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EXPRESSION OF GENES ASSOCIATED WITH ECONOMIC TRAITS OF BROILER CHICKENS (*Gallus gallus domesticus*), AS INFLUENCED BY VARIOUS PARATYPICAL FACTORS

(review)

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Abstract

Commercial production of broiler chicken meat is based on the use of early maturing high-yielding crosses created by geneticists and breeders. The original lines of modern broiler chickens were obtained through artificial selection, primarily in terms of feed efficiency, conversion and growth rate (W. Fu et al., 2016). Progressive genetic research, breeding and feeding techniques combined with effective veterinary control ensure production of high quality poultry meat (A.A. Grozina, 2014). From 1957 to 2001, the time for broiler chickens to reach market weight decreased 3-fold, while feed intake decreased too (M. Georges et al., 2019). Expression study of genes involved in broiler growth and development, nutrient assimilation, and resistance to pathogens is necessary for successful selection of birds with desirable qualities (K. Lassiter et al., 2019). The aim of the review is to analyze the diversity of genes and their activity in the formation of economically useful traits of broiler chickens and factors influencing their expression. The article presents an overview of the genes involved in growth and development (*GH*, *IGF-1*, *GHR*, *MYOD1*, *MYOG*, *MSTN*), nutrient assimilation (*SLC2A1*, *SLC2A2*, *SLC2A3*, *SLC2A8*, *SLC2A9*, *SLC2A12*, *SLC6A19*, *SLC7A1*, *SLC7A2*, *SLC7A5-7*, *SLC15A1*, *SLC38A2*), immune response (*IL1B*, *IL6*, *IL8L2*, *IL16*, *IL17A*, *IL18*, *TNF-α*, *AvBD1-AvBD14*). A somatotropic growth hormone (GH)—insulin-like growth factor 1 (IGF-1)—growth hormone receptor (GHR) axis is a pathway to regulate skeletal growth rate and body size (L.E. Ellestad et al., 2019). Analysis of the gene *GH, GHR,* and *IGF-1* expression and selection for high growth rate in broiler chickens can increase growth hormone binding activity, IGF-1 synthesis in the liver, and therefore body weight (S. Pech-Pool et al., 2020). Myogenesis is mediated by a number of factors and genes, including myogenic regulatory factors (*MRF*), myogenic differentiation factor 1 (*MYOD1*), myogenin (*MYOG*) the expression of which may vary depending on the feed ingredient and specific additives. Dietary proteases significantly increase the expression of *MYOD1* and *MYOG* genes in pectoral muscle, *GH* and *IGF-1* in liver and improve growth performance (Y. Xiao et al., 2020). Genes associated with nutrient absorption and their expression affect transport proteins, leading to accelerated nutrient delivery to the intestinal epithelium, circulatory system, and then to all organs and tissues. In turn, their expression can depend on various feed additives. Solute carrier family (SLC) proteins involved in amino acid transport comprises SLC6A19 (B0AT1) and SLC38A2 (SNAT2) sodium-dependent carriers of neutral amino acids; SLC7A1 and SLC7A2 carriers of cationic amino acids (cationic amino acid transporter — CAT: CAT1, CAT2); SLC7A5-7 L-type amino acid transporter (LAT: LAT1, gLAT2) (J.A. Payne et al, 2019; C.N. Khwatenge et al., 2020; N.S. Fagundes et al., 2020). Immunity gene expression (*IL1B*, *IL6*, *IL8L2*, *IL16*, *IL17A*, *IL18*, *TNF-α*, *AvBD1-AvBD14*) initiating the synthesis of immune response factors is affected by *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Clostridium perfringens*, *Listeria monocytogenes*, *Eimeria* spp. infections (G.Y. Laptev et al., 2019; T. Nii et al., 2019). The modulating effect of temperature on gene expression was also revealed. Increased rearing temperature (39 °C) leads to a significant increase in expression of *IL6*, *IL1b*, *TNF-α*, *TLR2*, *TLR4*, *NFkB50*, *NFkB65*, *Hsp70* and *HSF3* genes in spleen and liver tissues (M.B. Al-Zghoul et al., 2019). Various feed additives (prebiotics, probiotics, synbiotics, phytobiotics and amino acids) are being

sought that act via modulation of gene expression and may maintain the physiological condition of birds, prevent the development of diseases, promote faster growth without compromising health and thus improve poultry productivity.

