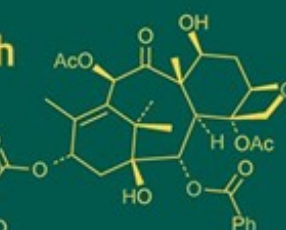


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The names and affiliations of all authors are provided below, following the references.

Role of diet in gene expression for functional outcomes in poultry: A review of nutrigenomic mechanisms and applications

Rishabh Sarwal¹, Ripusudan Kumar², Akriti Ballabh³, Anshu Rahal⁴, Bidhan C Mondal⁵, Aman Kamboj⁶, Manju Lata⁷

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ABSTRACT

Poultry production increasingly requires an understanding of the impact of nutrients and bioactive on biological processes that control growth, feed utilization, immune response, reproduction and meat and egg quality. Nutrigenomics approaches offer an opportunity to investigate diet-gene interactions by merging nutritional and genomic data along with transcriptional, epigenetic and metabolic parameters. The present review aims to describe mechanisms by which dietary factors can affect gene expression in poultry with examples of their application. Nutrigenomic research is exemplified by experimental results on dietary phytochemicals, lipids, methyl donors, trace minerals and methionine in terms of molecular, physiological and production responses. Also, the following areas where nutrigenomics can be used are described: precision nutrition, nutritional programming, phytochemical feed additives, biomarkers development, meat and egg quality improvement. Despite the possibility of diet-induced changes in gene expression and corresponding phenotypic responses, transcriptomic association does not imply direct molecular causation. There are still a number of important aspects to be clarified: protein level and functional validation, studies on genotype × diet interactions, causal epigenetic associations, multi-omics integration, biomarker validation and implementation into commercial practice. For future research in nutrigenomics, larger, replicable and integrative studies are needed to combine molecular responses with traditional nutrition and production economy.

Keywords: Nutrigenomics, Gene expression, Poultry nutrition

1. Introduction

Modern poultry nutrition is increasingly considered not just as a source of nutrients for energy provision, tissue development, and production, but also as a mediator of cellular signaling and regulation of gene expression by dietary factors. The interface between nutritional exposure and cellular/genetic processes is commonly described as nutritional genomics or nutrigenomics (Farhud *et al.*, 2010; Reen *et al.*, 2015) [1, 2]. Dietary factors and bioactives can regulate the activity of transcription factors, nuclear receptors, nutrient-sensing cascades, chromatin remodeling, and post-transcriptional regulators in order to modulate gene expression in relation to growth, metabolism, reproduction, antioxidant protection, and immune functions. This process is highly dependent on the context, which includes the genotype, dose, target tissue, developmental stage, physiological state, and environment, amongst others. As a result, even birds that receive apparently identical diets will have a different genetic and phenotypic outcome (Alagawany *et al.*, 2022) [3].

One of the key developments in this area is the use of omics technologies. Nutrigenomics may be considered as an approach that covers genomic diversity all the way through the transcriptome, proteome, and metabolome, with epigenomics and metagenomics as supplementary regulation and host-microbiome layers (Bordoni *et al.*, 2021; Brennan & de Roos, 2021) [4, 5]. Transcriptomics involves RNA expression, which may be assessed via targeted RT-qPCR and RNA sequencing; proteomics examines protein expression along with their post-translational modifications, when necessary; metabolomics assesses the presence of small molecule metabolites; and genomics studies the diversity of DNA sequences, including SNPs. Some of the available analytical methods include SNP genotyping for genomics; microarrays, RNA sequencing, and RT-qPCR for transcriptomics; 2D electrophoresis, high performance liquid chromatography (HPLC) and mass spectrometry for proteomics; and NMR spectroscopy or GC/LC-MS for metabolomics (Herrero *et al.*, 2010) [6]. In the poultry, transcriptomics, proteomics, and metabolomics are used for studying feed efficiency, nutrition, meat quality, and disease resistance, whereas integrated genomics and omics are increasingly used for exploring more complex traits of production (Zampiga *et al.*, 2018; Sukhija *et al.*, 2023) [8, 7]. Hence, using omics technologies, nutrigenomics goes beyond one-gene approach and helps to study the impact of diet at different biological levels.

Corresponding Author: Rishabh Sarwal

Department of Animal Nutrition, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

The omics layers act as complimentary, not substitutable, modules. Changes occurring at the transcriptome level do not automatically equate to changes in the levels of proteins or metabolites; hence, integrated measurements will be more informative when there is need for mechanistic interpretation of the biology problem at hand (Brennan & de Roos, 2021; Zampiga *et al.*, 2018) [5, 8].

