestock species, the microarray technology was discussed and reviewed as potential nutrigenomics tools, in context to its economic benefits and improvement of the food quality and safety in dairy and meat industries. However, the newly emerged nutrigenomics tools like - gene expression-based biomarker development still poses a major challenge. Finally, latest developments in the standardization of metabolomics data in relation to functional genomics, nutrigenomics and toxicology studies are discussed.

KEY WORDS: nutrigenomics, transcriptomics, proteomics, metabolomics, nutrition, feed evaluation

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## INTRODUCTION

The, albeit scanty, investigations into molecular interactions of foodstuffs have indicated that gene expression is modified by a number of dietary components, including macrocomponents (carbohydrates, proteins, fats and cholesterol), vitamins (e.g., A, B, E, D) minerals (e.g., Fe, Se, Ca) as well as phytochemicals, including flavonoids, isothiocyanates and indoles (Kaput and Rodriguez, 2004). Thus, a new era in nutritional sciences was initiated in the first year of the XXI century with the publication of the draft sequence of a human genome and related papers (Lander et al., 2001; Venter et al., 2001). The study of how genes and gene products interact with dietary chemicals to alter phenotype and, conversely, how genes and their products metabolize nutrients is called nutritional genomics or “nutrigenomics” (Kaput et al., 2005). It is expected that nutritional genomics will be a key area in nutritional science research over the next decade (Trayhurn, 2003) and that nutrigenomic studies will be very useful for elucidating the role(s) of food components in obesity (Chadwick, 2004), coronary heart diseases (Talmud, 2004) and cancer prevention (Davis and Hord, 2005). From a nutrigenomic perspective, nutrients are dietary signals, detected by the cellular sensor system, that influence gene and protein expression and, subsequently, metabolite production (Müller and Kersten, 2003). From the research perspective, to explore the effect of dietary components on the genome, the crucial stages of nutrigenomics are transcriptomics, proteomics and metabolomics. Application of these modern research tools, known as “omics” technologies, should yield new knowledge on the course of molecular processes in animal organisms and a more precise evaluation of the biological properties of feeds. This review presents the first results on the application of nutrigenomics tools (transcriptomics, proteomics and metabolomics) in experiments, which are important from the point of view of animal nutrition and food research.

## APPLICATION OF TRANSCRIPTOMICS IN ANIMAL AND FEED SCIENCES

The aim of transcriptomics is to determine the level of all or a selected subset of genes based on the amount of RNA present in tissue samples. Transcriptomics is concerned with the expression of over 30000 genes in humans (Müller and Kersten, 2003). In precise experiments conducted on animals, the scope of investigation is usually restricted, for example to assess the influence of dietary components on the transcript level of selected organs, as demonstrated in Table 1. The use of a microarray containing probes for the over 8000 genes present in the liver of rats demonstrated that about 33% of the genes of rats fed a soya protein diet differed from those of casein-fed animals (Takamatsu et al., 2004). Significant differences were observed in the gene cluster concerned with lipid

Table 1. Selected examples of applications of transcriptomics technologies in nutrition research

Gene expression bioarrays	The aim of investigations	Model of experiment	Authors
An Affymetrix rat genome array containing probes for over 8000 genes	To investigate the effect of soya protein on metabolism of liver and expression of the gene cluster concerned with lipid metabolism	Rats fed for 8 weeks a diet with casein or soya protein	Takamatsu et al., 2004
An Affymetrix rat genome array containing probes for over 8000 genes	To investigate the effect of soya protein on metabolism of liver and expression of the gene cluster concerned with lipid metabolism	Rats fed for 8 weeks a diet with casein or soya protein	Tachibana et al., 2005
Codelink DNA microarray containing 9028 rat colon-derived cDNAs	To recognize the mechanism of chemopreventive properties of n-3 polyunsaturated fatty acids	Rats fed a diet with different contents of PUFA	Davidson et al., 2004
Affymetrix gene chips with cRNA derived from rat duodenal mucosa	To examine the effect of iron-deficiency on known iron transport genes and to identify novel genes involved in intestinal iron transport	Rats at the age of 8 days and 12 or 36 weeks after feeding with a diet containing 198 or 3 ppm of iron	Collins et al., 2005
Microarrays containing about 2000 rat colon-derived cDNAs	To verify a hypothesis that the dietary heme (derived from red meat) and calcium are modulators of colon cancer risk	Rats fed for 2 weeks with high- and low-calcium diets with or without hem	Van der Meer-van Kraaij et al., 2005
cDNA microarray prepared at the Genome Centre Maastricht, containing 602 mouse genes	To recognize the genetic mechanism by which vegetable, in particular carrots, may prevent lung cancer risk	Mice fed a diet without or with different doses of vegetable	Breda et al., 2005