Keywords: broiler chickens, productivity, gene expression, growth, immunity, feed additives

Global demand for animal products is expected to increase by 70% by 2050. Meeting this demand will require the use of scientific advances, the use of modern technologies with minimal impact on the environment and improving the quality of raw materials of animal origin (mainly by genetic methods). Global livestock productivity, measured by carcass weight and egg production since the 1960s, has increased by 20-30% as a result of developments in nutrition, genetics, and disease control [1].

Farm animals are excellent model organisms for genetic studies of phenotypic evolution. Domestic animals have developed adaptations at the gene level to new environmental conditions and have been subjected to strict human selection, which has determined amazing phenotypic transformations in their behavior, morphology and physiology. The search for genetic changes that underlie phenotypic changes provides an opportunity to take a different look at the general mechanisms through which genetic variation determines phenotypic diversity.

Historically, domestic chickens were bred for two purposes — meat and eggs. Throughout the 20th century, specialized crosses of broiler chickens and laying hens were created and improved in order to improve productivity both in terms of growth and reproductive properties. This approach, coupled with the introduction of new genomic selection methods, has become most effective in achieving maximum productivity [2].

The original lines of modern broiler chickens are obtained through careful artificial selection, primarily for feed efficiency and growth rate, the traits that are of primary importance for the economic efficiency of the industry [3]. Progressive genetic research, breeding technologies and nutrition, combined with effective veterinary control, make it possible to produce high-quality poultry meat [4]. From 1957 to 2001, the time to reach market weight of broiler chickens decreased 3 times while feed consumption also decreased [1].

Feed conversion is an important genetic trait that determines economic efficiency, since 70% of the cost of raising animals is on feed. Determining the mRNA expression of genes involved in broiler growth and development, nutrient absorption, and pathogen resistance is essential for the successful selection of birds with desirable traits [5].

The purpose of this review was to analyze the diversity of genes and their activity in the formation of economically useful traits in broiler chickens and the factors influencing the expression of these genes.

Genes associated with growth of broiler chickens. Growth rate, weight and body parameters are determined by genotype and environmental factors, including nutrition. Along with genes (Table 1), the nervous and endocrine systems play a significant role in regulating broiler chicken growth [6].

Among the components of the neuroendocrine system, the somatotropic axis receives the most attention [7]. The main regulator of skeletal growth rate and body size is the growth hormone (GH)—insulin like growth factor 1 (IGF-1) pathway. These hormones stimulate tissue growth, regulate the metabolism of proteins, lipids and carbohydrates, and maintain homeostasis [6]. The effect of growth hormone on the body can be carried out directly through activation of the growth hormone receptor (GHR) or indirectly through its messenger IGF-1, produced in the liver and promoting the growth of muscle tissue [8].

1. Genes associated with growth of broiler chickens

Gene	Protein	Function	Biomaterial	Reference
GH	Growth hormone	Postnatal tissue growth, including skeletal muscle	Pituitary gland, liver, skeletal muscles	[6]
IGF-1	Insulin-like growth factor 1	Muscle and bone growth	Liver, skeletal muscles	[9]
GHR	Growth hormone receptor	Binding of growth hormone and activation of signal transduction leading to growth	Liver, skeletal muscles	[10, 11]
MYOD1 (MYOD)	Myogenic differentiation factor 1	Growth and development of skeletal muscles	Skeletal muscles	[12, 13]
MYOG	Myogenin	Growth and development of skeletal muscles	Skeletal muscles	
MSTN	Myostatin	Suppression of skeletal muscle growth and differentiation	Skeletal muscles	[14]

Analysis of the expression of *GH*, *GHR* and *IGF-1* genes in fast-growing broilers and slow-growing laying hens showed a significant difference. Thus, in slow-growing chickens, high expression of GH mRNA in the pituitary gland and low expression of *GHR* and *IGF*-1 mRNA in the liver and muscles were noted, while in broilers the values were opposite. Slow-growing chickens presumably had reduced GH binding activity in the liver, and this could be caused by downregulation of growth hormone receptors in the liver by increasing the amount of growth hormone in the plasma. Selection for high growth rate in broiler chickens could increase growth hormone binding activity, hepatic IGF-1 synthesis, and hence body weight [6].

Muscle growth, or myogenesis, is a complex, precisely regulated process [12]. Myoblasts participate in the formation of skeletal muscles of broiler chickens. Their differentiation is controlled by myogenic regulatory factors (MRFs) [11]. These factors are involved in the proliferation and differentiation of myoblasts (13), as well as in the regulation of skeletal muscle development and promote their growth. The MRF family includes myogenic differentiation factor 1 (MYOD1) and myogenin (MYOG) [12].