Nutrigenomics is a science that studies the molecular basis of how nutrition and bioactive compounds from food affect gene expression and its consequences, and nutrigenetics studies how hereditary genetic diversity affects individual reactions to diet (Alagawany *et al.*, 2022) [3]. Nutritional genetics encompasses both pathways of interaction where diet may change molecular phenotype and where genotype changes reaction to diet. Overall, these principles allow understanding of inter-individual differences in bird growth, feed efficiency, immune system, metabolic activity, and quality of produce. Molecular endpoints may include gene expression, methylation, miRNA profiles, proteins, and metabolites, alongside with traditional measures of performance and physiology. Whole-blood and tissue gene-expression profiling can provide useful molecular information, but interpretation requires attention to tissue specificity and the distinction between association and causation (Olsen *et al.*, 2015) [10].

The conversion of nutrients in the diet to growth, reproduction, immunity and quality meat and egg products is essential in modern poultry production systems. Breeding techniques have optimized the productivity of broiler and layer strains, yet the expression of this productivity depends on nutrient availability, metabolic status and the environment. This implies that in addition to acting as substrates, nutrients also act as signaling molecules which are capable of altering gene expression and metabolism in cells (Alagawany *et al.*, 2022; Brennan & de Roos, 2021) [3, 5]. Nutritional programming can also be investigated using poultry as an experimental model since maternal nutrition and in ovo treatments may affect the development of the embryo and resulting offspring (Andrieux *et al.*, 2022; Kadam *et al.*, 2013) [11, 12]. Nonetheless, the mechanisms of generation-to-generation transmission and sustainability of the phenomenon remain incompletely understood, and information about stable transgenerational effects of nutrition on commercial poultry production is still limited (Andrieux *et al.*, 2022) [11].

2. Mechanisms linking diet to gene expression

2.1. Direct nutrient–transcription factor interactions and signal transduction

Nutritional molecules or their metabolites may impact on transcription factors or nuclear receptors either by acting directly on these transcription factors or receptors or by means of intracellular signaling cascades. Nutrient-dependent transcription factors can act as mediators in the process of translation of nutritional signals into transcription: some activated transcription factors enter the nucleus and bind specific sites in the promoters of target genes (Osorio *et al.*, 2016) [13]. Fatty acids activate peroxisome proliferator-activated receptors (PPARs); sterol-dependent processes involve sterol regulatory element-binding proteins (SREBPs/SREBFs); carbohydrate-dependent metabolism can be affected by carbohydrate-responsive element-binding protein (ChREBP) and upstream stimulatory factors (USFs); vitamin A metabolites act via retinoic acid receptors (RAR/RXR); cellular zinc status is related to metal-responsive transcriptional regulation. Amino-acid availability might influence the transcriptional network that involves CCAAT/enhancer-binding protein (C/EBP) transcription factors and growth signaling pathways including insulin like growth factor-1 (IGF-1) axis. These represent selected examples illustrating the general principle that diet composition can influence transcriptional activity in the liver, muscle, adipose tissue, intestine, and reproductive tissues; nevertheless, the presence of nutrient-dependent

transcription factors alone cannot be considered evidence that a particular feed additive acts directly on those transcription factors (Reen *et al.*, 2015; Osorio *et al.*, 2016) [2, 13].

Signal transduction is an essential part of the direct interaction category. Nutrients can act as signaling inputs that are integrated by pathways such as mechanistic target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK). The pathway mTOR is responsive to amino acid levels and growth signals, encouraging anabolic activities like protein synthesis. On the other hand, AMPK pathway responds to low energy states and helps in conserving energy while inhibiting anabolic activities. In poultry, branched chain amino acids act as signals through the AKT-mTOR pathway, while the AMPK/mTOR pathway is associated with energy balance and control of feeding in chickens (Kim *et al.*, 2022; Volyanskaya *et al.*, 2024) [14, 15]. Nutrient signaling can hence act on the transcription factors and effectors, connecting the nutrient profile of feed with the changes in physiology of growth and metabolism (Naeem *et al.*, 2025) [16].

2.2. Epigenetic regulation: DNA methylation, histone modification, and non-coding RNA

Epigenetic regulation opens up another interface between nutrition and gene expression. Nutritional DNA methylation is considered a major epigenetic process that involves transferring methyl groups onto cytosine bases at CpG sites. Metabolism of methyl-donors such as methionine, folate, choline, and betaine affects the supply of one-carbon units and S-adenosylmethionine, but the nutritional provision of methyl donors does not cause the same methylation effect on different genome regions (Niculescu & Zeisel, 2002; Ju *et al.*, 2023) [18, 17]. DNA methylation in poultry birds is associated with growth, fat development, reproduction, health traits, and other production phenotypes. According to Sun *et al.* (2019) [19], methylation at certain CpG sites in the promoter region of PLIN1 gene correlates with its expression and accumulation of abdominal fat in the divergent chicken lines. Late-lay hens supplemented with dietary betaine had modified methylation-related and reproductive genes along with improved egg-laying and eggshell quality (Guo *et al.*, 2023) [20]. Methionine is a precursor of S-adenosylmethionine and can influence methylation-related pathways of growth regulation. Dietary methionine is linked to strain-dependent regulation of expression of myogenic genes, IGF-I, extracellular signal-regulated kinase (ERK) phosphorylation, and mTOR phosphorylation in broilers (Wen *et al.*, 2017) [21], and Felix *et al.* (2023) [22] demonstrated its effect on remethylation and homocysteine metabolism-related genes. These observations provide evidence for a nutritional epigenomics model, but they do not justify making an assumption that every transcriptional change means a methylation modification of a particular gene locus.