metabolism, and in the gene related to energy metabolism, transcription factor, and anti-oxidation enzymes. In similar experiments carried out by Tachibana et al. (2005), compared with casein, soya protein changed the expression of 120 genes involved in lipid metabolism, antioxidant activity and energy metabolism. Endo et al. (2002) also reported that various dietary protein sources resulted in a difference in expression of about 281 genes in rat liver, suggesting a nutritional function for protein components.

Davidson et al. (2004) used Code link DNA microarrays containing about 9000 genes to help decipher the global changes in colonocyte gene expression profiles in carcinogen-injected rats. The result obtained indicated that the chemopreventive effect of fish oil is due to direct action of n-3 PUFA and not to reduction in the content of n-6 PUFA. It was also noted that dietary fat composition alters the molecular portrait of gene expression profiles in the colonic epithelium at both the initiation and promotional stages of tumor development (Davidson et al., 2004). Use of a microarray was also allowed brain gene-expression changes in response to different amounts of omega-3 polyunsaturated fatty acids in diets of rats to be revealed (Kitajka et al., 2004). The authors concluded that PUFA-enriched diets lead to significant changes in expression of several genes in the central nervous tissue, and these effects appear to be mainly independent of their effect on membrane composition.

In the experiments of Collins et al. (2005), microarray techniques were used to examine changes in gene expression in the rat duodenum associated with iron-deprivation. The findings demonstrated that iron-deprivation results in a large spectrum of differentially expressed genes in the duodenal epithelium: the identification of these genetic changes is likely to increase our understanding of the complex physiology of intestinal iron homeostasis. The results of later experiments demonstrated similarities and provided evidence that more distal gut segments also may play a role in increasing iron absorption in iron-deficiency anaemia (Collins, 2006).

Microarrays containing about 2000 rat colon-derived cDNAs were used to verify that dietary haeme (derived from red meat) and calcium are modulators of colon cancer risk (Van der Meer-van Kraaij et al., 2005). The results obtained confirmed that dietary haeme increased the cytotoxicity of faecal water in the colon and elevated epithelial proliferation, a risk factor in colon carcinogenesis, moreover calcium-reduced cytotoxicity and inhibits haeme-induced effects.

A special cDNA microarray experiment, prepared at the Genome Centre Maastricht containing 602 mouse genes was used to investigate the genetic mechanism by which vegetables, in particular carrots, can prevent lung cancer risk (Breda et al., 2005). The results obtained indicate that vegetables affected the expression of genes involved in carcinogenic and anti-carcinogenic processes in the lungs of mice susceptible to lung cancer (Breda et al., 2005).

The experiments demonstrated the ability of cDNA microarray technology to study levels of gene expression in response to nutrition in an intact animal system. DNA microarray technology allows the simultaneous analysis of the expression of large numbers of genes, improving greatly the performance of traditional methods for gene expression analysis such as RT-PCR, northern blotting or more advanced techniques such as differential display, which have also enabled the discovery of novel differentially expressed genes (Spielbauer and Stahl, 2005). DNA array technology (or so-called DNA chip technology) is currently the most powerful tool in transcriptomics, which enables the measurement in parallel and in tens of samples, of the expression of up to 50,000 transcripts (Corthesy-Theulaz et al., 2005). The potency of DNA microarray technology in food and nutrition science is broadly recognized. This technique will surely continue to provide researchers and the public with valuable information on the beneficial and adverse effects of food factors. It should also be acknowledged, however, that there remain problems such as standardization of the data and sharing of results among researchers in this field (Kato et al., 2005).