Myostatin (MSTN) is a protein of the transforming growth factor beta (TGF-β) superfamily, which is secreted by skeletal muscles and acts as a potent inhibitor of muscle tissue growth and differentiation. Mutations in the *MSTN* gene cause myofiber hypertrophy which leads to increased muscle mass [15]. A striking phenotypic example of the manifestation of such a mutation are Belgian Blue cows, in which a natural mutation in the *MSTN* gene was identified [16].

Some ingredients in the diet can affect the expression of genes associated with body weight growth in broiler chickens. Thus, when protease was added to the diet, the expression of the *MYOD1* and *MYOG* genes in the pectoral muscles, as well as the *GH* and *IGF-1* genes in the liver significantly increased, along with improved growth performance [11]. Feeding chickens creatine in combination with pyruvate has been shown to reduce myostatin expression in breast muscles [14].

Genes associated with nutrient utilization. Broiler chickens exhibit rapid growth and development while meeting energy and nutrient requirements [17]. Poultry growth and productivity depend to some extent on the ability of the intestines to digest and absorb nutrients [18]. The main site of their absorption is the small intestine [19]. Transport of essential nutrients, the proteins, carbohydrates and fatty acids in the small intestine is performed by carrier proteins that are expressed in enterocytes. Improving the transport of nutrients due to the activation of genes encoding transport proteins can lead to an accelerated entry of these substances into the intestinal epithelium, the circulatory system, and then to all organs and tissues.

Once broken down into peptides and amino acids, proteins are transported to the small intestine [20]. Amino acid transport is carried out by carriers of the solute carrier family (SLC): SLC6A19 (B0AT1) and SLC38A2 (SNAT2), the sodium-dependent carriers of neutral amino acids; SLC7A1 and SLC7A2, the

cationic amino acid transporter (CAT); SLC7A5-7, the L-type amino acid transporter (LAT) [21].

Cationic amino acid transporters perform bidirectional transport to exchange cationic amino acids such as lysine, arginine and histidine between organs. CAT1 (cationic amino acid transporter 1), CAT2 (cationic amino acid transporter 2) and LAT2 (Y+L amino acid transporter 2) are involved in the transport of arginine and lysine. SNAT2 (sodium-coupled neutral amino acid transporter 2) transports L-glutamine to maintain homeostasis. LAT1 (L-type amino acid transporter 1) carries out the outflow of neutral amino acids (leucine, isoleucine, methionine) and the influx of aromatic ones (phenylalanine, tyrosine, tryptophan) [17, 22]. In turn, the peptides are transported to the small intestine via peptide transporter 1 (PEPT1), located within the membrane of epithelial cells [20].

Correction of the diet with various additives may affect the expression of genes associated with nutrient transport (Table 2). Thus, 0.5 g/kg cricket chitin added to the basal diet of Cobb 500 broiler chickens increased the relative expression of the *SLC15A1* gene mRNA on day 42 of growing, while 0.5 g/kg dietary cricket chitosan, on the contrary, decreased this parameter [18]. The addition of 0.03-0.09% protease to the basal diet of Ross 308 cross broilers increased the expression of the genes of the amino acid transporters *SCL6A19*, *SLC7A1*, *SLC7A2*, *SLC7A6*, *SLC7A7* and the peptide transporter *SLC15A1* [11].

Polysaccharides, broken down into glucose, fructose and galactose, are absorbed by enterocytes lining the microvilli of the small intestine [23]. Thus, in the form of monosaccharides, they enter the bloodstream and from there are transported into cells using membrane glucose transporter proteins (GLUT) [24]. In mammals, GLUT4 is a well-studied protein that serves as a major insulin-dependent transporter in skeletal muscle and adipose tissue [25] and is responsible for rapid glucose transport following insulin production by the pancreas [26]. However, the absence of GLUT4 has been revealed in chickens and broiler chickens, and only GLUT1, GLUT2, GLUT3, GLUT8, GLUT9 and GLUT12, the genes of which are expressed in skeletal muscles [27, 28], hypothalamus, liver, heart, adipose tissue, kidneys [23] and small intestine [20] have been partially described and characterized.

However, it has been established that in birds the insulin-induced glucose transporter protein GLUT12 may be an analogue of the GLUT4 transporter in mammals [27]. Expression of the transporter genes GLUT1, GLUT8 and GLUT12 depends on the stage of ontogenesis. Thus, during embryogenesis and within 5 days after hatching, the *SLC2A1* gene was expressed in the pectoralis major muscle, while the *SLC2A8* gene was expressed after hatching. The expression of *SLC2A12* gradually increased from day 12 of embryonic development to day 5 after hatching. In the sartorius muscle, the expression of *SLC2A1* and *SLC2A8* remained unchanged, while the *SLC2A12* expression also gradually increased during early muscle development after chick hatching [28].