Histone modifications affect the accessibility of chromatin by either adding or removing chemical groups such as acetyl, methyl and phosphate groups from the histone proteins. Dietary factors might influence the enzymes that regulate these modifications and thus the accessibility of the DNA regulatory regions to the transcription machinery. In this context, polyphenols, such as resveratrol, fatty acids, and vitamins have been suggested as diet components that may affect chromatin-regulation (Naeem *et al.*, 2025) [16]. In broilers, dietary resveratrol improved meat quality and antioxidant status and increased peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and nuclear respiratory factor 1 (NRF1) mRNA expression, consistent with altered mitochondrial and antioxidant regulation (Zhang *et al.*, 2018) [23]. Importantly, the mentioned study did not measure the degree of histone acetylation; therefore, it should be considered as an indication of the effect on transcription and mitochondrial regulation rather than the evidence of histone modification. The evidence concerning the regulation of histones in poultry remains less extensive than the one concerning the DNA methylation regulation.

Table 1. Omics Technologies in Poultry Nutrigenomics

Omics layer	Representative approaches	Nutritional relevance
Genomics	SNP genotyping; gene polymorphism analysis	Identifies genetic variation that may modify nutrient responsiveness.
Transcriptomics	RT-qPCR; microarrays; RNA-seq	Measures diet-responsive mRNA and pathway-level transcriptional changes.
Proteomics	2-D electrophoresis; HPLC; mass spectrometry	Assesses protein abundance and functional molecular phenotype.
Metabolomics	NMR; GC/LC-MS	Profiles metabolites and biochemical consequences of dietary interventions.
Epigenomics / ncRNA	DNA methylation assays; chromatin assays; miRNA/lncRNA profiling	Characterizes regulatory mechanisms linking nutrition to gene activity.
Metagenomics	Microbial community sequencing	Links diet, gut microbiota and host molecular/physiological responses.

Source: Dehau *et al.* (2022) [9], Zampiga *et al.* (2018) [8], and Herrero *et al.* (2010) [6]

Noncoding RNAs, especially microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), provide an extra posttranscriptional level through which nutrition can affect gene regulation. miRNAs usually act on complementary sequences within their targets' mRNAs, thereby facilitating mRNA degradation and translational inhibition. In contrast, lncRNAs may influence chromatin structure, transcription, RNA stability, splicing, and protein interactions. In chickens, miRNAs regulating lipids metabolism in liver and fat tissue have been discovered; for instance, miR-429-3p/LPIN1 pathway was found to be engaged in adipocyte formation in chickens (Chao *et al.*, 2020) [24]. It has been demonstrated that dietary intake of guanidinoacetic acid (GAA) can lead to changes in the miRNA profile of adipose tissues in broilers and particularly *Gallus gallus* microRNA-103-3p (gga-miR-103-3p) and *Gallus gallus* microRNA-107-3p (gga-miR-107-3p). These changes indicate potential relationships with stearoyl-CoA desaturase (SCD) and transmembrane protein 35B (TMEM35B) are associated with the accumulation of abdominal fat; the researchers emphasized the need for further functional validation (Liu *et al.*, 2026) [25].

2.3. Genetic variation and nutrient responsiveness

The genome encodes nutrient-responsive pathways that allow for birds to not always respond in the same way to the same dietary input. Genetic polymorphisms can influence coding regions of genes, promoter activity, transcription factor binding, receptor activity, or metabolic pathways of nutrients, causing changes in the response of the body to the same dietary inputs. The relationships between genetic variation in growth-related genes (IGF-1, IGFBP2, TGFβ3, Pit-1, MSTN) and phenotypes (growth, feed efficiency, carcass composition) and metabolic pathways (FASN- and PPAR-related pathways) and lipid metabolism/fat deposition have been identified in poultry (Naeem *et al.*, 2025) [16]. Thus, the idea of nutrigenetics, according to which the effect of the diet is determined by the genotype of the bird, is justified (Alagawany *et al.*, 2022) [3]. However, the presence of an SNP affecting production trait does not mean that the SNP influences the diet effect. Special genotype by diet experiments should be performed prior to using genotype-specific feeding regimes. Future nutrigenetic approaches will involve the combination of SNP genotyping and phenotyping using transcriptomics, proteomics, and metabolomics.

