Sel'skokhozyaistvennaya Biologiya [Agricultural Biology], 2013, № 4, p. 3-25

UDC 636.5:591.1:577.27

GUT IMMUNITY IN BIRDS: FACTS AND REFLECTIONS
(review)

V.I. Fisinin[1], P. Surai[2, 3]
[1] All-Russia Research and Technological Institute of Poultry Breeding, RAAS, Sergiev Posad 141311, Moscow province, Russia
e-mail: fisinin@land.ru
[2]Feed-Food. Ltd, Ayr, KA7 3BN, UK, Scotland
e-mail: psurai@feedfood.co.uk
[3]Scottish Agricultural College (SAC), Edinburgh, EH9 3JG, UK, Scotland

Received March 12, 2013

S u m m a r y

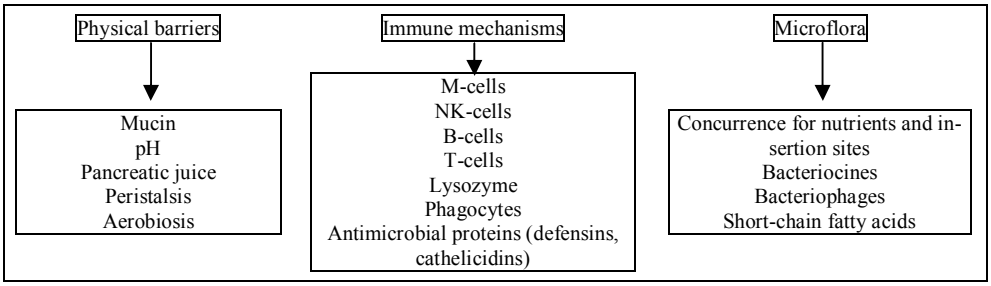
Gut immunity plays a crucial role in maintenance of the whole body immunity being the first and most important line of defence from various pathogenic organisms and substances consumed with feed and able to colonize host cells and tissues. The role of protective mechanisms in the gut is difficult to overestimate. For example, the process of learning distinguishing between «self and non-self» taking place in the gut are fundamental for the immunity development as well as for the development of nutrient tolerance. It is necessary to underline that structural changes in the gut, in particular at the mucosa level are responsible for decreasing efficacy of nutrient assimilation from the feed. Therefore, gut status determines the chicken health, utilization of nutrients and biologically active substances (FCR) and other important commercially relevant parameters of the poultry production. This review summarises recent knowledge about the development and functioning of protective immunological mechanisms in the gut. A particular attention is paid to the possibilities on the modulation of gut immunity by a mixture of biological active substances.

Keywords: chicken, stress, immunity, gut, vita-genes.

In post-hatch period a chick grows so fast that its weight increases 4.4 times in the 1st week of life, and by 5000 % after 5 weeks when it amounts 2 kg. Such high productive indices now are attainable owing to intense selection for growth rate, efficient healthcare and livestock management, and improved nutrition satisfying needs in all basic nutrients and bioactive substances. Growth period of birds is steadily reducing while feed conversion constantly improves, so maintenance of health and optimization of feeding are the priority tasks of poultry industry. Today, the production technology increasingly involves a subtle level of regulation and considers minor factors previously neglected in theory and practice. For example, changes in microstructure of the intestine, particularly in its mucosa, may reduce assimilation of nutrients, which affects the general health, the efficiency of utilization of nutrients and bioactive substances, and, therefore, growth, development, feed conversion, and other important economic parameters of poultry industry. The intestine is the first level of defense from exogenous pathogens that colonize host cells and tissues, and this is also the largest organ with immune properties.

In this regard, protective mechanisms in the gut including its immune system are so particular that deserve a detailed consideration. Any growing organism pays significant energy costs for immune protection. For example, the acute phase of immune response in chickens is associated with the decline in feed intake and productivity, and up to 10% extra consumption of nutrients that otherwise could be utilized in growth and development (1). In a fast-growing broiler about 12 % of newly synthesized proteins are spent on the maintenance of homeostasis in the digestive tract. Intense proliferation reduces the age and maturity of goblet cells, which may affect the quality of secreted mucin and thereby suppress the absorption of nutrients (2). Besides, quick renewal of these cells raises demands in energy for plastic and enzymatic processes in the intestine. Changes of the intestinal morphology may lead to hindered absorption, hypersecretion, diarrhea, reduced immunity and general productivity of a stock (3).

The purpose of this review was compilation of the latest scientific facts about gut immunity in birds.



Gut defenses in birds (explanation – see below).

General characteristics of protective mechanisms in the gastrointestinal tract (Fig.). The first group of factors includes physical barriers and specific conditions of the media. High rate of cellular renewal in the mucous layer prevents colonization of pathogenic microorganisms; protective properties of mucin inhibit penetration of microorganisms and their attachment to intestinal villi; low pH in the glandular stomach and small intestine is unfavorable for development of pathogenic microbes; oxygen suppresses proliferation of anaerobes; protective properties of pancreatic juice and bile (undigested food is a substrate for development of many microorganisms including pathogens); intestinal peristalsis and constant motility of the chyme reduce probability of attachment of pathogens to the villi. The second group are components of the immune system, such as antimicrobial peptides (defensins); lysozyme; mucin synthesized in response to cytokines, bacterial products, and growth factors; gut immunoglobulins facilitates successful antimicrobial defense; lymphoid tissue provides recognition of foreign antigens and immune responses associated with factors of congenital (phagocytic cells, NK-cells, etc.) and acquired immunity (B- and T-lymphocytes, their metabolic products). The third group of factors are synergistic microorganisms (gut microbiota), their bacteriophages, and products of their vital function (bacteriocins, short chain fatty acids).

In farm animals and poultry, intestinal mucosa is the greatest surface contacting with external environment and its foreign antigens, and protected by a special mechanism of effective defense against invasion. Different mucosal barriers have common

histological features with small local differences associated with anatomy of the tissue, its location, and physiological functions. For this reason immunological barriers of mucosa (MALT – mucosa-associated lymphoid tissue) are divided into subgroups: GALT (gastrointestinal-associated lymphoid tissue), BALT (bronchus-associated lymphoid tissue), NALT (nasal-associated lymphoid tissue), which represent lymphoid tissue associated with, respectively, the intestine, bronchi, nasopharynx, and also present in salivary glands and in the genitourinary system.

GALT is the main component of MALT; it is a complex infrastructure of immune cells and organs located in the epithelial layer and adjacent to lamina propria. GALT includes several types of cells including special inducers, immunoregulators, and effectors that are distinct from those involved in systemic immunity functions (4). Unlike mammals, chickens have no lymph nodes, so the induction and development of immune response occurs mainly in GALT and in the spleen. This fact shows why it's so important to understand the development and functional maturation of GALT as one of the fundamental immunological phenomena in birds including the immune response or tolerance to different antigens (4, 5). The most important task of the intestinal immune system is recognition "friend or foe" and adaptive tolerance to "friendly" antigens (e.g., nutrients or synergistic microbial populations), as well as active immune response to "foes". Any breach in this system may lead to allergic reactions for nutrients, tolerance to pathogens, and excessive consumption of feed nutrients and bioactive substances. So, gut immunity is regulated by fine fundamental mechanisms; peculiarities of these processes in birds and their distinctions from the ones described in other animal species, such as mammals, will be discussed below.