The addition of dried beer grains fermented by *Bacillus subtilis*, *Lactobacillus rhamnosus* and *Saccharomyces cerevisae* to the basal diet increased the expression of vector genes (*SLC2A1*, *SLC2A2*, *SLC7A1*, *SLC7A2*, *SLC7A5*, *SLC15A1*) in broiler chickens of the Ross 308 cross [20].

Different tissue specificity of glucose transporters was found in chickens, with mRNA expression of the *SLC2A1* gene being high in the hypothalamus, *SLC2A2* and *SLC2A9* in the liver, *SLC2A3* in skeletal muscle, and the *SLC2A8* gene was equally expressed in all tissues studied, including abdominal fat. Moreover, in chickens with high body weight, the expression of these genes was higher than in chickens with low body weight [25].

2. Genes involved in nutrient transport in broiler chickens

Gene	Protein	Function	Biomaterial	Reference
SLC2A1	Hexose transporter (GLUT1)	Transport of glucose, fructose, galactose	Skeletal muscle, small intestine, liver, hypothalamus, abdominal fat	[20, 25, 28]
SLC2A2	Hexose transporter (GLUT2)		Small intestine, liver, hypothalamus, abdominal fat	[20, 25]
SLC2A3	Hexose transporter (GLUT3)		Liver, hypothalamus, abdominal fat	[25]
SLC2A9	Hexose transporter (GLUT9)			
SLC2A8	Hexose transporter (GLUT8)		Skeletal muscles	[28]
SLC2A12	Hexose transporter (GLUT12)			
SLC15A1	Peptide transporter (PEPT1)	Peptide transport	Small intestine and pectoral muscles	[11]
SLC38A2	Amino acid transporter (SNAT2)	Transport of neutral amino acids		[22]
SLC6A19	Amino acid transporter (B0AT1)			
SLC7A1	Amino acid transporter (CAT1)	Transport of cationic amino acids		
SLC7A2	Amino acid transporter (CAT2)			[11, 17]
SLC7A5	Amino acid transporter (LAT1)	Transport of L-amino acids		[22]
SLC7A6	Amino acid transporter (γLAT2)	Transport of γ-L-amino acids		[11, 17]
SLC7A7	Amino acid transporter (LAT3, γLAT1)			[11]

Addition of 2% sugarcane bagasse to the basal diet of Ross 308 chickens upregulated the expression of *SLC7A1* (*CAT1*) in the duodenum, jejunum, and ileum, and *SLC6A19* (*B0AT1*) in the ileum only. Downregulation of the *SLC2A2* (*GLUT2*) gene in the small intestine was observed. Birds fed a coarse corn diet had increased expression of *SLC7A6* (*γLAT2*) in the jejunum and *SLC7A7* (*γLAT1*) in the ileum. However, feed additives did not affect the expression of the *SLC7A2* (*CAT2*), *SLC2A1* (*GLUT1*) and *SLC15A1* (*PEPT1*) genes [19].

Genes associated with immunity. The expression of immunity genes in broiler chickens (Table 3) is influenced by infection with microorganisms *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Clostridium perfringens*, *Listeria monocytogenes*, *Eimeria* spp. and others, initiating the synthesis of immune response factors [29, 30]. The poultry gastrointestinal tract is a major entry point for pathogens that can cause enteric infections, which result in significant economic losses due to treatment costs, reduced growth, and premature mortality [31]. The protective barrier functions in the intestine are provided by the mucous layer covering the epithelium, tight junctions between epithelial cells, and factors of innate (macrophages, cytokines and antimicrobial peptides) and acquired (T- and B-lymphocytes and secreted IgA) immunity [30].

3. Genes involved in the immune response in broiler chickens

Gene	Protein	Function	Biomaterial	Reference
IL1B, *IL6*, *IL8L2*, *IL16*, *IL17A*, *IL18*	Proinflammatory cytokines (interleukins: IL1β, IL6, IL8, IL16, IL17 и IL18)	Attraction of immune cells to the site of infection, development of inflammation	Spleen, small intestine, macrophages, cloacal bursa	[29, 33, 36, 39]
TNF-α	Proinflammatory cytokine — tumor necrosis factor	Development of an inflammatory response	Spleen	[29]
AvBD1-14	Gallinatsin (Gal-1-14)	Antimicrobial action	Bone marrow, respiratory tract, skin, small intestine, liver, genitourinary organs, spleen, thymus, cloacal bursa, red blood cells	[38, 41, 42]