Overall, these mechanisms are interconnected rather than distinct. For instance, a compound found in food may first affect the availability of nutrients or receptor functioning, initiate the activation of signaling pathways like mTOR and AMPK, alter the activity of transcription factors, and finally affect the regulation of chromatin and RNA levels. These alterations in the levels of transcripts, proteins, and metabolites can help explain the observed phenotype related to production. Omics studies are especially useful in deciphering these complex connec-

tions, although mechanistic conclusions are more reliable when supported with functional data (Brennan & de Roos, 2021; Zampiga *et al.*, 2018) [5, 8].

3. Evidence from experimental studies in poultry

3.1. Phytochemicals and the growth-hormone/IGF-1 axis: Curcumin under stocking-density stress

Research conducted by Hafez *et al.* (2022) [26] highlighted the interaction between a dietary bioactive component and gene expression in broiler chickens under stressful environment. This experiment used 200 broilers of Cobb 500 that were housed at moderate stocking density of 10 birds/m² and at high stocking density of 20 birds/m². At high stocking density, the birds were administered with dietary curcumin at concentrations of 100 and 200 mg/kg diet for 28 days. Growth performance, oxidative and antioxidant status, immunity responses, and liver gene expressions of four growth regulatory genes, GHR, IGF-1, MSTN, and leptin were assessed using real-time PCR.

The high stocking density led to stress-induced changes in the gene expression with significant down-regulation of GHR and IGF-1 ($p < 0.05$) as well as the elevated expression of MSTN and leptin along with poor growth, increased levels of corticosterone and malondialdehyde (MDA) as well as poor antioxidant and immune system response. Notably, curcumin feeding had profound effects on this stress-induced change in the gene expression. Feeding with 100 and 200 mg/kg curcumin resulted in a marked increase in the mRNA expression of GHR and IGF-1 ($p < 0.001$) along with a decrease in the expression of MSTN and leptin ($p < 0.001$) with dose-dependency of the effect with higher impact of 200 mg/kg curcumin.

These changes at the molecular level were reflected in considerable gains in body weight, body weight gain, feed intake and feed conversion ratio ($p < 0.0001$) as compared with the unsupplemented birds under high stocking density conditions. Furthermore, the administration of curcumin led to improvement in antioxidant defense by increasing the activities of superoxide dismutase, glutathione peroxidase and catalase enzymes and decreasing the MDA values while improving the concentrations of immunoglobulins and decreasing the inflammatory cytokines in serum of birds. The above observations indicate an important aspect of nutrigenomics in the field of poultry nutrition in which the dietary bioactive substances could modulate the transcriptional regulation of genes in the physiological pathways and these molecular changes could result in functional changes in growth, feed utilization, antioxidative ability and immunological status of birds. Thus, the present study demonstrates that the dietary phytochemical exposure in the form of curcumin had the potential to reverse the transcriptional alterations caused by high stocking density in growth-regulating genes and their phenotypic responses.

3.2. Dietary lipids and hepatic lipogenic gene expression: Flaxseed oil

Fatty acids in the diet can be considered a unique group of nutritional factors that could influence gene expression and lipid metabolism in birds. The study conducted by Yan *et al.* (2015) [27] focused on studying the effect of substitution of corn oil (CO) with flaxseed oil (FO) being a significant source of α -linolenic acid (18:3n-3) on growth parameters, fatty acid profile in the liver, enzymes activity, and genes involved in lipid metabolism in 240 Cobb-500 females. Experimental

groups were divided into 4 groups receiving 5% CO diet (LC), 3.75% CO + 1.25% FO (FO1), 2.5% CO + 2.5% FO (FO2), and 5% FO (FC) and reared from 1 to 42 days of age.

The dietary oil type had a strong effect on the liver fatty acid profile, which proved that the consumption of PUFA could alter the lipid metabolism in tissues. The 42-day-old birds showed an increase in the level of 18:3n-3 and 20:5n-3 fatty acids in the diet containing FO, indicating a high n-3 PUFA content with a lower n-6/n-3 PUFA ratio. The amount of 20:5n-3 fatty acid was much higher in the 5% FO diet group compared to corn-oil-fed rats ($p < 0.001$).

