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Review Article

NUTRIGENOMICS: NUTRIENT GENE INTERACTION IN POULTRY

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Abstract: Nutrigenomics is a science which study the effect of nutrients on the gene expression. 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.

Keywords: Nutrigenomics, Gene expression

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Introduction

Feed constitutes a major portion of total production costs, therefore, improvement in the efficiency of feed utilization will reduce the amount of feed required for growth, which would directly reduce production cost, increase profitability, and subsequently reduce the amount of manure produced. Genetic variation in feed efficiency still exists in broiler chickens and needs to be exploited for genetic gain [1]. However, current improvement methods for feed efficiency (FE) are limited in the rate of annual genetic improvement because the actual feed efficiency phenotypes (FEP) defined by genotypes or gene markers are unknown. Energy and protein are the principal dietary constituents affecting live performance and muscle development in poultry and other animals. Also, the expression of various genes responsible for vital metabolic and regulatory functions of the body is dependent on the calorie-protein status of an individual. Waldroup and Hellwig (1995) [2] reported that differences in the determination of methionine requirement for laying hensover the years is quite understandable due to major changesin genetics, nutrition and management which the birds are subjected to, besides the effects of age, type of diet and environmental conditions. The advancements in the knowledge of the nutritional requirements of birds, at their many phases, has constantly brought improvement to the quality of the diet; firstly, in the sense of reaching maximum production [3]. Thus, the great knowledge of the metabolism of protein in birds and the production of amino acids on a commercial basis have enabled the utilization of the concept of ideal protein for the formulation of diets. The growth rate is related to the feed efficiency and the deposition of muscle mass. The efficiency of an animal to convert food into muscle is related to the efficiency of energy production. Studies show that birds with lower ATP production because of lower efficiency of ATP production from substrate in the mitochondria have poor feed efficiency or feed conversion [4]. The efficiency in energy production depends not only on the perfect coordination among the complexes of the respiratory chain, but also on potent antioxidant system that protect mitochondria against the damage by products generated during ATP production. Methionine is required for the synthesis of glutathione, potent mitochondrial and cell antioxidant, further studies are needed to understand how the supplementation of methionine amino acid may influence the expression of the genes involved in energy production in mitochondria. Some important proteins were involved in the process of ATP production by the mitochondria: uncoupling protein (*avUCP*), adenine nucleotide translocase (*ANT*) and cytochrome c oxidase subunit III (COX III). Several researchers have demonstrated the relationship between the expression of genes encoding those proteins with feed efficiency in poultry. Genes that affect either feed intake or body weight gain (BWG) may or may not necessarily affect feed efficiency [5]. Thus, selection programs based on combinations of feed efficiency genotypes (FEG) or gene markers and the current traditional method will offer greater accuracy in breeding value estimation and consequently, a faster rate of genetic improvement. Some researchers have used quantitative trait loci (QTL) mapping to show complex genetic basis for feed efficiency. Feed efficiency QTL have been mapped in poultry [6]. However, confident interval of QTL regions is usually large and further fine mapping is required to narrow the QTL region and subsequently identify the underlying genes. Genomic profiling is the first critical step to comprehensive understanding of the mechanisms that underlie the interaction of nutrition and the genome. It is well known that nutritional perturbations affect gene expression [7], and these perturbations have been used to establish gene networks. However, gene networks established from genetic mutation perturbations would be useful for genetic improvement since such functional mutations are the genetic raw material needed to establish trait genotypes. Limited studies have only been made on global gene expression profiling on feed efficiency [8]. Microarray technology permits genome-wide differential gene expression analysis to uncover pathways and networks underlying feed efficiency. The expression of growth-related hormones, such as insulin-like growth factor I (*IGF-I*) and growth hormone receptor (GHR), may be influenced by other factors, such as nutrition [9,10]. According to Kimball and Jefferson (2004) [11], amino acids play a key role in regulating some cellular processes, such as the regulation of gene expression by mRNA modulation. Still, according to these authors, the cells are able to recognize the availability of amino acids and generate changes in translational signalling pathways, which are also regulated by hormones and growth factors. Characteristics governing animal production, such as feed and reproductive efficiency, are expressed as a function of the animal's genetics, the environment to which the animal is exposed and the interaction between these two factors. Selection for production traits in the poultry industry (broiler and layer) has resulted in a rapid improvement in animal performance. For broilers, the main selection pressure has been on growth rate, feed efficiency, and carcass traits, and in layers, the focus has been to increase egg production and quality.

However, although several traits have been genetically improved, phenotypic and genetic variations still exist among chicken populations due to differences in selection practices imposed by different breeding programs; therefore, improvements are required in this regard.

