

# Invited Speakers

## S6.5

### **Transcriptomics Based Approaches to Evaluating and Understanding Health and Nutritional Status of Poultry**

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Advances in the fields of nutrigenomics will enable avian researchers and producers to ask key questions about diet and its effects on poultry health and performance. By focusing on gene expression and functional genomics, it is very likely that we will soon be able to gain a more definitive understanding of the importance of dietary intervention in nutritional strategies. Whilst still in its infancy, this new scientific frontier will revolutionise our thinking about how dietary supplementation can have such dramatic and beneficial responses on whole body health. Functional genomic studies utilising real-time PCR and high-density microarrays are providing new ways for researchers to rapidly evaluate the effects of diet, environment and disease processes on the growth and performance of poultry.

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Key words: poultry, nutrigenomics, transcriptomics, microarray, review

#### **Introduction**

Over the last decade, the science of nutrigenomics has emerged. This new frontier of scientific research covers a wide range of technologies, the ultimate aim of which is to elucidate the influence of diet on the genetic programming of cells and tissues. Considering the current global issues that are impacting on poultry production, it is evident that a number of major challenges lie ahead. Issues such as the financial crisis, resource and energy strains, population growth and increasing food demand have placed a significant strain on production capacities. Additionally, the threat of a viral pandemic, consumer demands for antibiotic-free foods and the increasing consumer desire for functional and value-added foods will require changes in our approaches to production and efficiency. To meet these demands, producers will need to reappraise their approach to animal nutrition. This will not only result in even more maximisation of genetic potential through dietary and husbandry practices but also in the exploitation and maximisation of the genetic potential of the bird at the molecular level. Molecular potential exploitation is dependent on advances in the science of nutrigenomics, the main emphasis of which is the prevention of disease by optimizing and maintaining cellular, tissue, organ and whole-body equilibrium or homeostasis. This requires not only an understanding of, but the ability to manipulate a multitude of nutrient-related interactions at the gene, protein and metabolic levels. These new disciplines and their attendant technologies will redefine animal health and nutrition in the future.

#### **Poultry nutrition**

Traditionally speaking, classical genetics as applied to animal husbandry typically relied on the phenotypical appearance of an animal due to the ease with which one could judge the outcome of a breeding program. In some respects, this has successfully led to the development and exploitation of the genetic potential of a breed. However one must remember that it is the complex interaction between an animal's genotype, environmental factors and random variation that will ultimately determine phenotype. With respect to environmental influences, animal nutrition has played a pivotal role in further exploiting genetic potential. Dietary intervention to prevent disease through feed supplementation has become the norm, with producers realising that not only does an animal's health and whole body homeostasis affect its performance but that maternal health can dramatically influence the performance and health of its offspring.

Over the last century, modern selective breeding has significantly advanced egg and meat production traits (Burt 2002). However, these major advances have come at a cost with the introduction of a number of undesirable traits. For example in meat-type chickens, there has been an increase in the incidence of congenital disorders, such as ascites, reduced fertility and reduced resistance to infectious disease. Egg producers have experienced an increase in the incidence of osteoporosis associated with increased egg production among their flocks.

# Invited Speakers

The current rates of genetic progress in egg and meat production indicate that the industry will reach capacity within the next twenty years (Burt 2002). As such, the priority in the poultry industry will be to reduce costs and develop new products. Consumers however, want high-quality products and this leads to the requirement for greater uniformity and predictability in production. With an increased requirement for food safety, there will be a need to reduce the use of chemicals and antibiotics and increase genetic resistance to pathogens. These traits are difficult and costly to measure by conventional genetic selection but developments in poultry genomics in the last few years has provided new avenues of research to solve these problems.

## Avian genomics

In 2004, the first draft of an avian genome (the red jungle fowl) was published, elevating the chicken to model organism status and instantly provided a valuable resource for scientists studying a diverse set of issues related to avian health and production (International Chicken Genome Sequencing Consortium, 2004; Wallis *et al.* 2004).