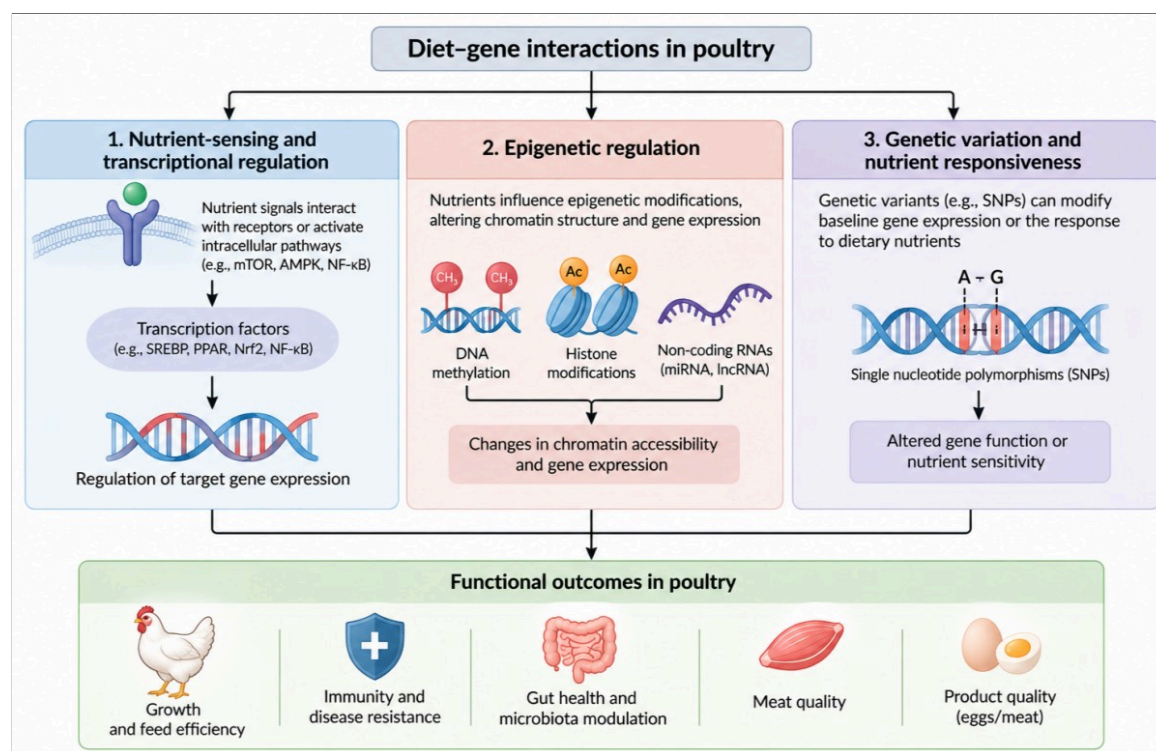


Figure 1. Major mechanisms of diet-induced regulation of gene expression and metabolic responses in poultry.

In terms of nutrigenomics, the highest response was noted in lipin 1 (LPIN1), the hepatic mRNA expression of which was increased significantly due to the diets with 2.5% and 5% flaxseed oil compared to both the corn oil and 1.25% flaxseed oil diets ($p < 0.01$). On the contrary, the mRNA expression levels of LPIN2, WDTC1, FADS2, and ND2 were not different from each other statistically. The protein encoded by the LPIN1 is lipin-1, an important enzyme for glycerolipid metabolism which acts as a phosphatidic acid phosphohydrolase playing an important role in the biosynthesis of triglycerides as well as interacts with transcription factors bound to the DNA molecules to influence the gene transcription process.

Therefore, the findings suggest that the structure of PUFA dietary components may serve as a nutrient signal to control the gene expression in the liver and lipid metabolism, at the same time modifying the structure of fatty acids in the tissues. The findings confirm the nutrigenomic theory stating that the effects of dietary fats extend their function of an energy source due to the ability of specific fatty acids to affect gene expression and lipid metabolism.

3.3. Methyl donors and reproductive function: Betaine in late-lay hens

Guo *et al.* (2023) [20] provided particularly relevant evidence for the nutrigenomic and epigenetic effects of a dietary methyl donor in laying hens. Specifically, in this study, 270 laying hens of the Jinghong-1 breed, aged 65 weeks, received a basal diet with 0.1% and 0.5% of betaine for seven weeks. Betaine is a methyl-donor nutrient that participates in the transmethylation cycle and can influence DNA methylation and, consequently, gene expression. The group of hens receiving 0.5% of betaine showed a significant improvement in laying rate

in weeks 6 and 7 ($p < 0.05$), as well as in eggshell thickness from 0.32 to 0.42 mm ($p < 0.01$).