Cytokines are small extracellular signaling proteins that play a significant role in the development of the immune system, as well as in the formation of the immune response to pathogens or environmental stressors, such as changes in the temperature of poultry rearing. It is known that in vertebrates, cytokines are secreted by all types of cells, the immune cells, blood cells, connective tissue, spleen, thymus, etc. In birds, tumor necrosis factor α (tumor necrosis factor-alpha, TNF-α) and interleukins (IL) 1β, 6, 8, 16, 17, and 18 act as proinflammatory cytokines, that is, they contribute to the development of the inflammatory response during bacterial, viral, and protozoal infections [32]. Moreover, IL8 is a chemokine that induces chemotaxis in immunocompetent cells such as macrophages and monocytes [30].

It has been proven that pathogenic microorganisms stimulate the expression of pro-inflammatory cytokines in broiler chickens. Thus, an increase in the expression of IL6 was found in the ileum and cecum of Ross 308 cross chickens infected with *Campylobacter jejuni* [33], and when chicken embryo fibroblasts were infected with the reticuloendotheliosis virus (REV) [34]. In broiler chickens, the *Eimeria tenella* indection increases in the expression of IL6 and IL8 in the spleen and cecum [35], *Salmonella enteritidis* increases IL1B and IL8 levels in enterocytes and macrophages, while exposure to sodium butyrate at a subinhibitory concentration reduces bacterial colonization due to gene suppression proinflammatory cytokines [36]. Addition of deoxynivalenol at a concentration of 5 mg/kg to the diet of Ross 308 chickens increased the expression of *IL6* and the tight junction protein *claudin 1* (*CLDN1*) in the duodenum [37].

A modulating effect of temperature on gene expression was revealed.

Increased poultry rearing temperature (39 °C) leads to a significant increase in the mRNA expression of *IL6, IL1β, TNF-α, TLR2, TLR4, NFκB50, NFκB65, Hsp70* and *HSF3* genes in spleen and liver tissues [38]. Reduced rearing temperature (gradual decrease to 20 °C) can lead to a slight increase in *IL2*, *IL6* gene mRNA expression in the spleen, indicating the ability to adapt to cold [39]. Antimicrobial peptides defensins divided into three classes, the α-, β- and θ-defensins, play a significant role in immunity; α- and θ-defensins are found in mammals, while β-defensins, also known as gallinacins (Gal), are found only in birds.

Defensins kill a wide range of bacteria, fungi and viruses and can stimulate the acquired immune response against pathogens. Currently, in chickens 14 β-defensins (from AvBD1 to AvBD14) have been identified the genes of which are expressed in the bone marrow, respiratory tract, skin, digestive tract, liver, genitourinary and immune organs (spleen, thymus, cloacal bursa), and also in erythrocytes. In addition, another group of defensins, the ovodefensins (gallins), was described in birds, which have antimicrobial activity against *E. coli*, are expressed in the oviduct and its membrane, and are present in egg albumen [40-42].

When chicken cells were infected in vitro with the intestinal commensal *Lactobacillus johnsonii*, *Bacteriodes doreii*, the expression of the *IL1B* and *IL6* genes increased, while the expression of *AvBD8-AvBD10* remained almost unchanged. These results suggest that commensal gut bacteria do not induce the *AvBD8-AvBD10* gene expression. However, when *E. coli* and *Enterococcus faecalis* were cultured with artificially synthesized defensins (AvBD6, AvBD9, and AvBD10), opportunistic bacteria were suppressed [40].

It was found that 14 *AvBD* genes are expressed depending on the bird breed and tissue type. The degree of acquired and innate immunity varies among breeds [39].

The influence of the feed factor on the expression of genes potentially significant when raising broiler chickens. Additional sources of nutrients (prebiotics, probiotics, synbiotics, phytobiotics, vitamins, enzymes, amino acids, minerals, fatty and organic acids) play an important role in maintaining the health of broiler chickens. They promote the growth and development of poultry, support the functioning of normal intestinal microflora, have an antimicrobial effect against pathogenic microflora, and strengthen the immune system [43]. Table 4 provides a summary of the effects of various feed additives on the expression of genes of potential importance in broiler chicken production.