The fully annotated sequence provides a framework for chicken breeders who want to understand how genetic variation influences traits that are important in the production of domestic chickens, by allowing these traits to be mapped back to precise genomic locations and genes.

An additional publication by the International Chicken Polymorphism Map Consortium (2004) described numerous single-base-pair differences (2.8 million) between three lines of domestic chicken (broiler, layer and Silkie) and the red jungle fowl. The map they developed has also allowed researchers to identify the genes, and the combinations of gene variations, that produce desirable traits in chicken breeding populations. It should also increase the odds of optimizing a particular trait in subsequent generations.

These key publications have allowed for the development of microarrays based on the completed chicken genome and opened the research options for avian scientists even further. However, one of the greatest challenges still in understanding the vast volumes of microarray data relates to the development of models to reconstruct functional gene networks and regulatory pathways.

To fully appreciate the effects of nutrients on poultry physiology, we must consider the effects at not only the transcript level (transcriptomics) but also at the protein (proteomics) and metabolite (metabolomics) levels. Obviously the full interpretation of such data requires the use of sophisticated computational approaches to identify metabolic pathways and co-regulatory mechanisms.

## Utilisation of genomic information

Studies of the basic biochemistry behind genetics, the genetic structure, and the basic flow of information in biological systems have fostered the development of a multitude of new genomic-associated disciplines. These are generally based on some basic molecular tools that were developed to increase our knowledge of the basic molecular structure of life. The sciences that make up functional genomics include transcriptomics, proteomics, and metabolomics, which study the quantitative relationships between the genome and gene expression, protein production, and metabolic processes, respectively. At its very basic level, biology can be defined by a central dogma that describes flow of biochemical information from DNA to RNA and then to protein. As a result, the information contained in the nucleotide base sequence in DNA determines the basic amino acid sequences in protein and plays a large role in determining the structural and functional nature of the encoded proteins.

All biological processes, are dependent on the regulation or control of the information flow in this pathway. While this process is carefully controlled by the basic genetic determinants, many external factors can also influence its regulation. Such factors include disease challenge, exposure to environmental toxins, and nutrient supply. The basic understanding of these complex regulatory processes has changed considerably with the delineation of the various animal, plant, and microbial genomes. It is now possible to understand these regulatory processes in extremely fine detail. One step in this pathway, the transcription of a gene sequence into mRNA, is currently being described by examining factors that influence the expression of specific genes and the transcription of their corresponding mRNA. The science of transcriptomics is based on the examination of gene expression patterns resulting from quantitative examination of the abundance of mRNA copied from a basic nucleic acid blueprint contained in the genome.