Specific and nonspecific defense factors. *Physical barriers in the intestine.* Intestinal mucosa is covered with microvillous simple columnar epithelium (enterocytes) that functions as a physical barrier between the external and internal environment of the body, that also provides digestion and absorption of nutrients. Enterocytes have specific apical membrane receptors that recognize bacterial antigens, which causes activation of local immune responses (6). Other cells of gut immune defense are goblet cells, M-cells, and Paneth cells. In avian gut only goblet cells were studied in detail: their main function is secretion of mucin and they develop from the same progenitor which enterocytes (7, 8).

Role of mucins in gut immunity. Mucus is synthesized and secreted by goblet cells in the intestinal mucosa where it acts as security barrier, lubricant, and a kind of transport system between gut contents and epithelial cells. Mucins are a group of glycosylated proteins with a molecular weight of 20 000 daltons that primarily prevent penetration of various pathogens through the mucosa (9-11). Mucin molecule is a complex glycoprotein biopolymer in which oligosaccharides (up to 50-80% its molecular weight) are connected to protein subunits via O-glycoside bond between hydroxyl groups of serine or threonine; the abundance of sulfate groups (sulfomucins) and carboxylate groups (sialomucins) contributes to a negative charge of the mucous layer.

The surface of enterocytes is covered by two layers of the secreted mucin – the inner dense layer and the outer thinned layer (10). Such layers significantly hamper adhesion of bacteria to epithelial cells. Moreover, mucin binds to bacterial receptors and thereby blocks their ability to adsorb on the cell surface before penetration (7). Activity of genes involved in the synthesis of mucins is controlled at the transcriptional level by cytokines, bacterial products, and growth factors. Biosynthesis of mucin also depends on conditions and/or agents that provide differentiation of cells into mature goblet cells. It is also affected by reactions of glycosylation and protein synthesis, as well as some other processes (12). Mucins in the layer covering the villa are composed of neutral and acidic molecules. The presence of charged groups in the molecule of mucin is often regarded as a reason of its ability to protect the epithelium from various pathogens and participate the absorption of nutrients. Along with it, mucin layer on the surface of mucosa is a source of nutrients for many intestinal organisms, and some species of intestinal bacteria can utilize polysaccharides of mucin.

Thus, there's a fine relationship between the gut microflora and the amount of mucin. The two types of mucin on the surface of cells interact with each other to form immiscible aqueous layer adjacent to mucosa, thereby to establish and maintain a constant level of specific pH. Molecular mechanisms of this phenomenon are not yet clear, though it is supposed to be associated with binding of hydrogen ions to bicarbonate secreted by the mucosal layer (7). It is believed that the local medium is slightly acidic or neutral (pH≈7). Acidity / alkalinity affects the growth of microorganisms, so it is quite important for the control of intestinal microflora, its number and composition. The immiscible aqueous layer serves as a molecular filter between gut contents and the surface of intestinal microvilli. The main strategy of digestion in the small intestine is treating feed particles by pancreatic enzymes up to a size small enough to penetrate such layer and complete digestion. The surface enzymes are protected from proteolytic degradation, while products of digestion are not available for gut microbes. An interesting fact is that goblet cells can change composition of secreted mucin in response to the presence of microorganisms (11). For example, *Clostridium perfringens* cause them to produce acid molecules instead of neutral ones, which can be seen as an attempt to maintain the optimum pH value in changed conditions. Mucin has low rate of degradation before it appears in anaerobic conditions of the large intestine.

Dietary factors, e.g. phytate and fiber, promote secretion of mucin. Anti-nutritional factors may cause increased production of mucin: their abrasive action removes mucin layer from the mucosa, which then is compensated by goblet cells that enhance the synthesis of mucin. Specific amino acids and proteins also affect the synthesis / secretion of mucin; they can interact directly with goblet cells or with nerve endings of the intestine. Amino acids threonine, serine, and cysteine significantly affect the production of mucin, as they are its components: e.g., threonine is about 11 % amino acid sequence of mucin. Suppressed synthesis of mucin in birds may be harmful for intestinal mucosa and reduce local utilization of nutrients (13).

Antimicrobial peptides. It's a known fact that avian heterophils do not contain the enzyme myeloperoxidase necessary for the formation of toxic nitrogen metabolites that cause death of pathogens. That's why bactericidal functions of heterophils are based mainly on non-oxidative mechanisms provided by enzymes and antibacterial substances of heterophilic granules (14, 15). Antimicrobial peptides (AMP) are the most important components of the innate immunity in all animals, from flies to mammals. They can disrupt the integrity of membranes of microorganisms (16). In vertebrates, there are two families of AMP: defensins and cathelicidins. Mammals have α- and β-defensins (17). Alfa-defensins are unique for mammals, while beta-defensins are more common; in chickens, four types of beta-defensins named gallinacins were discovered already at early stage of research (18-20). The homologous peptides were described in turkeys (18, 20). Gallinacins Gal-1, Gal-1α, and Gal-2 were isolated from chicken heterophils while Gal-3 was found in various epithelial tissues; induction of Gal-3 occurred during infection, e.g. in the trachea (20). Gallinacins are effective against many gram-positive and gram-negative bacteria species with varying specificity. Defensins Gal-1 and Gal-1α also showed the activity against yeast *Candida albicans* (14, 21, 22). Antibacterial β-defensins Gal-1 and Gal-2 found in avian heterophils are considered as major factors of birds' immunity to pathogenic bacteria and fungi (23).

Then in chickens were described seven other gallinacins (from Gal-4 to Gal-10) (24). The authors observed a moderate expression of Gal-1and Gal-2 in the bursa Fabricii and in the gut. Expression of the others (new discovered) gallinacins varied in

different tissues including the small and large intestines. Gallinacins play an important role in adaptive evolution of animals and birds being involved in adaptation to changed environment and expanded diversity of pathogens. In chickens, a significant production of beta-defensin Gal-6 occurred in the esophagus and craw, moderate – in the glandular stomach, and low – in other parts of the gut (25). Gal-6 showed a significant antibacterial activity against *Campylobacter* sp., *Salmonella* sp., *Clostridia* sp., and *Escherichia coli*; it suppressed their growth and caused dose-dependent changes in their morphology (intracellular granulation, cytoplasm retraction, irregular formation of cell walls in dividing cells), and lysis. Anti-salmonella effect of gallinacins Gal-9, Gal-4, and Gal-7 can be ranked as: Gal-9 ≥ Gal-4 > Gal-7 (26), moreover, Gal-7 and Gal-9 have synergistic effect against *S. enteriditis*. The authors found a notable expression of Gal-11 in the small intestine, liver, gallbladder, and spleen of chickens, Gal-13 was detected in the colon, while other beta-defensins were not identified in the gut (24, 27, 28). Studying the properties of synthetic Gal-11, it was found to be effective against enteric pathogens including *S. typhimurium* and *Listeria monocytogenes* (27). Eight of the 14 recently studied beta-defensins of chickens are tissue-specific: AvBD1, AvBD7, and AvBD9 were found in the craw, AvBD8, AvBD10, and AvBD13 – in the gut, AvBD1 and AvBD7 – in the spleen (29).