To obtain considerable genetic gain in a selection program, it is necessary to understand the population structure and the genetic architecture of the traits to be selected for, in order to avoid deleterious effects. With the advantage of DNA investigation technologies, the ability to identify molecular markers that are used to construct linkage maps has improved, allowing the detection of hundreds of quantitative trait loci. Several studies using microsatellite markers have identified QTLs associated with production traits across the chicken genome [12]. Other studies that have used single nucleotide polymorphism (SNP) markers have identified genetic associations and linkage with production, health, and behavioural traits in farm animals [13].

Important QTLs have been identified on chicken chromosome 4 (*GGA4*) between the markers *MCW0240* and *LEI0063*, which are associated with body weight [14,15].

Initial investigations of this region have resulted in the identification of a polymorphism located at 76,163,331 bp G>A on *FGFBP1* (protein binding growth factor fibroblast 1), which is associated with eviscerated carcass weight in a commercial broiler line. Single-marker studies cannot precisely identify regions that harbor causative mutations. To increase our knowledge of this important QTL on *GGA4*, in this study, three additional genes positioned between markers *MCW0240* and *LEI0063* were sequenced in a F1 population in order to detect SNPs, and their associations with growth and carcass traits was analysed.

Gene expression due to alterations in energy and protein levels

The transcription level of *mucin* gene was found to be modified by dietary protein and energy levels, and age of the bird [16]. mRNA expressions of *MUC-2* and *β-actin genes* in intestinal mucosa were quantified at the age of first, third and fifth week by real time PCR, using *β-actin* gene. *Mucin* expression in duodenum was higher in all treatment groups compared to control diet at early age but reduced as the age progressed in broilers.

Gene expression due to alterations in amino acid levels

The cDNA was amplified using primers specific for the target genes, and expression was analyzed using the real-time polymerase reaction (qRTPCR). mRNA *avUCP* expression in the muscle, showed significant difference among treatments. No statistical difference was observed among treatments with regard to the expression of the *COX III* and *ANT genes* in the muscle and liver. There was no significant effect of methionine supplementation on the expression of mRNA *avUCP* in liver [17].

Dietary supplementation of methionine on intestinal amino acid/peptide/monocarboxylic acid transporter gene expression was studied by Zhang *et al*. (2015) [18]. Duodenum, jejunum, and ileum were collected and mRNA abundance was assayed by real time PCR and expression data were calculated. *ATB0,+, b0,+AT, B0AT, LAT1, rBAT, SAT1, SAT3, y+LAT1* and *y+LAT2* had their highest expression in ileum, while *SAT2, MCT1, NHE3* and PepT1 had the highest expression in duodenum. When analyzed within each segment, there was no significant difference between different levels or sources of methionine. In conclusion, dietary supplemental methionine sources altered minimally the expression of intestinal nutrient transporter genes in broiler chickens.

In ovo administration of amino acids: lysine, arginine, threonine or methionine plus cysteine (Methionine+Cystine) in broiler chicken embryos. Insulin like growth factors (IGF) I and II, and *mucin*) and immunity related genes (*IL-2, IL-4, IL-6, IL-12, TNF-α, and IFN-γ*) [19]. Arginine and threonine enhanced the expression of growth-related genes, while threonine and Methionine and Cystine modulated expression of immune genes in broiler chickens.

cGH and mucin gene expression-lysine, threonine, arginine or Methionine+Cystine

IGF-II expression - threonine, arginine or Methionine+Cystine

cGH, IGF-I, IGF-II and *mucin gene* - threonine or arginine

IL-6 and *TNF-α* - Threonine or Methionine+Cystine

TNF-α – arginine higher *IL-2*, but lower of *IL-12* and *IFN-γ* gene - Lysine, threonine or

Met+Cys

GHR mRNA expression in the liver and muscle and increased *IGF-I* mRNA expression in the liver was influenced by methionine supplementation. *UCP* mRNA expression in the muscle was higher in methionine deficient diet [20].

