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Overview of Genes Associated with Egg Productivity and Resistance of Domestic Hen.

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ABSTRACT

At present, the most poorly understood factor affecting the quality of the eggs is the condition of the intestinal microflora and its changes under the influence of various feed additives. As candidate genes for expression analysis in real-time under different feeding regime were chosen genes influencing immunity and productivity of poultry largely associated with egg productivity. To study the expression profiles in the tissues of the oviduct and intestine were selected genes that are positional candidates for performance traits of laying hens, such as egg weight, egg production, elastic deformation of the eggshell, eggshell thickness encoding the following proteins : apocalixis-32 (OCX32), vocalising-36 (OCX-36), ovocleidin-116 (OC-116), the receptor of very low density lipoproteins (VLDLR), vitellogenin (VTG), Riboflavin-binding protein (RBP), cellular retinol-binding proteins (CRBPs), avidin(AVD), ovalbumin, CaBP-D28k. To study the effects of probiotics and phytobiotic on the immune system of laying hens has been selected genes encoding defense peptides (HDPS), comprising a large group of natural broad-spectrum antibiotics and play an important role in the immune response in almost all forms of life.

Keywords: gene expression, polymerase chain reaction in real time, hens, egg quality, protective peptides, probiotics.

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At the present stage of development of industrial poultry egg one of the main objectives is to reduce the costs of production and improving its quality. It is necessary to create the conditions of keeping and feeding poultry for maximum implementation of genetically determined potential capacity of the organism.

The microflora of the gastrointestinal tract (GIT) of poultry, especially resident and symbiotic, affects the health of birds (primarily on the immune system), on productivity and thus on the term of productive use. Characteristics of the microflora of chickens affect the sanitary-hygienic requirements for poultry products (meat, eggs). For example, many pathogens of food toxiinfectio and toxicosis in humans, especially campylobacteriosis, are caused due to the contamination of meat and eggs with bacteria, which is the normal abode of the gastrointestinal tract in chickens. In this regard, relevant is the development of new molecular genetics technologies to evaluate the expression of genes associated with productivity and resistance to adverse factors for maintaining the health of poultry improving biosecurity, productivity, and quality of poultry products. System correction of the microflora based on the use of safe for human feed additives (probiotics, phytobiotic) and monitoring system microflora using molecular-genetic methods of analysis, for example, methods of terminal length polymorphism restriction fragments (T-RFLP).

One of the main directions of this research is to study the profile of expression of genes encoding proteins of the eggs of domestic chicken and involved in the immune response in connection with the feature of the power bird. It will also explore the relationship between the composition of the microflora components of the diets of chickens, health, and productivity of hens of egg breeds and resistance to diseases.

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To study the effects of probiotics and phytobiotic on immunity has been selected genes encoding defense peptides (HDPS), which are a large group of natural broad-spectrum antibiotics and play an important role in the immune response in almost all forms of life. Protective peptides of the host (HDP) represent a diverse group of small cationic peptides present in a variety of organisms, both animals, and plants [1]. HDP is an important "first line" of defense, especially those species that have an adaptive immune system is absent or rudimentary. Most HDPs are strategically synthesized in phagocytic cells and mucosal epithelium of the owners, who meet regularly with microorganisms from the environment. Mature HDPs are widely active against gram-negative and gram-positive bacteria, mycobacteria, fungi, viruses and even cancer cells [2]. AMP proposed several schemes of classification. However, most AMPs are usually divided into four clusters based on their secondary structures: linear peptides with α-helical structure [3], cyclic peptides with the structure of β-sheet [4], peptides with β-conifers structure [5], and peptides with linear structure [6]. The increased synthesis of endogenous HDPS can be a promising antibiotic alternative approach to combating the disease. In the foreign study, the authors [7] tested the hypothesis that exogenous administration of butyrate a major type of short-chain fatty acids obtained in the process of bacterial fermentation of undigested dietary fiber) that can cause the induction of HDPS and enhancing disease resistance in chickens. Discovered that butyrate is a potent inducer of several, but not all, chicken HDPS such as HD11 macrophages and primary monocytes, bone marrow cells, the jejunum and the caecum. Furthermore, treatment with butyrate increases the antibacterial activity of chicken monocytes against *Salmonella Enteritidis*, with minimal impact on inflammatory cytokine production, phagocytosis, and oxidative stress of cells. In addition, the country supplementation with 0.1% butyrate led to a significant increase in HDP gene expression in the intestinal tract of chickens. More importantly, this strategy of feeding led to a nearly 10-fold reduction in bacterial titer in the cecum of experimental infections caused by *S. Enteritidis* [7]. Together, the results showed that endogenous HDPS are induced by butyrate is phylogenetically conservative mechanism of innate protection of the host mammals and birds and that dietary supplementation of butyrate has a potential for further development as a convenient antibiotic-alternative strategy to enhance innate immunity and disease resistance of poultry [7].