Cathelicidins are the another family of antimicrobial proteins found mainly in non-peroxidase granules of neutrophils. Cathelicidins are encoded in the genome as propeptides, which then are activated by proteases to form bioactive peptides of 12-97 amino acids (30). Most of cathelicidins show different occurrence in tissues, antimicrobial activity against a number of Gram-positive and Gram-negative bacteria, fungi, protozoa, and viruses (31). They also bind and neutralize endotoxins (32), induce chemotaxis of neutrophils, T-cells, and monocytes (33). Cathelicidins were found in various mammals (34) and in chickens (24). The main producers of cathelicidins are neutrophils.

Recently it was described a new cathelicidin of chickens – myeloid antimicrobial peptide 27 (CMAP27). The highest expression of this peptide was observed in myeloid / lymphoid tissues and testes. The active synthesis of mRNA of CMAP27 was detected in bursa Fabricii and in the bone marrow. CMAP27 is intensely produced in cecal amygdales and in the lymphoid tissue localized near the ileocolic junction, and less intense – in other parts of the gut including the glandular stomach (35). Cathelicidin-2 often presents in heterophils in the form of rod-shaped granules, though it is absent in other cells of the peripheral blood and in the intestinal epithelium of chickens. It was shown that lipopolysaccharides of *Salmonella* stimulate heterophils to release cathelicidin-2 with time-dependent effect (36). Thus, cathelicidin-2 showed antibacterial and fungicidal activity against various pathogenic microorganisms including *Salmonella*. Along with it, cathelicidins are immunoregulators that affect production of cytokines and renewal of the immunocompetent cellular pool, they bind endotoxins and reduce endotoxin-mediated inflammatory response (37).

Lysozyme activity. Lysozyme (EC 3.2.17) is the relatively low-molecular enzyme that catalyses specific cleavage of polysaccharides (peptidoglycans and chitodextrin) in the cell wall of bacteria. Three types of lysozyme are classified according to peculiarities of their amino acid – c (chicken), g (goose), and i (invertebrates) (38). Egg white of most of bird species including hens contains c-type lysozyme, though geese and ostriches have g-type lysozyme. Lysozyme of egg protein is a polypeptide with a molecular weight of 14 300 daltons including 129 amino acid residues. Lysozyme molecule consists of two domains with the active center located between them (39). Lysozyme amounts 3.5 % of total egg protein; its highest content was found in the egg white and in the embryo whose adaptive immunity is yet underdeveloped and provided by non-specific mechanisms; therefore, unless embryo and a chick starts own synthesis of immunoglobulins, it is protected by egg lysozyme. In all animals lysozyme are the key effectors of innate immunity. They hydrolyze β-glycoside bond between N-acetylmuramic acid and N-acetylglucosamine of peptidoglycan – the basic component of the bacterial cell wall (40).

Lysozyme is found in phagocytic and secretory granules of neutrophils, it is synthesized by monocytes, macrophages, and epithelial cells. Significant amounts of lysozyme present in saliva, respiratory mucosa, milk, and other body secretions; it is regarded as the first line factor of antibacterial defense. Many Gram-positive bacteria are rapidly killed by lysozyme in vitro, but Gram-negative bacteria are resistant to it due to the outer membrane that prevents direct access to the peptidoglycan. However, in vivo Gram-negative bacteria become recognizable and sensitive to lysozyme as they are attacked by the components of innate immunity – specific antimicrobial peptides defensins and the complement system disrupting the outer membrane. Since lysozyme is very common in the animal kingdom, bacteria have developed counter measures of own security: in particular, Gram-negative bacteria may produce specific inhibitors of each of the three lysozyme types (41).

Vertebrates have genes encoding both c- and g-type enzymes, but their expression is species-specific. For example, chicken genome contains genes for one c-type and two g-type lysozymes. C-type lysozyme is actively expressed in the oviduct under the control of steroid hormones. C-type lysozyme is also synthesized in macrophages, and this process is enhanced by bacterial lipopolysaccharides. In the gut of young chickens, c-type lysozyme is expressed up to the age of 8 days, g-type – to the 38th day. In chicks, g-lysozyme was also found in the liver, kidney, bone marrow, and lungs (38). Lysozyme is undoubtedly important for gut immunity, but this assumption yet lacks the experimental evidence.

Immunoglobulins. As already mentioned, gut immunity includes both innate and acquired components. The best preventive strategy against infections is avoiding any contact with pathogens and absorbing them by enterocytes (42), as performed by non-specific antibacterial substances or specific neutralizing antibodies secreted by epithelial cells into the intestinal lumen (42). Antibodies provide target suppression of a particular pathogen, while the general "antibacterial arsenal" may kill them both with symbiotic microflora. At the same time, many non-pathogenic bacteria have common antigenic determinants with pathogens, and, therefore, the action of neutralizing antibodies may lead to cross-reactivity.

Neutralization of bacteria through inhibition of their binding to epithelial cells is seen as the main defense mechanism of gut immunity realized by antibodies – IgG, as well as monomeric or dimeric IgA (5, 43). IgG and monomeric IgA are excreted with bile into the foregut or, via the bursal duct, into the hindgut (44). Plasma cells-producers of IgG or dimeric IgA are located in the intestinal wall and, according to some data, in the bone marrow and spleen (45, 46). Dimeric IgA are locally synthesized in plasma cells and secreted via enterocytes (43): they bind to polymeric Ig-receptors (not yet described in birds) on the basal membrane of enterocytes to be transported to the apical membrane and excreted into the lumen (6). This type of IgA is protected from proteolysis by a so-called secretory component (SC), a peptide fragment of polymeric Ig receptor responsible for transcytolysis of dimeric IgA from the lamina propria into the gut lumen (6). Similarly to mucin, dimeric IgA inhibits binding of bacteria to cell surface. IgA is innate immune factor with specific action caused by the primary contact with antigens in the intestinal lamina propria (43, 47).

Gut associated lymphoid tissue (GALT). Unlike other components of the immune system associated with body cavities, GALT deals with two types of antigenic molecules – harmless (usually nutrients, which normally don't cause immune response) and harmful (substances of endogenous or exogenous pathogens). The balance between immune response and tolerance is finely regulated depending on the interaction between immune cells and cells of the gut parenchyma. In other words, any antigenic molecule absorbed

via enterocytes (intracellular transcytosis) is tolerogen, while all antigens transferred via the paracellular pathway or phagocytic cells (M-cells) are immunogens (42, 48).