4. Expression of genes potentially significant in raising broiler chickens as influenced by various substances

Feed additive	Gene	Function	Reference
Oligosaccharides of the raffinose family	*CD3*, *chB6*	Increased expression	[44]
Lactobacillus plantarum and oligosaccharides of the raffinose family	*IL1β*, *IL6*, *IL8*, *IL18*	Increased expression	[45]
Mannanoligosaccharides	*PEPT1*	Increased expression	[46]
Thyme extract	*GH*, *IGF-1*	Increased expression	[47]
β-Glucan	*IL1*, *IL18*, *TNF-α*	Increased expression	[48-50]
	AvBD1, *AvBD2*, *AvBD4*, *AvBD6*, *AvBD9*, *AvBD4*, *AvBD9*	Decrease in expression	
Galactooligosaccharides	*IL17A*, *IL1β*, *AvBD1*, *GLUT1*	Increased expression	[51]
	IL10, *GLUT2*	Decrease in expression	[52, 53]
Inulin	*GHR*, *IGF-1*	Increased expression	[54, 55]
	IL6, *IL8*, *IL18*	Decrease in expression	
Lactobacillus spp.	*IL1β*, *IL6*	Decrease in expression	[56]
ДНК *Lactobacillus acidophilus*	*IL18*	Increased expression	[57]
Bacillus amyloliquefaciens	*IL1β*	Increased expression	[58]
Lactobacillus salivarius and galactooligosaccharides	*IL1β*, *IL6*, *IL18*	Increased expression in the spleen	[45]
	IL1β, *IL8*	Decreased expression in the cecum	

Continued Table 4

Lactococcus lactis subsp. *lactis* 2955 and inulin	*IL6*, *IL8*, *IL18*	Decrease in expression	[55]
Lactobacillus reuteri, *Enterococcus faecium*, *Bifidobacterium animalis*, *Pediococcus acidilactici* and fructooligosaccharide	*IL1β*	Decrease in expression	[59, 60]
	IL10	Increased expression upon infection *Clostridium perfringens*	
	IL1β, *IL10*	Reduced expression during infection *Salmonella enterica* ser. *Enteritidis*	
Essential oils of garlic, lemon, thyme, eucalyptus (Intebio, BIOTROF, Russia) (infection of *S. enterica* ser. *enteritidis*)	*AvBD10*, *IL6*, *IL8L2*	Increased expression followed by decrease	[29]
	AvBD9	No influence	
Thymol (infection of *S. enterica* ser. *Typhimurium*)	*IL10*	Increased expression	[61]
	IL6	Decrease in expression	
Chestnut tannins	*IL6, IL10*	Increased expression	[62]
	IL1β, IL8	No influence	
Licorice extract (infection of *Campylobacter jejuni*)	*IL1β*	Decrease in expression	[63]
Essential oils of mint, star anise, cloves	*IL18*	Decrease in expression	[64]
Basil	*GH*	Increased expression	[65]
	GHR	No influence	
Sage, chamomile, wall germander, marjoram	*IGF-1*	Increased expression	[66]
Dogwood cherry extract	*GLUT-1*, *GLUT-2*	Increased expression	[67]
Methionine	*LAT1*	Reduced expression in methionine deficiency	[22]
	SNAT2, *CAT1*	Increased expression in methionine deficiency	
	B0AT1	Increased expression under methionine deficiency and normal levels	
	MYOD, *MYOG*	No influence	[68]
Methionine and cysteine	*IGF-1*	Increased expression	[69]
L-arginine	*MYOD*, *MYOG*	Increased expression	[70, 71]
	IL8, *TNF-α*	No influence	

Prebiotics. Prebiotics improve and support the functioning of the intestine by stimulating the growth of the number and biodiversity of beneficial microorganisms and reducing the spread of pathogenic microorganisms, and also have a positive effect on lymphoid tissue and innate immunity of the intestine. Prebiotics are fructooligosaccharides (FOS), galactooligosaccharides (GOS) and raffinose (RFO) family of oligosaccharides, extracted from various plants; mannanoligosaccharides (MOS) from the cell walls of the yeast *Saccharomyces cerevisiae*; β-glucan from the cell walls of yeast or fungi [45, 49, 72].

Injection of RFO into Cobb 500 broiler embryos at increasing concentrations (1.5, 3.0, and 4.5 mg) directly proportionally increased the expression of *CD3* and *chB6* which serve as markers of T- and B-cells [44].

Some polysaccharides, such as β-glucans, affect genes associated with immunity. Thus, the introduction of 0.1% β-glucan into feed induces the expression of the *IL1*, *IL18* and *TNF-α* genes. In turn, the increased content of TNF-α in poultry stimulates the appearance of CD8+ lymphocytes (T-killer cells) [48, 49], and such a response may depend on infection. In Cobb cross broiler chickens infected with *Salmonella enteritidis* and consuming β-glucan, the expression of *AvBD1*, *AvBD2*, *AvBD4*, *AvBD6*, *AvBD9* genes increases while in birds free from infection, on the contrary, the *AvBD4* and *AvBD9*expression in the spleen decreased. Consequently, β-glucan exhibits immunostimulant properties, providing protection against pathogen infection [50].