Defensins and cathelicidins are the two main families of HDPS vertebrates [8]. While defensins are classified according to the presence of the six conservative cysteine residues in the C-terminal Mature sequence, all cathelicidins consist of conservative katelyn domain (Cathelin) in the Pro-sequence with a very

divergierende the C-terminal sequence. The chicken genome encodes a total of 14 B-defensins known as AvBD1-14 (9), and four cathelicidin, namely fowlicidin 1-3 (fowlicidins) and cathelicidin-B1 (10). All AvBDs tightly grouped on chicken chromosome 3q, while cathelicidin genes are located on chromosome 2R. Both chicken AvBDs and cathelicidins are expressed in various tissues, but most strongly in the bone marrow or Bursa [9], and defensins in the liver and the entire digestive, respiratory, and reproductive systems. HDPS exhibit a wide spectrum antimicrobial activity against bacteria, protozoa, covered in viruses, and fungi, mainly due to direct binding and lysis of microbial membranes [10].

Pathogens are extremely difficult to develop resistance to HDPS because with such physical interactions, Many HDPS such as chicken AvBD9 (formally known as gallinacins-6) and cathelicidin B1 have a strong antibacterial activity against a broad spectrum of bacteria, including Salmonella. In addition to direct bactericidal activity, HDPS have a strong capacity to modulate the innate immune response by inducing chemotaxis and activation of various types of leukocytes. Pleiotropic effect of HDPS actively investigated as a new class of therapeutic agents against antibiotic-resistant bacteria and against other inflammatory diseases (1.10). In connection with the foregoing data, it is planned to study the effect of food additives on the expression of genes encoding the protective peptide that can facilitate the selection of rational nutrition with an addition of probiotics and vitabiotics to enhance resistance to infectious disease.

To study the expression profiles in the tissues of the oviduct were selected genes that are positional candidates for performance traits of laying hens, such as egg weight, egg production, elastic deformation of the eggshell, eggshell thickness encoding such proteins as: apocalixis-32 (OCX32), vocalising-36 (OCX-36), ovocleidin-116 (OC-116), the receptor of very low density lipoproteins (VLDLR), vitellogenin (VTG), Riboflavin-binding protein (RBP), cellular retinol-binding proteins (CRBPs), avidin (AVD), ovalbumin, CaBP-D28k.

Calcification of the shell occurs in the organic matrix associated with the fibers of the outer membrane shell, which leads to the formation of the mastoid (inner) and spongy (external) layer. The last column consists of rhombohedral calcite with the preferred orientation. This degree of structural and crystallographic organizations can arise as a result of competition for crystal growth between adjacent foci of calcification [11] or from the control of the matrix components of calcite crystal shape, size, and orientation [12]. The size and orientation of calcium carbonate crystals affect the strength of the eggshell. Markers ovalbumin and ovotransferrin were associated with crystal size, and -116 markers ovocleidin and vocalising of-32 (RARRES1) were associated with crystal orientation. The localization of these proteins in the eggshell is consistent with different phases of the process of formation of eggshell. In addition, protein matrix of the shell affects the variability of the crystals and, in turn, on the thickness of the eggshell that can be used to create breeding programs to improve this trait. [13]. The matrix of the eggshell contains such proteins like glycoproteins and proteoglycans. Data matrix proteins were identified after decalcification and dissolution of the egg shell [14]. Three of them were previously identified in egg white: ovalbumin [15], ovotransferrin [14], and lysozyme [16]. The osteopontin, phosphorylated glycoprotein, contains into bones, also available in eggshell, expression of which is significantly higher in the cells of the surface epithelium of the uterus and is regulated in response to mechanical stress in contact with eggs in the uterus.