The integration of microarray analysis into basic and applied nutrition and food research provides new insights into the effects of nutrition and food ingredients like fats, carbohydrates, proteins, carotinoides, vitamins, minerals, flavonoids and xenobiotics at the molecular level (Müller and Kersten, 2003). The schematic overview of integration of potential applications of transcriptomics tools in nutrition and food research is presented in Figure 1. The figure shows the concept and methodical approach of functional genomics at whole

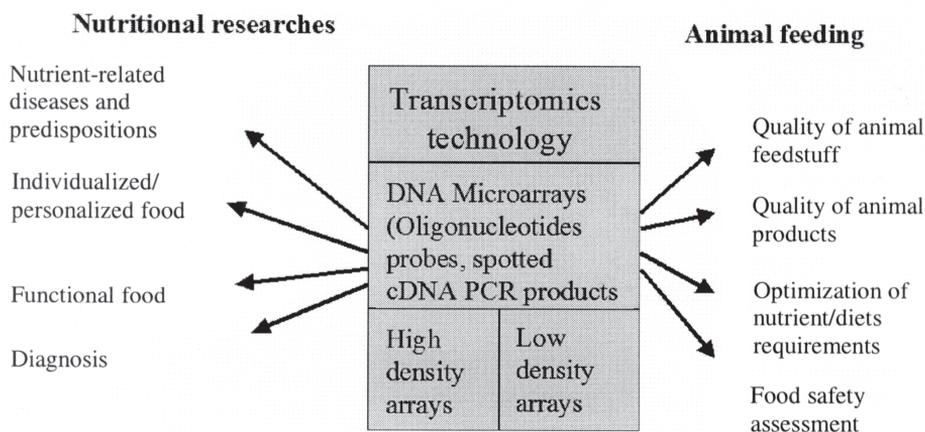


Figure 1. Schematic overview of integration of omics technology in nutrition and food research - a transcriptomics prospective view

genome level using microarrays and other potential techniques. The results of such high-throughput screening approaches can change our fundamental understanding of cellular processes on the molecular level. The relationship between specific nutrients or diet and gene expression, may help to identify these effects and facilitate the prevention of common diet related diseases.

In the context of nutrition and micronutrient research in livestock species, transcriptomic methods have been popularly applied; however, it has been widely discussed albeit primarily in other studies using cell lines and animal models. Under such type of approach, a multitude of genes regulated at the mRNA level by dietary components has been identified and this, in turn, has provided new insights into the biological processes affected by nutritional parameters. In livestock species, the major application of nutrigenomics tools is to how effectively being utilized for dairy and meat industries.

In dairy industry, an effective utilization of microarray technology was beneficial to study mammary gland tissues (milk production and udder health), muscle growth and development and myogenesis process (beef production) and the role of gut microflora on nutritional diet intake in ruminants (health and food safety). Study of Ron et al. (2007) has effectively been hybridized Affymetrix microarray (MG-U74v2) in identification of 249 differentially expressed probe sets common to the three experiments along the four developmental stages of puberty, pregnancy, lactation and involution. In context to candidate genes for milk production traits, a total of 82 expressed genes were identified in mammary gland tissue with at least 3-fold expression over the median representing all tissues tested in GeneAtlas.

The bovine cDNA microarray for beef industry was mainly investigated for muscle fibre number and fibre composition of muscle is largely determined during prenatal development. Study of Lehnert et al. (2007) provided a detailed description of molecular events accompanying skeletal muscle differentiation in the cattle, as well as gene expression profiling for muscle growth and development and developmentally regulated in bovine foetal muscle. Their study also highlights the developmental expression pattern of *FSTL1* and *IGFBP5*, which have previously been implicated in myogenesis regulation, as well as describing the changing representation of a recently-described ncRNA (*NEAT1* orthologue) in developing cattle muscle. In context to animal health and food safety prospectives in birds and ruminant, microarray technology was successfully utilized (Paustian et al., 2008) by analysing comparatively genome of *Mycobacterium avium* subspecies obtained from multiple host species. Study showed several polymorphic regions within the genomes of *M. avium* subspecies obtained from a variety of host animals. They further concluded that genome diversity in *M. avium* subspecies appears to be mediated by large sequence polymorphisms that are commonly associated with mobile genetic elements.

In pork industries, the impact of advanced nutrigenomics tools has been discussed leveraged for the economic benefits and to improve human nutrition and health. In pig, nutrigenomics tools were effectively utilized in analysis of regulation of myogenesis and its biochemical pathways (Te Pas et al., 2007). Combination of biochemical pathway and microarray results revealed the biological insight of porcine myogenesis process is controlled by two distinct waves, i.e. Notch signaling pathway and the WNT signaling pathway. Recent study on porcine microarray expression profiling in 16 tissues revealed the interaction between gene and tissue for differentially expressed genes targeted differentially for each tissues's transcriptome (Ferraz et al., 2008). Evidence from the recently published transcriptomics based nutritional studies performed in livestock species suggests that, with appropriate study design, it is feasible to apply transcriptomic methods successfully in animal feed and nutritional research. However, newly emerged nutrigenomics tools like - gene expression-based biomarker development still poses a major challenge and the use of expression profile 'signatures', rather than single genes, may provide an eventual solution for this.