In chickens, GALT is represented by individual immune cells distributed in the intestinal epithelium and in underlying layers (49, 50), as well as by lymphoid aggregates and structures located focally in some parts of the digestive tract. The highest segment of the intestine outgoing from the gizzard contains few lymphoid structures – esophageal amygdales located at esophagogastric junction (51), and aggregates on lamina propria of the gizzard (52). The gizzard around ileocecal junction contains Peyer's lymphoid follicles (53, 54); a lymphoid aggregate was found in Meckel's diverticulum (55). Histological study revealed a significant amount of lymphoid tissue in the initial segment of duodenum (56) focused around the site of transition of intestinal crypts into almond-shaped crypts with lymphoepithelial lining. This so-called pyloric tonsil is a new discovered lymphoepithelial organ of the avian digestive tract. Therefore, birds have a reliable immunological defense in the small intestine, which distinguishes them from mammals.

Formation and functioning of B- and T-lymphocytes were described in detail in the earlier authors' work (57). Now it should be mentioned only the formation of antigen-recognizing T-cell receptor as a crucial event in development of T-lymphocytes. Obviously, recognition of any particular antigen in a whole pool is assessed by millions of antigen-recognizing receptors that have unequal specificity. чThese numerous receptors are the result of genetic transformations that occur during proliferation and differentiation of progenitor cells. Antigen-recognizing receptors and other molecules that mediate interaction with antigen-presenting cells appear on the surface of T-lymphocytes during their maturation. These surface molecules of T-lymphocytes are regarded as markers of particular subpopulations of cells and stages of their differentiation; sets of these molecules are denoted by clusters of differentiation (CD) associated with a surface cellular phenotype. Along with T-cell receptor, CD4 and CD8 molecules are involved in recognition of own molecules belonging to the major histocompatibility complex (MHC). T-lymphocytes at later stages of differentiation retain one of these markers allowing to segregate mature T-lymphocytes into CD4+ (T-helpers) and CD8+ (cytotoxic T-lymphocytes).

Lymphoid follicles consist of B-cells immersed in the network of follicular dendrite cells with a small number of $CD4^+$ T-cells and macrophages. Interfollicular space is filled mainly by $CD4^+$ and $CD8^+$ T-cells (58-60). Population of T-lymphocytes includes αβ- and γδ-T-cells with, respectively, heterodimeric αβ- and γδ-receptors. These αβ-T-cells recognize antigenic peptides in MHC class I or II by means of CD8 and CD4 molecules. A trimeric complex formed by αβ-receptors of T-cells (TCR-αβ), peptide, and MHC, was found to be a crystalline structure driven by the mechanism of accurate recognition at the molecular level (61-64). In turn, γδ-T-cells recognize non-peptide antigens (e.g., pyrophosphate monoesters and alkylated amines of microbial pathogens) with no participation of MHC (65-68). Defense mechanisms against pathogens capable to disrupt the epithelial lining are based on activation of interepithelial lymphocytes such as natural killers (NK), αβ- and γδ-T-cells (62). It's notably that the unique population of $CD4^+$ T-cells – producers of interleukin-17 (T_h17-cells) – is distinct from ordinary T_h1- and T_h2-cells, and it is located mainly in the gut lamina propria of healthy animals (69, 70). It is still unclear whether T_h17-cells are pathogenic or protective; their specialization and development also need to be further clarified (71).

Compact lymphoid structures (caecal tonsils, Peyer's patches) include $CD4^+$ αβ-T-cells and B-cells (58, 60), while diffuse lymphoid components of epithelium and lamina propria – mainly γδ-T-cells (49). The bursal sac known as a primary source of IgM-forming M-cells contains much less CD4+ and CD8+ T-cells (60).

Leukocytes belonging to the cellular link of innate immunity, γδ-T-lymphocytes, and NK-cells are located mainly in the epithelium (72); their development and functions are similar to such cells of mammals (49, 73). In chickens, differentiation of γδ-T-lymphocytes occurs in the thymus (74, 75); intestinal origin of these cells was shown in mice (76), but this hypothesis is yet insufficiently investigated. Functionally, γδ-T- and NK-cells can response to stimulation immediately after activation, which is controlled by exogenous cytokines and antigen (77, 78). Sources of cytokines are activated CD4- or CD8-lymphocytes at immune response (77) and, possibly, stressed enterocytes at early phase of inflammation (79, 80). So, activated cells – participants of innate immune responses in the intestinal lining, contribute to defense along with activation of cells in the lamina propria.

Peculiarities of adaptive immune responses are associated with the presence of lymphoid follicles formed by B- and T-lymphocytes where specialization and cell division lead to formation of effector lymphocytes. Dividing cells are selected according to specific binding to the antigen transferred by follicular dendritic cells. Then, selected cells differentiate into effector cells or memory cells capable to migrate in body tissues. Scanty amounts of such primary follicles can be found in the small intestine of chickens (54). Possibly, immune response can be generated in the foregut as well, though primary responses most likely start in the hindgut, bursal duct, bursal sac, and spleen. The importance of cloacal (Fabricius) bursa for primary immune response in birds is the proven fact (81).

Consequently, immunological activity in the foregut is mainly a secondary process caused by functioning of lamina propria or by immune molecules excreted in the gut lumen with bile or delivered by enterocytes via transcytolysis, whereas primary responses to antigens are generated mainly in the hindgut. These primary responses cause systemic effects, because locally synthesized antibodies are carried away by plasma and effector cells to be then outspread in farther parts of the intestine, spleen, bone marrow, and other organs. A key factor in formation of primary immune responses in the hindgut is its capacity to "select" antigenic material from the environment. This assumption was indirectly evidenced by retrograde (backward) intestinal contraction in birds. In the authors' view, such retrograde motility of the middle intestine improves reabsorption of urine and caecal contents, and provides better absorption of cellulose degradation products and crude protein (82-84). Such reverse peristalsis between the middle part of jejunum, duodenum, and even gizzard, was observed in both newly hatched chicks and in adult chickens (85). So, it is possible that external material enters immunoactive caecal appendices, and, moreover, that antibodies may uplift from the bursal sac into the small intestine. The bursal sac is assumed to participate in immune responses of GALT, although many scientists checking this statement were focused mainly on differentiation of B cells (86, 87). At the same time, there is a growing evidence for immune functions of the bursal sac and its duct – for example, the active transfer of antigens through the duct into the lumen of bursal sac (88).

Morphology of GALT. Inner gut lining is formed by intestinal villi and crypts. The villi are covered with simple columnar epithelium with brush-like suction surface containing secretory goblet cells and many intraepithelial leukocytes. In the crypts occurs differentiation and maturation of enterocytes, goblet cells, enteroendocrine cells, and Paneth cells (the latter are still poorly studied in birds) (89, 90).

Intraepithelial lymphocytes of chickens are represented by a diverse population of cells – NK-cells (91), αβ- and γδ-T-cells (49), and heterophils (the latter were found in caecal appendices). In mammals, Paneth cells located in intestinal crypts produce lysozyme, defensins, and other antimicrobial agents (92, 93). The description of epithelial defensins of birds (including chickens) lacks the data about cells-producers (94). Since Paneth cells are quite rare in birds, antimicrobial substances are secreted probably by macrophages or heterophils and not by typical cells similar to Paneth cells of mammals (23, 95).