Apocalixis-32 (OCX32) - is a protein matrix contained in the outer layers of the eggshell and the cuticle. Numerous reports in the literature indicate a relationship between the OCX32 gene variants encoding the protein, and different quality of the eggshell. Thus, the OCX32 gene is a candidate for selection on the grounds of the eggshell in commercial poultry populations. Studies have shown significant effects of OCX32 Association signs the color of the shell, the height of the protein in the egg, egg weight, and yolk weight [17]. Apocalixis-32 (OCX-32) has high and specific expression level in the uterus (shell's gland), isthmus regions of the oviduct of G. gallus and is concentrated in the eggshell [14]. OCX-32 is a protein matrix, the eggshell, which enhances the antimicrobial properties of the eggshell, providing protection for the developing embryos of birds [18]. RARRES1 is expressed in different tissues and presumably a tumor suppressor in humans [19].

Apocalixis-36 (OCX-36) is a protein matrix of the eggshell, which is highly secreted in uterine glandular cells during the active phase of calcification and is contained in an eggshell and the yolk membrane. Apocalixis-36 belongs to lipopolysaccharide-binding proteins and bactericidal family (BPI), which is well known in mammals for their involvement in defense against bacteria. These proteins belong to the BPI fold superfamily containing PLUNC / PSP / BSP30 / SMGB [20]. Members of this family are associated with lipids and lipopolysaccharides of the cell wall of gram-negative bacteria, leading to the death of bacteria. Apocalixis-

36 (OCX-36) was identified as a specific protein of the eggshell of G. Gallus. It is detected only in those regions of the oviduct, where the formation of the shell included in the shell membranes, mainly in the inner part of the calcified eggshell [20].

Egg yolk, known as vitellogenin (VTGs) is the main source of amino acids, structural and non-structural proteins consumed by the embryo (21). VTGs are synthesized in the maternal liver in response to the hormone estrogen and is selectively absorbed by the developing oocytes, where the Mature polypeptide is cleaved into three polypeptides: lipovarin (VTG1), is highly phosphorylated phosvitin (VTG2), and a smaller peptide (VTG3) [22].

Education eggs include oocyte development and ovulation process. In this process, oocytes take essential nutrients from the General circulation for the formation of the yolk and grow from 6-7 mm to 35 mm to ovulation. VLDLR (receptor of very low-density lipoproteins) and VTG (vitellogenin) are the two key precursors of yolk and are involved in the transport of lipids, which make up more than 30% by weight of the yolk. The receptor of very low-density lipoproteins receptor (VLDLR) is a key medium VTG and VLDL, suggesting that changes in expression and function of the VLDLR gene can affect the development of oocytes and egg laying. This conclusion is supported by the study showing that chickens have a nonsynonymous mutation in this gene is not able to lay eggs and have signs of hyperlipidemia. The gene expression levels of VLDLR are also correlated with egg weight and size and interval of laying eggs [22].

Riboflavin-binding protein (RBP), synthesized in the oviduct (egg-white RBP), and liver (yolk RBP) laying hens, and plays an important role in the development of oocytes/embryos. It binds Riboflavin (vitamin B2) and is involved in the transport of serum in oocytes/embryos from the oviduct and in egg white [23].

Ovalbumin is the main protein egg protein and is synthesized in the oviduct of the chicken that is consumed by developing embryo in the extra yolk to the power source. Two types of ovalbumin protein called ovalbumin X and Y, and are also concentrated in egg white (24). The analysis revealed that these three proteins belong to the ovalbumin - related serpin family of proteins (SERPINB) [25].

Other specific protein associated with the formation of the eggshell, ovocleidin-116 (OC-116) is associated with shell calcification in birds (26). OC-116 was isolated from osteoblasts and osteocytes of cortical bone (27). The structure of the OC-116 clearly shows that this gene belongs to the family of the secretory calcium-binding phosphoproteins (SCPP), which are associated with mineralization of tissues in vertebrates.

There is a homology between the OC-116 and other proteins of the family of SCPP. This hypothesis finds wide support in several studies [28, 29] and analysis sintenie shows that *G. gallus* (also in M. gallopavo, and T. guttata) gene, OC-116 is localized in a specific gene cluster associated with mineralization of tissues (including bone sialoprotein and dentin matrix acidic phosphoprotein) on chromosome 1.