Gene expressions in correlation with body weight

The gene expressions study of *b0, +AT, EAAT3, PepT1, LAT4, NHE2, NHE3,* and *y+LAT2* in the small intestine had positive correlations with both body weight and intestinal weight of the domestic pigeon [21]. However, mRNA expression levels of *CAT1, CAT2, EAAT2, SNAT1*, and *SNAT2* in the small intestine had the opposite. Single markers were used and identified - *KLF3* gene was associated with weight gain, PPPARGC*1A* - was associated with liver and wing-parts weights and yields, *SLIT2* - was associated with back yield and fat traits, *PPARGC1A* and *SLIT2* - were associated with body weight [22]. Growth traits are under the control of multiple genes and understanding the genetic information of related genes is helpful for the selection and breeding course through marker assisted selection [23]. A single nucleotide polymorphism was identified using PCR-RFLP technique and confirmed by sequencing. The *A287G SNP of BMPR-1B* gene was associated significantly with body weight and may be considered in Marker Assisted Selection program to improve chicken growth performance. Adiponectin receptor 2 (*ADIPOR2*) is a receptor for both globular and full-length adiponectin [24]. In ovo administrated carbohydrates on the expression pattern of growth in broiler chicken embryos [25]. In ovo injections were carried out on the 14th day of incubation into the yolk sac/amnion of the expression of growth-related genes: chicken growth hormone (cGH), insulin-like growth factor-I & II (*IGF-I* & II) were studied in hepatic tissues and *mucin* were studied in jejunum tissues of late-term embryo and early post-hatch chicks.

Gene expressions due to alterations in feed intake

Chickens selected for improved RFI achieve efficiency by reducing feed intake with a nominal or no change in weight gain by either up-regulating *CD36, PPARα, HMGCS2, GCG* or down-regulating *PCSK2, CALB1, SAT1,* and *SGK1* genes within the lipid metabolism, small molecule biochemistry, molecular transport, cell death, and protein synthesis molecular and cellular functions.

Leptin plays a role in the regulation of appetite, energy expenditure, and maintenance of body weight through its actions at specific hypothalamic sites as part of a negative feedback control system [26]. The signalling function of leptin was subsequently found to require the expression of specific leptin receptors and melanocortin receptors Richards *et al.* (2003) [27]. Two types of signals produced by the gastrointestinal tract have been proposed: those that stimulate feeding behavior such as ghrelin and those that inhibit it such as CCK and bombesin [28].

In mammals, changes in the circulating level of leptin and possibly insulin signal the hypothalamus to effect long-term changes in energy balance by activating or inhibiting specific anabolic and catabolic efferent pathways. Leptin protein levels in plasma and tissue (liver and fat) samples from chickens have been analyzed using specific immunoassay techniques.

Gene expression on organ weights

The data revealed that the g.34490C>T mutation in intron 3 was significantly associated with liver weight and globulin in chicken (Wang *et al.* 2015). The g.34490C>T mutation might play an important role in regulating liver weight. but it is uncertain whether it could be a molecular marker for liver disease.

Gene expressions on feed conversion ratio/feed efficiency

Single nucleotide polymorphisms and their associations with important economic traits (Pertille *et al.* 2015). Using multiple markers, *SLIT2* gene was associated with feed conversion. Increase in breast muscle content indicated high feed efficiency in chickens [29]. Tissue samples from extreme high and low feed efficiency in broiler chickens were used to identify genes and pathways differentially regulated in breast muscle, providing important information towards understanding the biological basis of variation in FE in broiler chickens.

RNA was isolated, RNA sequencing, mapping of the genes, expression of the genes was analysed, the gene expression was verified by Nano Stringn Counter technology. *IGF-I* mRNA gene expression and *GHR* mRNA gene expression was high and *UCP* mRNA expression in the liver was lower in high FE in Japanese quails (Gasparino *et al*. 2013). Total RNA was extracted from the liver and breast muscle of each quail, and cDNA was amplified using specific primers for the target genes. Expression was analyzed using quantitative real-time PCR (qRT-PCR). Haplotype analyses of genome-wide significant *SNVs in PGM2, PHKG1, DGKZ,* and *SOD2* were associated with FCR. This finding facilitates the discovery of causative variants for FCR and contribute to marker-assisted selection [30]. Functional variants in FCR trait was analyzed by coding and non-coding single-nucleotide variants (SNVs) across the genome by exome sequencing in broilers with divergent FCR and with a sequence coverage at an average depth of four-fold.

Gene expression on immunity

In ovo administrated carbohydrates on the expression pattern of growth in broiler chicken embryos (Bhanja *et al.* 2015). In ovo injections were carried out on the 14th day of incubation into the yolk sac/amnion of the broiler chicken embryos. In ovo glucose could modulate humoral-related immunity, while fructose or ribose might help in improving the cellular immunity in broiler chickens.

Gene expression on egg parameters

Egg weight influenced the amino acid transporter genes in the yolk sac membranes and small intestines of pigeon embryos. expression of amino acid transporter genes in the yolk sac membranes and small intestines of the domestic pigeon (*Columba livia*) was based on the egg weight [31]. The gene expression of *EAAT2* in the intestine of high egg weight producing birds, whereas the expression of *EAAT3* was lower in the high egg weight producing birds.

Application of review: The role of genes in manipulating the role of nutrient in various production performance like body weight, body weight gain, feed consumption, feed efficiency, meat yield, immunity, egg production and egg weight.

Review Category: Gene expression

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