In both birds and mammals, the intestinal lamina propria contains all types of immune cells including plasma cells, effector T-lymphocytes and memory lymphocytes, macrophages, and granulocytes (14, 73, 96). Another morphological distinction of avian gut is underdevelopment of Peyer's patches. In the small intestine of chickens, the side opposite to the mesenteric edge demonstrates up to six Peyer's patches (54) (so-called intestinal glands; similar to those of mammals). One of them is located in the ileum (54). Lymphoid tissue of intestinal glands is submerged in the lamina propria and penetrates the submucosa; it consists of primary and secondary lymphoid follicles that contain mainly lymphocytes separated by interfollicular areas with significant amount of T-lymphocytes (51, 54, 97). Peyer's patches are covered with epithelium formed by undifferentiated enterocytes with inclusions of lymphoid cells. This lymphoepithelium mediates the contact between chyme and the gut immune system. Intestinal glands develop very quickly. New hatched chicks have no visible Peyer's patches in histological preparations which though have detectable lymphoid cells; 10 days later the patches can be seen by the naked eye and then they increase in volume up to the age of 3 months. After the 12th month of life this development is reversed by atrophic processes, and chickens retain the only Peyer's patch in the ileocaecal junction (54, 98).

So, in avian gut, lymphoid follicles are the most numerous in ceca, crodeum, proctodeum (54), and in the bursal canal. These follicles are smaller in size than Peyer's patches of mammals while a similar structure: specialized lymphoepithelium including M-cells (97, 99, 100) and follicular structures that divide and differentiate into T- and B-cells, which both are quite frequent as well. The border zones of these follicles contain all types of macrophages and effector cells (73). Consequently, in the gut lymphoepithelium of birds (similar to mammals) the main function of M-cells with small rounded apical microvilli is capturing antigens from the gut lumen, processing and transferring them across the epithelial barrier to underlying immune cells, where occurs antigen presentation and initiation of the immune response (97). M-cells are not involved in absorption and digestion as they have undeveloped brush fringe and enzymatically inactive apical part. In caecal appendices lymphoid follicles are quite frequent: so-called cecal tonsils in the proximal part of cecum, numerous lymph nodes around the appendix, and an aggregate in its apical part (101, 102, 103). The colon of birds has no lymphoid follicles, but they occur in its terminal part opening into the cloacal bursa – a lymphoid organ located in proctodeal part of the cloaca and involved in primary and secondary immune responses. Mucosa and submucosa of the bursal duct contain many lymphoid follicles (85). There are single lymphoid nodes in procdeum (hindgut) and urodeum (part of cloaca near urethral orifices) (54). The structure of the bursal duct is similar to the digestive tract: a columnar epithelium with underlying lamina propria, submucosal layer with exocrine glands, and the muscle layer promoting bursal secrets into the cloaca. Along with lymphoid follicles located in mucosa and submucosa of the bursal sac, there are quite numerous leukocytes in the intestine, as well as many intraepithelial lymphocytes and lymphoid cells in lamina propria (49, 50). So, the bursa of Fabricius is an important immune organ of birds with functions of a peripheral lymph node and a source of differentiation of B-lymphocytes.

Post-hatch development of GALT in chickens. The digestive system of a new chick undergoes successive changes immediately after hatching. There significantly increases weight, number, and length of villi, number of enterocytes, crypt depth, and number of dividing cells. GALT develops rapidly and simultaneously with development of digestive structures and processes. This lymphoid system acts in a close connection with the intestinal parenchyma.

Before hatching, only few leukocytes and lymphocytes responsible for adaptive (specific) immunity can be observed in a chick's gut. These cells represent an early wave of embryonic cell migration from the thymus and the bursal sac; later, such processes occur from the 4-days-age throughout ontogeny. Along with changes in number of lymphocytes, new chicks acquire adaptive immunity that achieves a complete functionality by the 10th-14th day of life (104). From hatching to maturation of own adaptive immune system, gut immunity of a chick depends solely on innate defenses and maternal immunoglobulins of the egg (however, molecular regulation of these processes needs further explanation). In mature immune system of birds its elements are mainly concentrated in distal part of the intestine, including cecal tonsils and the bursa of Fabricius, from where the corresponding cells move to other parts of the small intestine.

The development of GALT in chickens before and immediately post-hatch is an insufficiently studied matter; there are only few reports about studying B-lymphocytes in the bursal sac (105, 106). In post-hatch chicks, histological investigation of the intestine reveals a small number of lymphoid structures providing both innate and acquired immunity. However, more sensitive molecular techniques, e.g. assessing the expression of genes encoding receptors of B- and T-cells, reveal lymphocytes in the intestine of hatching chicks (107). Number of lymphocytes changes simultaneously with waves of migration of T-cells from the thymus (74) and emergence of peripheral B-cells (86, 108). These lymphocytes are functionally inactive due to low expression of cytokines (IL-2 and IFN-γ) at this period (107).

The major (second) migration wave of immune cells (after the 4th day of post-hatch life) coincides with development of intestinal parenchyma under a similar trend for both T- and B-cells (107). Intestinal and cecal populations of lymphocytes are almost equal in size, but in the intestine it emerges somewhat earlier. Rapidly increased expression of cytokines (IL- 2 and IFNγ, respectively, activation factor and effector factor) indicates completely mature lymphocytes; they appear at this period after stimulation by activation (107). Enhancement of lymphoid function in the intestine depends on contact with bacteria and occurs simultaneously with development of enterocytes and the villi (109). The large intestine (ceca and colon) functionally maturate earlier than the small intestine (107, 109). According to present data, there's a correlation between functional maturity of the intestine and complete development of local immune system.

Maternal antibodies are well known as quite important for prevention of diseases in post-hatch chicks (110), but their role is yet poorly studied. It was shown in chickens that maternal antibodies protect from colonization by *Campylobacter jejuni* (111), though they are less effective against intracellular parasitic bacteria such as *Sallmonella* spp. (112). Relations between the activity of innate immunity in the 1st week of life and the efficiency of defense against pathogenic enterobacteria need further investigation as well. At the same time, it was demonstrated that experimental oral infestation of 1-day-old chicks with *Salmonella* induce local chemotaxis of phagocytes and subsequent phagocytosis (113).

The development of GALT was observed in healthy chicks in the 1st week of life (114) along with studying the expression of three genes encoding proinflammatory cytokines and chemokines IL1β, IL8 and K203A. IL1β is the major mediator of inflammation in mammals and birds; it is synthesized primarily by monocytes and tissue macrophages (115, 116), as well as by other cells including enterocytes (117-120). In chickens, IL1β participates in inflammatory responses caused by bacterial infection or LPS stimulation; IL1β induces fever (high body temperature) and chemokine production (115, 116). Comparing the synthesis of IL1β mRNA immediately post-hatch and two days later, it remained almost unchanged in the colon and in ceca, while increased almost twice in the duodenum on the 2nd day of life (probably, due to the first feeding and bacterial colonization in the gut) (121, 122).