The nutrigenomic implications of the research were demonstrated by the gene expression changes regarding the response of reproductive hormones, apoptosis, eggshell formation, and epigenetic modifications. The expression of the luteinizing hormone receptor (LHR) and follicle-stimulating hormone receptor (FSHR) genes was significantly increased in ovarian tissue in response to the supplementation with 0.5% dietary betaine ($p < 0.05$). The LHR and FSHR genes code the receptors that enable modulation of follicular development, steroidogenesis, and reproduction by luteinising hormone and follicle-stimulating hormone; therefore, their increased expression may indicate increased sensitivity of ovaries to these hormones. Betaine decreased the expression of pro-apoptotic genes Caspase-3, Caspase-8, and Caspase-9; however, these changes were described as tendencies instead of significant results ($P = 0.16, 0.07, 0.08$, respectively). In the uterine tissue, betaine increased the expression of the ovalbumin, ovotransferrin, and carbonic anhydrase 2 (CA2) genes ($p < 0.05$). These genes are involved in the process of eggshell formation: ovalbumin binds calcium, ovotransferrin is active during the initial phases of shell formation, and CA2 catalyzes CO₂ hydration and provides bicarbonate for eggshell mineralization.

More importantly, the study provided evidence that diet-induced changes in gene expression were associated with epigenetic modification. It was shown that betaine reduced expression of DNA methyltransferases (DNMT1, DNMT3a, and DNMT3b) by 47%, 86%, and 51%, respectively ($p < 0.05$) but increased TET2 expression ($p < 0.05$). DNMTs are the key enzymes responsible for methylation of CpG sites, while TETs have opposite activity, being involved in DNA

demethylation process. Indeed, the data obtained suggest that betaine significantly decreased the 5-methylcytosine (5mC) methylation at the promoter region of the ovarian LHR gene ($p < 0.01$), while there were no changes in the FSHR promoter methylation. Therefore, the authors suggested that LHR expression is regulated by betaine through DNA methylation. Summarizing, this research provides strong evidence of the diet $\rightarrow$ epigenetic modification $\rightarrow$ gene expression $\rightarrow$ functional phenotype axis of nutrigenomics in poultry, since it revealed the effect of dietary betaine supplementation on the regulation of reproductive gene expression and function, eggshell formation, and laying efficiency.

3.4. Trace mineral source and innate immune gene expression

In a study by Jain *et al.* (2021) [28], dietary trace minerals were found capable of regulating nutritionally responsive genes' expression involved in the immune system of broiler chickens, highlighting the presence of a clear interaction between diet and genes in poultry. In a feeding experiment lasting 35 days, 216 Cobb 500 broilers were divided into groups according to the intake of organic or inorganic form of Zn, Se, and Cr, either separately or in combinations. The expression of *chTLR4* gene in the bursa of Fabricius and the spleen was measured by real-time PCR technique. Being a pattern recognition receptor in innate immunity, TLR4 is involved in the process of detection of pathogen-derived stimuli and subsequent immune reactions, such as cytokines production and activation of the adaptive immune response. It was found that mineral supplementation significantly increased the expression of the gene in the bursa (Organic Se supplemented at 0.30 mg/kg, 3.02-fold) and spleen (Organic Zn supplemented at 40 mg/kg, 3.21-fold).

The organic-mineral combination diet produced significant up-regulation of TLR4 gene expression in the two tissues, resulting in an increase of 2.89-fold in the bursa of Fabricius and 3.01-fold in the spleen. This transcriptional modification occurred in association with an improved humoral immune response, evidenced by increases in plasma IgG levels, where the organic-mineral combination group showed significant increases in IgG levels compared to the control at day 21, day 28, and day 35 ($p < 0.05$). Further, the organic-mineral combination group had lower heterophil-lymphocyte ratios ($p < 0.05$) and heavier lymphoid organs. From the results, it can be concluded that the diet mineral source and its availability can influence the transcriptional regulation of the innate immunity receptor, which may be related to changes in immune function. Importantly, while the study establishes a diet-associated change in TLR4 gene expression, it does not demonstrate an epigenetic mechanism; therefore, its findings primarily support the diet–gene expression–immune function component of nutrigenomics rather than the epigenetic pathway.

3.5. Dietary methionine source and level: Remethylation-related gene expression

Felix *et al.* (2023) [22] showed that dietary methionine source and concentration could affect the transcriptional regulation of genes related to the metabolism of methionine in broiler chickens as a specific case of gene regulation triggered by nutrients in the nutrigenomics concept. For 42 days, 450 Cobb-700 male broilers were administered with diets with deficient, requirement or excess concentrations of either DL-methionine (DL-Met) or DL-methionine hydroxy analogue (DL-HMTBA). The jejunal expression of methionine adenosyltransferase 1 (MAT1), 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR), betaine-homocysteine S-methyltransferase (BHMT), 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (MTRR), cystathionine beta-synthase (CBS), and glutathione synthetase (GSS) was evaluated using real-time PCR.