## APPLICATION OF PROTEOMICS IN ANIMAL AND FEED SCIENCES

Proteomics is the study of all the proteins in a particular cell, tissue or compartment (Banks et al., 2000). The major tools of proteomics are two-dimensional (2D) gel electrophoresis and mass spectrometry (MS). Proteomics is concerned with over 100 000 proteins in humans (Müller and Kersten, 2003). In experiments on animals, the scope of the investigations is usually restricted to assessment of the influence of dietary components on the proteome of selected organs, for example, the liver. The proteome represents the protein equivalent of the genome, which is determined by the sequence, the type and number of its nucleotides. In contrast to this static nature of the genome, the proteome represents a tremendously dynamic object, which is influenced by a variety of parameters. However, arraying of proteins is more difficult than the arraying of DNA, because they have to maintain their correctly folded conformations. The fabrication of protein arrays is, therefore, particularly challenging and protein arrays have lagged behind so far in development because of the more complex coupling chemistry, the instability of the immobilized protein and the far weaker detection signals (Chipping Forecast, 1999). In contrast to these technical problems, genome-wide screens for protein function are of biological importance for many applications such as: analysing protein expression profiles, monitoring protein-protein interactions, identifying protein posttranslational modifications, screening the substrates of protein kinases, examining the protein targets of small molecules, and proteomic analysis as a function of bioprocess cultivation conditions. A schematic overview

of integration of potential application of such protein array analysis in basic and applied nutrition and food research is presented in Figure 2.

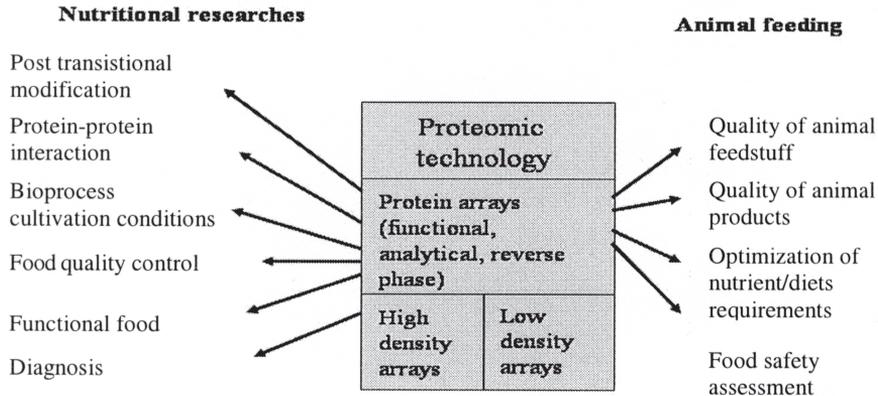


Figure 2. Schematic overview of integration of Omics technology in animal feeding and nutritional research - a proteomic perspective view

Epidemiologic and experimental studies have demonstrated that the risk of degenerative diseases in humans is increased by dietary folate deficiency. In experiments by Chanson et al. (2005), two-dimensional electrophoresis and matrix-assisted laser desorption/ionization time-of-flight MS were used to detect the changes in the proteome of liver of rats fed diets with or without folic acid. Special attention was directed on liver, because it is the main tissue of folate storage and metabolism. The results obtained indicate that folate deficiency modifies the abundance of several liver proteins consistently with adaptive tissue response to oxidative and degenerative processes. Nine spots (compared with controls) were identified corresponding to differentially expressed proteins in the liver of folate-deficient rats (Chanson et al., 2005).

In the last decade, incidences of contamination of animal products (chickens, eggs, some pigs and cattle, and dairy products) with BCB- and dibenzofuran (Schechter et al., 2001) were reported in Europe and USA. The mechanism of dioxin toxicity is not clear. For this reason, differential protein expression in Long-Evans and Han/Wistar rats exposed to dioxin was detected by two-dimensional gel electrophoresis, supported by computerized gel image analysis, in-gel digestion and mass spectrometry (Pastorelli et al., 2006). Significant changes were noted in the abundance of several proteins, which may contribute to strain-specific sensitive differences in dioxin toxicity. Furthermore, a difference in basal proteome profile between the rat strains as potential contributors to divergent sensitivity to dioxin toxicity, was also observed.