IL8 and K203 are major chemokines belonging to subfamilies, respectively, CXC (one amino acid between two N-terminal

cysteines) and CC (two N-terminal cysteines not separated by any amino acids) (123, 124). CXC chemokines are involved in activation of neutrophils: they increase chemotaxis and expression of adhesive molecules and promote adhesion to endothelial cells. They also increase exocytosis of lysosomal enzymes of angiogenesis and expression of complement receptors. CC chemokines induce migration of monocytes and other cells (e.g., NK and dendritic cells). Local production of chemotactic signals (chemokines and their receptors) regulates infiltration of leukocytes in tissues. It was shown in chickens that IL8 and K203 are involved in recruitment of mononuclear cells and development of myeloid precursors (125, 126). In different parts of the intestine expression of K203 and IL8 varies: increased expression of K203 mRNA occurs in both duodenum and colon, while IL8 mRNA – mostly in ceca and a slightly increased expression in the colon. Such different expression of chemokines in the studied regions can be explained by unequal distribution of cells providing innate immunity. For example, the abundance of heterophils in the colon is correlated with increased expression of IL8 (127, 128). It's yet unclear if IL8 is synthesized by these cells (128) or it is the product of some other cells that just attracts heterophils (128). Recently it was shown in chickens that natural bacterial colonization of ceca stimulates increased expression of IL8 and IL17 cytokines in the 1st week of life (129), which modifies their immune response and enhances resistance to *Salmonella* infection. Supposedly, varying expression of chemokine genes may be caused by innate immune mechanisms responding to individual differences in microbial population. Undoubtedly that normal microbiota may vary in both chickens (121) and mammals, and it modulates variable expression of genes in intestinal tissues (130-132).

As regards mRNA for β-defensin, its synthesis is enhanced already at hatching in all parts of the gut, and then it declines in the 1st week of life owing to the two possible reasons. Firstly, β-defensins can be produced not only (and not so much) by heterophils but also in many other tissues including the intestinal epithelium; a significant level of these antimicrobial peptides in a new hatched chick may reflect preparation of its enterocyte lining for upcoming bacterial invasion (however, expression of gallinacins in the intestinal epithelium wasn't observed). Secondly, in the intestine gallinacins bind solely to heterophils or to granulocyte granules while, according to available data, maturation of protein granules occurs during preparation of the gut to postnatal development (regardless of bacterial colonization). Therefore, both these explanations show the production of defensins as related with preparation to bacterial invasion. Increased expression of defensins in newly hatched chicks may indicate maturation of heterophils at this period proved by histological evidence. Enhanced synthesis of defensins in chicken gut after the 1st week of life probably reflects completing of maturation by immature heterophils (both migrants and ones formed in the intestine after differentiation).

The expression of *PS1* gene affecting early stages of granulopoiesis was studied in the intestine of chickens (133, 134). In mammals, it is associated with azurophil granules of polymorphonuclear leukocytes (133). In duodenum and ileum of chicks, active synthesis of *PS1* gene product occurs before and immediately post-hatch (which presumes enhanced granulopoiesis), and later it declines. In the large intestine the expression of *PS1* gradually increases during the first week of life, which may be caused by attraction of extra granulocytes under bacterial stimulation.

Consequently, some findings (114) indicate maturity of the innate immune system in the developing gut including two groups of defense mechanisms. The first group is represented by local processes of extramedullary granulopoiesis independent from food and bacteria; they are confirmed by histological evidence and observed expression of several genes (PS1, genes of β-defensins). Extramedullary changes occur before hatching in the small intestine and do not occur in the colon. The second group of mechanisms is associated with expression of pro-inflammatory cytokines and chemokines caused by the contact with feed and microbiota. This response arises immediately after hatching, it is most pronounced in the large intestine as major region of bacterial colonization. Rapid increase in expression of these genes shows the readiness of the intestinal immune system for immediate response to external stimuli. Along with it, in the first two weeks of life, in all parts of the gut (especially in ceca) increases the number of polymorphonuclear cells (114).

So, in early post-hatch period of life, a chicken experiences stimulation of innate immunity in the gut with participation of pro-inflammatory mediators, resident leucocytes, recruited blood leukocytes, and, later, lymphocytes. Chicks start eating immediately after leaving the egg shell, so their immune system is initially focused on acquiring a tolerance; however, colonization of the intestinal microbiota meets immunologically mature GALT pre-programmed for the immune response. Notably that the maintenance of antigen-specific tolerance induced in the first 4 days of life depends on re-exposure to specific antigens; without a repeated contact during 4-6 weeks, the tolerance is replaced by immune response to oral administration of this antigen. Today, it is yet unknown the source of this induced tolerance – whether it is the intestine, central or peripheral immune organs.

In contrast to food-dependant maturation of enterocytes, functional activity of GALT is stimulated by microflora. This results in earlier maturation of GALT in the hindgut compared with the small intestine whose microbial population is much smaller than in the large intestine. Practically this means that chicks should be given a feed as soon as possible after hatching, because it improves their tolerance to nutrients and provides early start of immune responses, better immune defense and development of the digestive system. Stimulation of the muscle tissue development is another important aspect discussed in the earlier authors' work (135). Of course, immature lymphoid system may be not ready for the impact of external agents harmful for birds' health; this threat is partially overcome due to innate maternal antibodies – that's why vaccination of hens is so important. Along with it, early contact of chicks with droppings stimulates development of their immunity through the contact of the hindgut with external microflora.

Modulation of gut immunity. The intestinal immune system experiences stresses as well as general immune system of an organism (57, 136, 137), and its immunomodulation obeys to the same principles as descried earlier. In particular, the central role in gut immunity belongs to prooxidant-antioxidant balance (138-140) that largely determines reliability of immune mechanisms. Maintenance of this balance is associated with vitamin E recycling system along with Zn and Mn necessary as components of the active site of superoxide dismutase, the main antioxidant enzyme. These components, along with ascorbic acid and Se, are available in the new generation antistress medication Feed-Food Magic Antistress Mix™ whose target is formation of efficient gut immunity.

Intestinal mucous membranes prevent penetration of pathogens in the gut, so it's important to ensure their integrity by an optimum osmotic balance. That's why Feed-Food Magic Antistress Mix™ contains osmotic betaine in combination with a mixture of electrolytes. Betaine is quite important for normal structure of the chicken gut, especially under stress (141, 142). This substance is osmoprotector for macrophages that enhances their chemotaxis and release of nitric oxide in response to the stress caused by coccidia (143). A broad spectrum protective action of betaine includes inhibition of inflammation, lipid peroxidation (LPO), prevention of oxidative stress in the endoplasmic reticulum, and apoptosis (144-147). Betaine also inhibits the synthesis of toll-like receptor 4 (TLR4, or CD284 – a membrane protein involved in innate immune responses) thereby providing defense under stress (148).