Cellular retinol-binding proteins (CRBPs) are members of a multigenic family of intracellular lipid-binding proteins (iLBP). CRBPs have a small molecular weight (~15 kDa) that bind retinol and retinoic acid. CRBPs play an important role in the absorption of retinol and its cellular transport in the gut as well as regulate the metabolism and homeostasis of retinoids through interaction with metabolic enzymes. Currently, there are four known cellular retinol binding proteins (CRBPs): (CRBP I, CRBP II, CRBP III and CRBP IV). It is best characterized by CRBP I and CRBP II. Cellular retinol-binding protein II (CRBP II) belongs to the family of cellular retinol-binding protein and plays an important role in the absorption, transport, and metabolism of vitamin A and its active derivatives (the retinoids) play a fundamental role in embryonic development of vertebrates, organogenesis, tissue homeostasis, cell proliferation, differentiation and apoptosis [30]. Vitamin a-active retinoids are vital for many aspects of avian reproduction. Vitamin a deficiency and excess have a significant impact on the number of eggs, egg weight, embryo survival and hatchability of eggs [31].

The level of expression of this gene is very high ($p<0.05$) in the jejunum intestine and liver, medium in kidney, ovary, and oviduct, and lowest ($p<0.05$) in heart, hypothalamus, and pituitary. Due to the important role of CRBP II in the absorption and transport of retinol, this gene may be an important gene candidate in reproductive performance of laying hens.

The gene encoding the protein of chicken eggs - avidin (AVD), acts as an antimicrobial agent by depriving invading microorganisms of Biotin. Avidin is expressed in skeletal muscle and cartilage of the developing embryo of a chicken. The results of studies indicate that interfering with the metabolism of fatty acids, avidin is involved in the differentiation of chondrocytes and myoblasts embryo chicken. This gene affects the reproductive function of the chicken and carries the protection against pathogens, expression of which was increased in the intestine under the influence of pathogenic microorganisms [32].

Gene CaBP-D28k is expressed in the tubular gland cells of the uterus of a chicken and plays a critical role in the transport of calcium ions Ca_2+ in the formation of the eggshell [33]. In addition to the vital role in the transport of calcium, has the function of protecting cells from high concentrations of calcium preventing cell death by apoptosis, acting in this case as a buffer. Savr-D28K has expressed also in the kidney, brain, intestine and shell gland of the chicken [33]. Birds have a high concentration of CaBP was detected in the tissues, which are characterized by mass transport of Ca_2 +, such as intestine and shell gland. In both tissues, CaBP and calcium transport are closely linked, calbindin present in the intestine of hens before the onset of oviposition, and its level increases at the beginning of oviposition in order to create a high concentration of Ca_2 + is required for calcification of eggshell.

In the eggshell gland, calbindin appears during the formation of the first eggshell and disappears within 3 days after the cessation of mineralization. Concentration calbindin into eggshell's gland is proportional to the rate of deposition of Ca_2 + in the shell [33].

In a recent study, Xuefeng Q., 2016 studied the effect of subtype H9N2 of avian influenza (AIV) on the egg production and the study of gene expression of CaBP-D28k affect the calcification of the shell. H9N2 subtype AIV has a close correlation with changes in the level of mRNA in the uterus CaBP-D28k mRNA, leading to reduced egg production and quality of eggshell of laying hens [34].

Upon completion of this project, it is planned to study the relationship between genes associated with the quality of the eggs and the immune resistance of chickens egg breeds feed and as microbial background the digestive tract of a bird. Increasing the knowledge about the States of the microbiome of the digestive tract of a bird allows you to create a Foundation for the prevention of many diseases of bacterial origin (especially mycoplasmosis. The work will be used methodological approaches to determine the total number of bacteria, archaea, and fungi on the basis of T-RFLP analysis, real-time PCR and NGS - sequencing for determining the structure of microbiome dynamics.

Obtained in the course of the project, knowledge can help create the potential for the development of new approaches for the extension of the productivity of laying hens, and accordingly, to reduce losses in the poultry industry associated with increasing the productivity of poultry, reduced feed costs.

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