# Invited Speakers

## **Transcriptomics and the use of microarrays for evaluating gene expression**

In the last 10 years, our knowledge of nucleic acid sequences, nucleic acid hybridization, and cloning techniques has provided tools that can be used to gain a clearer understanding of overall gene expression at the transcriptional level. While techniques to study the expression of individual genes have been available for many years, oligonucleotide and cDNA microarrays have provided powerful tools that will allow rapid evaluation of gene expression on an unprecedented scale. These techniques are based on a quantitative assay of the relative concentrations of specific RNA messengers (mRNA) in tissue samples. The relative amount of individual mRNA molecules present in a given tissue or cell directly reflects the level of gene regulation and can be used to quantitatively examine the factors that regulate the gene expression. The amount of the mRNA transcript present in tissues can be measured indirectly after it is extracted and then used to create a complimentary labeled strand of DNA. This labeled material can be hybridized with a complimentary strand on an array containing a known set of gene sequences that are attached to a solid glass slide or nylon substrate. The sequences are often organized as an array of small spots on the solid matrix and are generally referred to as probes. The intensity of the color that results during the hybridization process is directly related to the amount of target mRNA present and reflects the level of gene expression. In this way, it is possible to determine which gene is up-regulated or down-regulated as a result of specific biological manipulations or during normal tissue development. Comparison studies of gene regulation can be carried out using subtractive hybridization procedures that use contrasting color labels on complimentary DNA from two sets of messengers from different tissues (Moody, 2001). As a result, it is possible to quantitatively compare gene expression in two contrasting groups of tissue or animals. By using robotic techniques for producing arrays on a minute scale and laser techniques to discern the color of specific spots, it is possible to examine the expression of thousands of genes at one time. This is an extremely powerful tool that can be used to study metabolic processes at a very basic level and lends itself well to the complex understanding of interactions that regulate gene function. Since gene transcription is only one step in the regulatory pathway that leads to functional protein formation, it is not always possible to correlate the increased presence of mRNA in the tissue with phenotypic or protein changes in tissues (Moody, 2001; Muller and Kersten; 2003). While studies of gene transcription may have many drawbacks in this respect, the ability to globally evaluate the initial regulatory steps in gene expression provides many tools for elucidating the key processes in metabolic regulation. Powerful screening methods are now available to identify the key gene expression patterns that are influenced by environment, disease, and nutrition or simply during the process of tissue development. In the past, microarray studies have depended on specific arrays with relatively few nucleotides and limited amounts of information. These arrays were often generated to examine specific metabolic functions or immune responses. Recent work has reported the development of arrays that can be used to examine gene expression in a variety of species. These arrays range in size from a few hundred probes to systems that have over 40,000 elements. While the use of smaller, more defined arrays to examine regulation of specific tissue response have been useful, the development of standardized systems for examining the expression of large numbers of genes will greatly enhance our ability to understand basic metabolic and physiological functions.

## **Current applications for transcriptomics in poultry health and nutrition**

Transcriptomics based studies utilizing not only cDNA microarrays but also quantitative real-time PCR (qRT-PCR) are being increasingly used to evaluate the health and nutritional status of poultry (Cogburn et al. 2007). Notable among the very many research applications are studies designed to utilise these technologies to identify the immunological response of poultry to infectious disease. Studies carried out on Marek's disease virus (MDV) by Liu *et al.* (2001) found that MDV infection was linked to expression of TSA-1, a gene important for T-cell differentiation and activation. Munir and Kapir (2003) evaluated gene expression patterns in response to respiratory pathogens. Microarray analysis of *Eimeria* has also been reported (Min *et al.* 2003) as has the transcriptional response of chickens to *Salmonella* infection (van Hemert *et al.* 2006). Oncogene induced lymphomagenesis has also been examined by a number of groups (Neiman *et al.* 2003; Black *et al.* 2004; Morgan *et al.* 2001) and they have illustrated the common mechanisms that exist within transformation pathways in poultry. Degen *et al.* (2006) used microarray technology to study the immune response to vaccination with avian influenza. Such studies provide strong insights into host-virus interactions. Similarly, Ruby *et al.* (2006) examined the differential inflammatory response between resistant and infectious lines following exposure to infectious bursal disease.

# Invited Speakers

Ultimately, the identification of gene expression patterns and pathways in response to infection may play a role in the development and implementation of effective immunomodulators and vaccines.

With regard to the study of nutrition and metabolism using a transcriptomics based approach, a large number of research groups are currently engaged in this area. There are numerous studies detailing differential gene expression patterns in tissues such as the liver. Whilst some of these focus on differential gene expression in the abrupt embryo-to hatchling transition period (Cogburn *et al.* 2003; 2004), additional studies have focused on altered nutritional states such as fasting and refeeding (Cogburn *et al.* 2004; Duclos *et al.* 2004; Feige and Auwerx, 2007). Bourneuf *et al.*, (2006) used a focused cDNA microarray to study differential hepatic mRNA expression in genetically fat (FL) and lean (LL) poultry lines. Results from this work indicated that numerous genes involved in lipogenesis such as *APOA1*, *SREBP1* and *MDH2*, were overexpressed in the FL line. Overall, the results strongly suggest that regulation of adiposity in lean and fat chicken lines is linked to regulation of genes involved in lipogenesis. Given that excessive adiposity has become a major drawback in meat-type chicken production; this type of analysis will ultimately lead to a better understanding of the role of nutrition in effecting changes at a molecular level and allow for far greater control of problem areas such as adiposity.