Intestinal mucosa is the location of quite dynamic metabolic processes, a physical and chemical barrier to foreign agents in the digestive tract; its epithelial cells mediate interaction between the immune system and various proteins in the gut lumen some of which aren't safe for an organism. Carnitine is another component of the new antistress medication; it is considered as the key factor

in regulation of these interactions, along with oxidative stress, activation of immune cells, and integrity of epithelial barriers (149). Carnitine allows better control over formation of superoxide radicals in neutrophils (150) and macrophages (151) isolated from old rats, and enhances their chemotaxis. This substance showed a strong protective effect in gastric disorders of rats (152). As found in mice deficient in specific carnitine transporters, the lack of carnitine leads to spontaneous atrophy of epithelial cells in the small intestine and colon inflammation (153). In old rats, dietary administration of carnitine for 21 days contributed to significant reduce in formation of LPO products and improved activity of antioxidant enzymes – superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. Along with these positive changes, the animals exhibited enhanced proliferation of T-cells in response to various agents, decline in rate of DNA damage and apoptosis of lymphocytes (154). In old animals, carnitine normalizes the function of neutrophils, immunoreactivity, and increases the levels of IgA and IgG (i.e. restores their immunocompetence associated with aging) (155). Dietary supplemented carnitine enhances humoral immunity of growing chicks (156) and the synthesis of acute phase proteins (157). Carnitine provides a significant positive effect in chickens under heat stress (158; watering it for the first 3 weeks of life significantly improves growth and feed consumption (159). Carnitine-supplemented diet of parental flocks positively affects the development of immunity in their offspring (160).

Vitamin A is another important component of the new antistress drug; after metabolic conversion to retinoic acid, vitamin A participates in regulation of activity of T-lymphocytes in the intestine (161, 162). Intestinal endothelial cells and dendritic cells have retinaldehyde dehydrogenase activity necessary for the conversion of vitamin A to retinoic acid. Retinoic acid plays a key role in development of immunotolerance in the gut (163), i.e. effective recognition of “friend or foe” and prevention of allergic reactions. Besides, retinoic acid is necessary for formation of intestinal dendritic cells (164).

Folic acid is important for normal functioning of regulatory T-cells. They have a strongly expressed folate receptor 4 (165), while folic acid is regarded as a limiting factor for survival of regulatory T-lymphocytes (166). An experimentally proved fact is that regulatory T-cells differentiate from naive T-cells and immediately die under the deficit of folic acid. The deficit of folic acid leads to reduce in number of regulatory T-lymphocytes in the gut.

Organic acids included in the proposed antistress medication cause a positive effect on histological structure of the small intestine (167), which, in turn, increases functional reactivity of the immune system.

First days of post-hatch life are quite important for development of the gut immunity in chickens, so providing them the new antistress drug at this period ensures better development of the gut and its immune system (135, 168). It also relives the harmful effect of feed mycotoxins seen as major stressors and suppressors of the general immune system and gut immunity (169). Watering the antistress drug reduces negative effects of mycotoxin DON (170, 171), ochratoxin (172, 173), and T-2 toxin (174, 175). Besides, the concept of nutrients affecting the activity of vitagenes and increasing adaptive capacities of the organism under stress is applicable to the gut immunity as well (176).

Thus, analysis of the scientific literature clearly shows that gut immunity plays the essential role in maintenance of health, productive and reproductive qualities of farm animals and poultry. Certainly, mechanisms of the immune tolerance to nutrients and beneficial microorganisms (“friend or foe” recognition), and immune response expressed at this level, need further exploration. At the same time, successful experiments with watering of the new developed immunomodulatory antistress medication to farm animals show significant future prospects of the proposed concept.

REFERENES

1. Klasing K.C. Nutrition and the immune system. *Br. Poult. Sci*., 2007, 48: 525-537.
2. Hampson D.J. Alterations in piglet small intestinal structure at weaning. *Res.Vet. Scie*., 1986, 40: 313-317.
3. Nabuurs M.J., Hoogendoorn A., Van der Molen E.J., Van Osta A.L. Villus height and crypt depth in weaned and unweaned pigs, reared under various circumstances in the Netherlands. *Res. Vet. Scie*., 1993, 55: 78-84.
4. Friedman A., Al-Sabbagh A., Santos L.M., Fishman-Lobell J., Polanski M., Das M.P., Khoury S.J., Weiner H.L. Oral tolerance: a biologically relevant pathway to generate peripheral tolerance against external and self antigens. *Chem. Immunol*., 1994, 58: 259-290.
5. Klipper E., Sklan D., Friedman A. Response, tolerance and ignorance following oral exposure to a single protein antigen in gallus domesticus. *Vaccine*, 2001, 19: 2890-2897.
6. Brandtzaeg P. The mucosal immune system and its integration with the mammary glands. *J. Pediatr*., 2010, 156: S8-15.
7. Deplancke B., Gaskins H.R. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. *Am. J. Clin. Nutr*., 2001, 73: 1131S-1141S.
8. Specian R.D., Oliver M.G. Functional biology of intestinal goblet cells. *Am. J. Physiol*., 1991, 260: C183-193.
9. Chadee K., Petri W.A. Jr., Innes D.J., Ravdin J.I. Rat and human colonic mucins bind to and inhibit adherence lectin of Entamoeba histolytica. *J. Clin. Invest*., 1987, 80: 1245-1254.
10. Kim Y.S., Ho S.B. Intestinal goblet cells and mucins in health and disease: recent insights and progress. *Curr. Gastroenterol. Rep*., 2010, 12: 319-330.
11. Forder R.E., Howarth G.S., Tivey D.R., Hughes R.J. Bacterial modulation of small intestinal goblet cells and mucin composition during early posthatch development of poultry. *Poult. Sci*., 2007, 86: 2396-2403.
12. Smirnov A., Sklan D., Uni Z. Mucin dynamics in the chick small intestine are altered by starvation. *J. Nutr*., 2004, 134: 736-742.
13. Horn N.L., Donkin S.S., Applegate T.J., Adeola O. Intestinal mucin dynamics: response of broiler chicks and White Pekin ducklings to dietary threonine. *Poult.Sci*., 2009, 88: 1906-1914.
14. Harmon B.G. Avian heterophils in inflammation and disease resistance. *Poult.Sci*., 1998, 77: 972-977.
15. Lillehoj H.S., Min W., Dalloul R.A. Recent progress on the cytokine regulation of intestinal immune responses to Eimeria. *Poult. Sci*., 2004, 83: 611-623.
16. Satchell D.P., Sheynis T., Shirafuji Y., Kolusheva S., Ouellette A.J., Jelinek R. Interactions of mouse Paneth cell α-defensins and α-defensin precursors with membranes. Prosegment inhibition of peptide association with biomimetic membranes. *J. Biol. Chem*., 2003, 278: 13838-13846.
17. Liu L., Zhao C., Heng H.H., Ganz T. The human β-defensin-1 and α-defensins are encoded by adjacent genes: two peptide families with differing disulfide topology share a common ancestry. *Genomics*, 1997, 43: 316-320.
18. Evans E.W., Beach G.G., Wunderlich J., Harmon B.G. Isolation of antimicrobial peptides from avian heterophils. *J. Leukoc. Biol*., 1994, 56: 661-665.
19. Harwig S.S., Swiderek K.M., Kokryakov V.N., Tan L., Lee T.D., Panyutich E.A., Aleshina G.M., Shamova O.V., Lehrer R.I. Gallinacins: cysteine-rich antimicrobial peptides of chicken leukocytes. *FEBS Lett*., 1994, 342: 281-285.
20. Zhao C., Nguyen T., Liu L., Sacco R.E., Brogden K.A., Lehrer R.I. Gallinacin-3, an inducible epithelial β-defensin in the chicken. *Infect. Immun*., 2001, 69: 2684-2691.
21. Evans E.W., Harmon B.G. A review of antimicrobial peptides: defensins and related cationic peptides. *Vet. Clin. Pathol*., 1995, 24: 109-116.
22. Evans E.W., Beach F.G., Moore K.M., Jackwood M.W., Glisson J.R., Harmon B.G. Antimicrobial activity of chicken and turkey heterophil peptides CHP1, CHP2, THP1, and THP3. *Vet. Microbiol*., 1995, 47: 295-303.
23. Sugiarto H., Yu P.-L. Avian antimicrobial peptides: the defence role of beta defensins. *Biochem. Biophys. Res. Commun*., 2004,323: 721-727.
24. Lynn D.J., Higgs R., Gaines S., Tierney J., James T., Lloyd A.T., Fares M.A., Mulcahy G., O’Farrelly C. Bioinformatic discovery and initial characterisation of nine novel antimicrobial peptide genes in the chicken.*Immunogenetics*, 2004, 56: 170-177.
25. Van Dijk A., Veldhuizen E.J., Kalkhove S.I., Tjeerdsma-van Bokhoven J.L., Romijn R.A., Haagsman H.P. The beta-defensin gallinacin-6 is expressed in the chicken digestive tract and has antimicrobial activity against food-borne pathogens. *Antimicrob Agents Chemother*., 2007, 51: 912-922.