Dietary galactooligosaccharide (GOS) modulated the immune response by increasing the expression of the cytokine IL17A, improving growth performance [51], and decreasing *IL10* expression in the ileum and cecum of Ross 308 chickens [52]. Administration of GOS activated the *IL1β*, *IL10*, *AvBD1*, *GLUT1* and *GLUT2* genes in the jejunum and cecum of Ross 308 broilers while suppressing the glucose transporter gene *GLUT2* expression in the duodenum [53].

In broiler chickens fed postbiotics (metabolic products of *Lactobacillus plantarum* RG14 and *L. plantarum* RI11) and inulin (fructooligosaccharide), hepatic *GHR* and *IGF-1* mRNA expression and final body weight increased [54].

Probiotics. Probiotics are live strains of microorganisms that, when administered in adequate quantities, have a positive effect on health, intestinal functioning, prevent the proliferation of pathogenic microflora, and generally promote the growth of the macroorganism. Probiotics are widely used in feeds, especially those intended for animals with a simple monogastric stomach. Probiotic strains include microorganisms of the genera *Bacillus*, *Enterococcus*, *Lactobacillus*, *Bifidobacterium*, *Pediococcus*, *Streptococcus*, *Saccharomyces* and *Kluyveromyces* [73].

Probiotic based on *Lactobacillus* spp. is able to reduce the expression of *IL1β* and *IL6* in the cecum of Arbor Acres cross broiler chickens infected with *Salmonella typhimurium*. Suppression of expression by probiotics is most likely due to reduced intestinal colonization by pathogens. Treatment of chicken cecal cells with *Lactobacillus acidophilus* DNA increased *IL18* expression [56, 57, 74].

A probiotic consisting of three strains of *Bacillus amyloliquefaciens* increased *IL1β* expression in the ileum on day 21 in Cobb 500 broilers infected with *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* oocysts [58].

Synbiotics. Synbiotics are a combination of pre- and probiotics that have beneficial effects on the health, create a protective barrier in the digestive tract and promote the growth of beneficial intestinal microorganisms [73].

Administration of *Lactobacillus salivarius* in combination with GOS to Cobb broiler chicken embryos on day 12 of incubation significantly increased the expression of *IL1β* in the spleen on day 7 and *IL6* and *IL18* on day 21. In the cecum, this combination led to a decrease in the expression of *IL1β* and *IL8* on day 42 [45]. Inulin enriched with 1000 CFU of *Lactococcus lactis* subsp. *lactis* 2955, as well as the prebiotic alone, can reduce the expression of genes *IL6*, *IL8*, *IL18* associated with immunity. Moreover, the suppression was more pronounced in the cecum than in the spleen, and increased with the age of broilers [55].

A dietary synbiotic containing four live strains *Lactobacillus reuteri*, *Enterococcus faecium*, *Bifidobacterium animalis* and *Pediococcus acidilactici* and the prebiotic fructooligosaccharide reduced the content of *IL1β* mRNA, while increasing the *IL10* mRNA in Cobb 500 broiler chickens infected with *Clostridium perfringens* [59]. This supplement reduced the *IL1β* and *IL10* mRNAs when chickens were infected with *Salmonella enterica* ser. *enteritidis* [60].

Phytobiotics. Phytobiotics are bioactive substances of plant origin, including phenolic, nitrogen-containing and organosulfur compounds, alkaloids, phytosterols and carotenoids. They are found in fruits, vegetables, grains and legumes, nuts, herbs and essential oils. Phytobiotics can be used as antimicrobials for protection against pathogenic bacteria, viruses and fungi. They are added to feed to improve health, productivity, the quality of poultry meat and eggs and as growth stimulants. Phytobiotics can also act as prebiotics and provide nutrients for beneficial gut bacteria [74, 75].

A phytobiotic containing a mixture of garlic, lemon, thyme and eucalyptus essential oils increased the *AvBD10*, *IL6* and *IL8L2* expression in Ross 308 chickens on day 1 after infection with *S. enterica* ser. *enteritidis* followed by a decrease in expression. At the early stages of infection, the phytobiotic stimulated the immune response to the pathogen and then suppressed the inflammatory response [29]. Feeding broiler chickens of the Ross 308 cross with 1% thymol nanoemulsion (a phenolic compound from thyme essential oil) increased the expression of *IL10*, decreased the expression of *IL6* and improved growth during infection with *S. enterica* ser. *typhimurium* [61]. Thyme extract had no effect on the expression of *GH* and *IGF1* genes in Cobb 500FF broilers [47].