Methionine is particularly important in terms of nutrigenomics since it is converted to S-adenosylmethionine (SAM), which is one of the main donors of methyl groups involved in cellular methylation reac-

tions. As for the genes studied, the source of dietary methionine significantly affected the expression of BHMT and MTRR, with DL-HMTBA upregulating the expression of both genes (BHMT: $P = 0.0072$; MTRR: $P = 0.0003$), and with methionine deprivation increasing the expression of MTRR ($P = 0.0018$). The gene BHMT is involved in the remethylation of homocysteine to methionine through the choline-betaine pathway, while the gene MTRR recycles the methionine synthase involved in folate-dependent remethylation pathway. Hence, the modulation of the expression of those genes shows the impact of nutrition on regulation of the pathways maintaining homocysteine and methionine homeostasis. It is worth noting that the interaction of methionine source and methionine level is also statistically significant for BHMT ($P = 0.0332$) and MTRR ($P = 0.0021$).

Furthermore, CBS responsible for utilizing homocysteine for cysteine production and GSS involved in the synthesis of glutathione were responsive to the source or amount of dietary methionine. This implies a possibility of a link between sulfur-containing amino acid metabolism and antioxidant activity. These changes were associated with changes in the productive performance: methionine deficiency led to decrease in weight gain and poor feed conversion ($p < 0.0001$), while excess methionine promoted increase in weight gain and improvement of feed conversion. Overall, these data imply that the source and amount of dietary amino acids can serve as metabolic cues controlling the expression of genes involved in the process of metabolism. Though there is no information on DNA methylation or any other epigenetic modification in this study, the findings can be especially interesting from the standpoint of nutrigenomics as SAM produced by methionine serves as the biochemical bridge between availability of methyl-donors, gene expression, and metabolism.

4. Applications in poultry production

4.1. Precision nutrition

Precision nutrition aims to move from population-average recommendations toward feeding strategies that consider production stage, physiological status, environment, and eventually genotype and molecular phenotype. Nutrigenomics can contribute by identifying nutrient-responsive genes and pathways that are associated with feed efficiency, growth, metabolic adaptation, and health. However, commercial genotype-tailored diets remain an emerging application rather than a routine practice. Current evidence supports the biological rationale, but large-scale validation and cost-effective biomarkers are still required before individualized genomic feeding can be widely implemented (Alagawany *et al.*, 2022; Souf *et al.*, 2026) [3, 29].

4.2. Nutritional programming and early-life interventions

Maternal and in ovo nutrition provide opportunities to influence developmental trajectories through metabolic and epigenetic mechanisms. Early-life nutritional programming may affect immune competence, metabolism, growth, and later production traits. Such interventions are promising, but durability under commercial conditions and the consistency of responses across genetic lines remain important research questions. Evidence of an offspring phenotype should not be described as transgenerational inheritance unless effects are demonstrated beyond the directly exposed generation.

4.3. Phytogetic feed additives and antimicrobial reduction

Phytogetic compounds, organic trace minerals, prebiotics, and related feed additives are being investigated as components of strategies to support gut health and immunity while reducing reliance on antimicrobial growth-promoting approaches. Nutrigenomic measurements can help identify whether such additives alter immune or antioxidant pathways. Nevertheless, gene expression changes should be interpreted alongside microbial, immunological, physiological, and production outcomes rather than treated as evidence of efficacy by themselves.

Table 2. Summary of Key Dietary Intervention Studies

S. No.	Title of the research paper	Dietary component	Genes regulated (↑/↓)	Final outcome	Reference
1	The Impact of Curcumin on Growth Performance, Growth-Related Gene Expression, Oxidative Stress, and Immunological Biomarkers in Broiler Chickens at Different Stocking Densities	Curcumin (100 or 200 mg/kg feed)	GHR ↑; IGF-1 ↑; MSTN ↓; Leptin ↓	Improved body-weight gain and feed conversion; improved antioxidant status and immune responses under high stocking density	Hafez <i>et al.</i> (2022)
2	Effect of Different Dietary Oil Sources on the Growth Performance, Blood Characteristics, Fatty Acid Profiles, and Expression of Lipogenic Genes in the Liver of Broiler Chickens	Flaxseed oil replacing corn oil (1.25–5%)	LPIN1 ↑ at higher flaxseed-oil inclusion; LPIN2, FADS2, WDTC1 and ND2: no significant change	Improved early growth/feed efficiency at 1.25%; increased hepatic n-3 PUFA and improved n-6: n-3 ratio	Yan <i>et al.</i> (2015)
3	Effects of Dietary Betaine on the Laying Performance, Antioxidant Capacity, and Uterus and Ovary Function of Laying Hens at the Late Stage of Production	Betaine (0.1 or 0.5%)	LHR ↑; FSHR ↑; CA2 ↑; ovalbumin/ovotransferrin ↑; DNMT1/DNMT3A/DNMT3B ↓; TET1/TET2 ↑	Higher laying performance and thicker eggshells; altered reproductive and methylation-related gene expression	Guo <i>et al.</i> (2023)
4	Effects of Different Concentration of Organic and Inorganic Trace Minerals (Zinc, Selenium, and Chromium) Supplementation on Expression of chTLR4 Gene and Humoral Immune Response in Broilers	Organic/inorganic Zn, Se and Cr	chTLR4 ↑ (maximum 3.02-fold in bursa and 3.21-fold in spleen in specific organic-mineral treatments)	Higher IgG, lower heterophil: lymphocyte ratio and greater immune-organ weights with organic trace-mineral supplementation	Jain <i>et al.</i> (2021)
5	Methionine Sources at Different Dietary Levels Alters the Growth and Expression of Genes Related to Homocysteine Remethylation in the Jejunum of Broilers	DL-Methionine vs DL-HMTBA at deficient, requirement and excess levels	BHMT ↑; MTRR ↑; GSS tended ↑ with DL-HMTBA; methionine level altered MTRR and other remethylation genes	Methionine deficiency impaired growth/feed conversion; DL-Met at requirement level was preferable for performance	Felix <i>et al.</i> (2023)