Proteomic analysis was quite effective and useful to evaluate the effect of dietary methionine on breast-meat accretion and protein expression in skeletal muscle of broiler chickens (Corzo et al., 2006). *Via* a tandem mass spectrometer, a total of 190 individual proteins were identified from *Pectorali major* muscle tissue; three of them were recognized which differed distinctly between the treatment proteome and could be considered as potential biomarkers regulated by a methionine deficiency in broiler chickens.

The use of chromatographic fractionation and/or mono- and two-dimensional electrophoresis associated with mass spectrometry allowed the identification of new minor proteins from hen egg white (Guerin-Dubiard et al., 2006). Until recently, the total number of egg proteins characterized was less than 50 and was limited to the most abundant ones. The recent development of nutrigenomics tools and the availability of the chicken genome sequence have already allowed the identification of hundreds of novel minor components of the egg (Gautron et al., 2006)

In other experiments two dimensional electrophoresis gels were used in order to study molecular proteins of high quality meat from Label Rouge turkey and a conventional line, BUT9 (Molette et al., 2006). After gel image analysis, 101 protein spots were identical in both genetic types, 53 spots were only found in the Label line and 13 were only present in the BUT 9 line. In all identified spots, 53% belonged to metabolic contractile proteins, 13% were cell defense protein and 20% corresponded to other functions.

As pointed out by some authors (Kuiper et al., 2003; Bender, 2005; Spielbauer and Stahl, 2005), nutrigenomics tools are very useful tools in the evaluation of transgenic crops. In recent years the majority of the soyabean meal and a considerable part of the maize used in animal feeding have originated from transgenic crops.

As shown in Table 2, two different approaches and techniques can be used to identify the unintended effect of genetic modification. Nutrigenomics technology, as a non-target approach, is a global analysis of all the proteins and metabolites and is, most probably, the way to exclude an unintended effect of genetic modification. In recent years, the applicability of proteomic techniques has been investigated within European multidisciplinary projects for the food safety evaluation of GM crops (GMOCARE, 2003). Results of the first published papers indicate that the microarray detection system can identify GM soyabean seeds as well as processed food made of those seeds, with 100% accuracy (Chen et al., 2004).

Table 2. Safety assessment of GM food (according to Kuiper et al., 2003)

Type of analysis	Range of analysis	Methods of analysis
Targeted approach	Macronutrients, micronutrients, anti-nutrients, toxin, secondary metabolites	Chemical and physical detection of selected ingredients
Non-targeted approach	Nucleotides Protein in a cell, tissue or biofluid Metabolites in a cell, tissue or biofluid	DNA microarray Proteomics Metabolomics

The application of recently developed proteomics tools is of interest of a wide audience of researchers because of the utility derived from understanding how genomics and proteomics function in various organisms. Applications such as xeno-transplantation, increased livestock productivity, bioengineering new materials, products and even fabrics are several reasons for thriving farm animal genome activity. Currently mined in rapidly growing data warehouses, completed genomes of chicken, pig, cows and other livestock species are available but are largely stored in decentralized data repositories. More recently, protein microarray technology has been developed for the parallel identification, quantification, and functional analysis of different proteins. In principle, these applications will allow the substitution of single-plex systems. However, for acceptance, microarray approaches should meet certain prerequisites, such as robustness, reliability, appropriate pricing, low complexity, lower demands of experimental time and manpower, comparable sensitivity and specificity, and the possibility for high-throughput use (Stoll et al., 2005). One such system, which has been developed recently and designed specifically for routine diagnostic laboratories, is the ArrayTube (AT) platform (CLONDIAG Chip Technologies GmbH, Jena, Germany). These miniaturized arrays are mounted on the bottom of standard 1.5-ml micro-reaction tubes. Hybridization and analysis are performed using standard laboratory equipment. The hybridization signals are amplified by an enzyme-catalysed precipitation reaction, and the kinetic measurement of the precipitation reaction at each spot is detected by specific changes in red light transmission, which is recorded using a photo-imager (Korczak et al., 2005). Among livestock species, the application of protein array has not been cited in context to its application towards animal nutrition and feed science research. However, it has effectively been utilized in identification of novel bacterial antigens/pathogens and in early diagnosis of *M. paratuberculosis* infections in cattle (Bannantine et al., 2008).