Adding dietary chestnut tannins significantly increased the expression of the cytokines *IL6* and *IL10* in Ross 308 chickens on days 2 and 6 of feeding, while no significant increase was observed for the proinflammatory cytokines *IL1β* and *IL8*. This phytobiotic product has the potential to support growth and feed conversion efficiency [76]. The *Glycyrrhiza glabra* (licorice) extract added to the diet of Ross 308 broilers increased bodyweight gain and improved feed conversion. Moreover, infection of chickens with *Campylobacter jejuni* led to a decrease in the *IL1β* expression [63]. A supplement containing the essential oils of *Mentha arvensis* (mint), *Illicium verum* (star anise), and *Syzygium aromaticum* (clove) also increased bodyweight gain and improved feed conversion but decreased the *IL18* mRNA content in Ross 308 broilers [64].

In Rose cross broiler chickens fed basil, the expression of the *GH* gene in the liver increased significantly with an increased weight and improved feed conversion. Broiler productivity was higher due to stimulating synthesis and release of growth hormone. However, basil had no effect on the growth hormone receptor GHR [65]. The use of a powder preparation from medicinal plants *Salvia officinalis* (sage), *Matricaria chamomilla* (chamomile), *Teucrium polium* (felty germander) and *Origanum majorana* (marjoram) led to an increase in the expression of the *IGF1* gene in Ross 308 broiler chickens, which may favor the development of immunity [66]. Ross 308 broiler chickens fed 200 mg/kg cornelian cherry extract showed increased expression of the glucose transporter genes *GLUT1* and *GLUT2* and the highest weight gain [67].

Amino acids. Amino acids perform the main physiological function in the body, they participate in protein synthesis necessary to construct tissues and organs. The use of amino acids as a feed additive has a positive effect on poultry productivity [43].

The essential amino acids methionine and arginine must be present in the chicken diet. Methionine is involved in the DNA methylation, the elimination of reactive oxygen species, and affects growth performance and breast yield in broilers [68, 69]. Methionine deficiency (0.28% methionine) slowed down the growth of broiler chickens of the Arbor Acres and Cobb 500 crosses, decreased feeding efficiency and *LAT1* expression in the kidneys, and activated the expression of the *SNAT2* and *CAT1* transporter genes [22, 68]. Co-injection of methionine and cysteine in ovo increased *IGF1* expression in newly hatched Ross 308 chicks [69].

Arginine is involved in maintaining the immune system, improves growth performance and reduces abdominal fat percentage in chickens [43]. Injection in ovo of L-arginine (100 $\mu g \cdot ml^{-1} \cdot egg^{-1}$, 1000 $\mu g \cdot ml^{-1} \cdot egg^{-1}$ and 2500 $\mu g \cdot ml^{-1} \cdot egg^{-1}$) increased the *MYOD* and *MYOG* expression in Ross 1040 broiler chickens, but has no significant effects on the *IL8* and *TNFα* expression [70]. A mixture of palm and sunflower oils together with L-arginine and vitamin E fed at a dosage of 50 mg/kg, increased productivity and changed the expression of cytokines in Cobb 500 broiler chickens which may have a positive effect on immune function [71].

Thus, the commercial production of broiler chicken meat is based on the use of early maturing, highly productive crosses. However, such a bird has weak resistance and is exposed to various environmental factors that can affect growth rate, weight, appetite, feed digestion and may cause various diseases. Currently, there is a search for feed additives for poultry to maintain its physiological state, prevent diseases, help accelerate growth without compromising health, and improve productivity performance by changing the transcriptional activity of various genes. Many works have shown that the expression of genes involved in the poultry growth and development (*GH*, *IGF1*, *GHR*, *MYOD1*, *MYOG*, *MST*), nutrient utilization (*SLC2A1*, *SLC2A2*, *SLC2A3*, *SLC2A8*, *SLC2A9*, *SLC2A12*, *SLC6A19*, *SLC7A1*, *SLC7A2*, *SLC7A5*-7, *SLC15A1*, *SLC38A2*), immune response (*IL1β*,

IL6, *IL8L2*, *IL16*, *IL17A*, *IL18*, *TNF-α*, *AvBD1-AvBD14*) are influenced by various factors, including prebiotics, probiotics, synbiotics, phytobiotics and amino acids as feed supplements.

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