4.4. Molecular biomarkers

Expression of genes such as GHR, IGF-1, chTLR4, and adipogenic genes, together with methylation patterns and miRNA profiles, may eventually serve as biomarkers of nutritional status, stress, metabolic phenotype, or production potential. For a biomarker to be useful in commercial systems, however, it must demonstrate reproducibility, sensitivity, specificity, biological relevance, and predictive value across breeds, farms, diets, and environmental conditions.

4.5. Meat and egg quality

Nutrigenomic approaches may also support manipulation of meat and egg characteristics. Dietary fatty acids can alter tissue lipid composition, while phytochemicals and antioxidants can influence oxidative stability and meat quality. In layers, nutritional modulation of reproductive and eggshell-related pathways may contribute to shell quality and laying persistence. Integration of molecular data with conventional quality measurements will be necessary to distinguish mechanistically meaningful effects from isolated transcriptional changes.

5. Knowledge gaps and future directions

Despite increasing understanding about the effects of diet-gene interactions on poultry, certain limitations have been noticed. The use of mRNA expression has remained dominant in almost all the studies; however, protein expression and functional analysis have been less emphasized. In future research, an approach that combines the information from gene expression along with protein expression and biochemical activity of enzymes needs to be considered. Further experimental studies need to include more replication and a range of doses and consideration of tissue, age and physiological status.

Interactions between genotype and diet call for increased academic research, since a link between genetic variants and production traits does not necessarily demonstrate differential responses to specific diets. At the same time, studies on the epigenetics of nutrition require separation of correlation from causality through application of locus-specific methylation testing, chromatin testing, and functional analysis, especially with regards to heritability between generations. The use of genomic, transcriptomic, epigenomic, proteomic, metabolomic, and microbiome data together may give an even more complete picture of diet-genotype interactions, but standardized sampling, adequate experimental power and robust bioinformatic approaches are required.

At last, implementation of nutrigenomic research in poultry farming is an uphill task. The biomarkers have to be verified among different breeds, feed types, and other environmental parameters, and the nutrigenomics intervention needs to be evaluated in tandem with traditional performance indicators, health, quality of products, and cost factors. Hence, future studies need to move away from isolated molecular relationships towards practical precision nutrition systems.

6. Conclusion

Diet can influence poultry phenotypes through a network of transcriptional, signaling, epigenetic, post-transcriptional, and genetically dependent mechanisms. Experimental studies involving phytochemicals, dietary lipids, methyl donors, and trace minerals demonstrate that nutritional interventions can modify genes associated with growth, lipid metabolism, reproduction, and immunity and can be accompanied by measurable changes in production traits. However, the strength of evidence varies, and gene-expression changes should not automatically be interpreted as proof of direct molecular causation.

The next phase of poultry nutrigenomics will require integration of molecular measurements with conventional nutrition, phenotyping, and production economics. Multi-omics approaches, nutritional programming, molecular biomarkers, and nutrigenetic information may ultimately support more precise and sustainable feeding strategies. At present, the field is best viewed as a complementary layer over established nutritional science rather than a replacement for conventional feed formulation. Progress toward commercial precision nutrition will

depend on larger, longer-duration, multi-omics studies and rigorous validation across poultry genotypes and production environments.

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Authors

Rishabh Sarwal

Department of Animal Nutrition, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Ripusudan Kumar

Department of Animal Nutrition, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Akriti Ballabh

Department of Animal Nutrition, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Anshu Rahal

Department of Animal Nutrition, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Bidhan C Mondal

Department of Animal Nutrition, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Aman Kamboj

Department of Veterinary Physiology and Biochemistry, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Manju Lata

Department of Animal Nutrition, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India