## APPLICATION OF METABOLOMICS IN ANIMAL AND FEED SCIENCES

Metabolomics represents the final step in understanding the function of genes and their proteins. The aim of metabolomics is to determine the sum of all metabolites (other substances than DNA, RNA or protein) in a biological system: organism, organ, tissue or cell (Müller and Kersten, 2003). Techniques employed to investigate the metabolome include nuclear magnetic resonance (NMR) spectroscopy, high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). These methods are capable of resolving and quantifying a wide range of compounds in a single sample. The main characteristics of these new technologies were miniaturization, automation, high throughput and computerization (Corthesy-Theulaz et al., 2005).

Metabolomics is concerned with several thousands of metabolites in humans (Müller and Kersten, 2003). As in the case of transcriptomics and proteomics, the scope of metabolomic analysis is mainly restricted to the assessment of the influence of dietary components on the metabolome of selected organs or tissue in animal nutrition studies. In experiments performed by Bertram et al. (2006) metabolomic analysis was implemented to detect the changes in the biochemical profiles of plasma and urine from pigs fed with high-fibre rye bread. Two diets with similar levels of dietary fibre and macronutrients, but with contrasting levels of wholegrain ingredients, were prepared from whole rye and fed to pigs. Using an explorative approach, the studies disclosed the biochemical effects of a whole-grain diet on plasma betaine content and excretion of betaine and creatinine.

In another experiment, proton nuclear magnetic resonance microscopy (H-1-NMR) was used to determine the metabolite profiles in the liver of rats used as a animal model to characterize the toxicity of triazole fungicides (Ekman et al., 2006). Triazole fungicides, which exhibit their antifungal activity by inhibiting fungal ergosterol biosynthesis, are economically important agricultural chemicals as they are widely used on crops such as wheat, barley and orchard fruits (Filipov and Lawrence, 2001). For this reason animal feed can be, sporadically, contaminated with these fungicides. The results of above quoted report support the possible application of a metabolomics approach to assess the toxicity of triazole fungicides and identifying biomarkers of exposure and/or effect.

The results of another study, indicate that hierarchical metabolomics is useful in evaluating potentially undesirable changes in the overall metabolite composition of transgenic plants (Catchpole et al., 2005). Comparison of total metabolites in tubers of GM and conventional potatoes indicated that GM potatoes with increased content of inulin-type fructans were substantially equivalent to traditional cultivars.

In the past decade, a large amount of metabolomic data has been produced concerning the versatility of metabolomics/metabonomics as high through-put functional genomic tools for monitoring disease processes, drug toxicity and phenotypic genetically modified mammals nutrigenomics, clinical trails for human studies (Raamsdonk et al., 2001). In most of the mammalian metabolomic data, it is important to identify, develop, and disseminate a core set of reporting requirements necessary for the minimal description of biological samples and procedures. Most of the biological samples used in metabolomics experiments are currently being developed by the metabolomics society (<http://www.metabolomicssociety.org/>). In a very recent publication Griffin et al. (2007) proposed the foundation of a metabolomics standards initiative-mammalian context working sub-group (MSI-MCWSG) as a part of the wider standardization initiative led by the Metabolomics society. They divided the reporting requirements for metabolomics experiments into two, first as pre-clinical studies (for example: toxicology, func-

tional genomics experiments, drug efficacy and disease intervention) and second as clinical studies (for example: clinical trials, nutritional studies, human disease investigation). According to Griffin et al. (2007), most of animal researches in the fields of nutrigenomics and functional genomics can be classified as pre-clinical studies.

## CONCLUSIONS

Application of nutrigenomics tools, i.e. transcriptomics and metabolomics can be utilized to efficiently investigate molecular events taking place in a genome receiving nutritional signals and responding to them through characteristic metabolic processes in the organism. The cumulative application of different molecular biological techniques in transcriptomics, proteomics and metabolomics discussed in this review paper can lead to the essential survey of multi-factorial, nutritional influences on humans and livestock species. In last decade, microarray technology has been extensively utilized in livestock species as nutrigenomics research tool to improve food production, quality and their safety in dairy and meat industries. This widely utilized microarray or DNA chip technology in nutrigenomics research enables not only the screening of large numbers of genes simultaneously, giving a comprehensive picture of the variation of gene expression patterns, but will also provide explanations for complex regulatory interactions, such as those between diet-nutrients and genes.

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