Yang *et al.* (2007) used a cDNA microarray approach to examine the differential expression of mRNA transcripts in shell glands from low and high egg production chicken lines. The authors identified 85 differentially expressed genes and proposed that these differentially expressed transcripts may aid in the development of potential molecular markers associated with high rates of egg production and provide the basis of a rapid and early breed selection method, operating prior to the egg-laying stage.

Recent unpublished work (Dawson, personal communication) has utilised a cDNA microarray based strategy to compare broiler gene expression in a tissue specific fashion in response to organic selenium in the form of Sel-Plex<sup>®</sup>, selenomethionine and inorganic sodium selenite supplementation. One of the most striking benefits of improved selenium status was the overall reduction in cellular stress associated with oxidation response. Genes encoding the key antioxidant proteins GSH-Px 1 and Thioredoxin reductase were upregulated by over 4 and 2 fold respectively when compared to control groups. Other genes which were shown to be upregulated following selenium addition included Thioredoxin 2 and Iodothyronine deiodinase, both of which play key roles in fertility (Dawson, 2006). Additional studies looked at the expression of genes in tissues such as cerebral cortex, intestinal liver and skeletal muscle. One of the main genes studied encoded the GADD45 $\beta$  (Growth Arrest and DNA Damage-Inducible) protein. This gene is involved in the regulation of cell cycle and apoptosis (programmed cell death) and has been shown to be induced in response to oxidative stress and, in particular, DNA damage. Expression of this gene is now recognized as an excellent marker for these stressors. The studies found that GADD45 $\beta$  expression levels were significantly decreased across all tissues but only in the Sel-Plex<sup>®</sup> treatment group. This indicates lower endogenous oxidative stress and DNA damage throughout the entire animal.

More recent work (Dietrich, personal communication) has utilised cDNA microarrays to examine differential gene expression in broilers fed a diet with moderate deoxynivalenol (DON) contamination (1.0, 2.5 and 5.0 mg DON/kg). This group found that in the liver, 368 genes were upregulated and 114 genes were downregulated in groups with DON-contaminated diet compared to the control group. The genes were primarily related to mRNA stabilization, signaling, apoptosis, DNA and protein repair and protein modification. The genes AKR1B1, EIF2AK3 and MIA2 were downregulated and gene IFT57 was significantly upregulated due to DON contamination in the feed. The authors concluded that even at low levels (1mg/kg DON) of contamination, alterations in gene expression occurred in the liver of broiler chickens.

Ultimately it will be through the use of nutrigenomics based tools that we will be able to fully understand how nutrition can both positively and negatively influence homeostasis and how we can develop strategies for the prevention and treatment of disease. Microarray based studies could also potentially play a role at a regulatory level in assisting governmental agencies in defining maximum permissible feed limits for contaminants and toxigenic agents such as mycotoxins.

# Invited Speakers

## Avian research beyond the genome

Following on from the completion of the avian genome sequence, the main challenge facing poultry scientists is the utilization of this information to improve all facets of poultry production. The coordination of existing resources such as genomic and phylogenetic data, quantitative trait loci (QTL) markers, expressed sequence tag (EST) libraries, the ever improving microarrays, and the so called 'omics' tool sets will allow for an understanding of the complex and interconnected molecular pathways controlling cellular and molecular biochemistry. The genome sequence has already begun to facilitate the study of genes and their regulatory elements, the subsequent gene products and the gene expression patterns for various metabolic processes. The avian genome will be essential to predict the amino acid sequences of encoded proteins/peptides, thus facilitating the development and utilization of proteomics based research approaches. Ultimately, the completed avian sequence has allowed for a rapid and simplified tool allowing scientists to search for candidate genes that are in close proximity to a marker linked to a desirable trait thus accelerating and increasing the breeding potential.

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# Invited Speakers

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