26. Milona P., Townes C.L., Bevan R.M., Hall J. The chicken host peptides, allinacins 4, 7, and 9 have antimicrobial activity against Salmonella serovars.*Biochem. Biophys. Res. Commun*., 2007, 356: 169-174.
27. Higgs R., Lynn D.J., Gaines S., McMahon J., Tierney J.T., James T., Lloyd A.T., Mulcahy G., O'Farrelly C. The synthetic form of a novel chicken beta-defensin identified in silico is predominantly active against intestinal pathogens.*Immunogenetics*, 2005, 57: 90-98.
28. Xiao Y., Hughes A.L., Ando J., Matsuda Y., Cheng J.-F., Skinner-Noble D., Zhang G. A genome-wide screen identifies a single β-defensin gene cluster in the chicken: implications for the origin and evolution of mammalian defensins. *BMC Genomics*, 2004, 5: 56.
29. Hong Y.H., Song W., Lee S.H., Lillehoj H.S. Differential gene expression profiles of β-defensins in the crop, intestine, and spleen using a necrotic enteritis model in 2 commercial broiler chicken lines. *Poult. Sci*., 2012, 91: 1081-1088.
30. Ramanathan B., Davis E.G., Ross C.R., Blecha F. Cathelicidins: microbicidal activity, mechanisms of action, and roles in innate immunity. *Microbes Infect*., 2002, 4: 361-372.
31. Zaiou M., Gallo R.L. Cathelicidins, essential gene-encoded mammalian antibiotics. *J. Mol. Med*., 2002, 80: 549-561.
32. Giacometti A., Cirioni O., Ghiselli R., Mocchegiani F., D'Amato G., Circo R., Orlando F., Skerlavaj B., Silvestri C., Saba V., Zanetti M., Scalise G. Cathelicidin peptide sheep myeloid antimicrobial peptide 29 prevents endotoxin-induced mortality in rat models of septic shock. *Am. J. Respir. Crit. Care Med*., 2004, 169: 187-194.
33. Yang D., Chen Q., Schmidt A.P., Anderson G.M., Wang J.M., Wooters J., Oppenheim J.J., Chertov O. LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. *J. Exp. Med*., 2000, 192: 1069-1074.
34. Skerlavaj B., Scocchi M., Gennaro R., Risso A., Zanetti M. Structural and functional analysis of horse cathelicidin peptides. *Antimicrob. Agents Chemother*., 2001, 45: 715-722.
35. Van Dijk A., Veldhuizen E.J., Van Asten A.J., Haagsman H.P. CMAP27, a novel chicken cathelicidin-like antimicrobial protein. *Vet. Immunol. Immunopathol*., 2005, 106: 321-327.
36. Van Dijk A., Tersteeg-Zijderveld M.H., Tjeerdsma-van Bokhoven J.L., Jansman A.J., Veldhuizen E.J., Haagsman H.P. Chicken heterophils are recruited to the site of Salmonella infection and release antibacterial mature Cathelicidin-2 upon stimulation with LPS. *Mol. Immunol*., 2009, 46: 1517-1526.
37. Van Dijk A., Molhoek E.M., Bikker F.J., Yu P.L., Veldhuizen E.J., Haagsman H.P. Avian cathelicidins: paradigms for the development of anti-infectives. *Vet. Microbiol*., 2011, 153: 27-36.
38. Sahoo N.R., Kumar P., Bhusan B., Bhattacharya T.K., Dayal S., Sahoo M. Lysozyme in livestock: a guide to selection for disease resistance: a review. *J. Anim. Sci. Adv*., 2012, 2: 347-360.
39. Myers F.A., Lefevre P., Mantouvalou E., Bruce K., Lacroix C. Developmental activation of the lysozyme gene in chicken macrophage cells is linked to core histone acetylation at its enhancer elements. *Nucleic Acids Res*., 2006, 34: 4025-4035.
40. Callewaert L., Michiels C.W. Lysozymes in the animal kingdom. *J. Biosci*.,2010, 35: 127-160.
41. Lesnierowski G., Cegielska-Radziejewska R. Potential possibilities of production, modifi cation and practical application of lysozyme. *Acta Sci. Pol., Technol. Aliment*., 2012, 11: 223-230.
42. Sanderson I.R., Walker W.A. *Mucosal barrier: an overview*. London, 1999: 5-18.
43. Mestecky J., Moro I., Underdown B.J. Mucosal immunoglobulins. In: *Mucosal Immunology* /P.L. Ogra, J. Mestecky, M.E. Lamm, W. Strober, J. Bienenstock, J.R. McGhee (eds.). London, Academic Press, 1999: 133-152.
44. Klipper E., Sklan D., Friedman A. Immune response of chickens to dietary protein antigens. *Vet. Immunol. Immunopathol*., 2000, 74: 209-223.
45. Fagerland J.A., Arp L.H. Distribution and quantitation of plasma cells, T lymphocyte subsets, and B lymphocytes in bronchus-associated lymphoid tissue of chickens: age-related differences. *Reg. Immunoly*, 1993, 5: 28-36.
46. Sharma J.M. Overview of the avian immune system. *Vet. Immunol. Immunopathol*., 1991, 30: 13-17.
47. Muir W.I., Bryden W.L., Husband A.J. Investigation of the site of precursors for IgA-producing cells in the chicken intestine. *Immunol. Cell Biol*., 2000, 78: 294-296.
48. McGhee J.R., Mestecky J., Dertzbaugh M.T., Eldridge J.H., Hirasawa M., Kiyono H. The mucosal immune system: from fundamental concepts to vaccine development. *Vaccine*, 1992, 10: 75-88.
49. Lillehoj H.S., Chung K.S. Postnatal development of T-lymphocyte subpopulations in the intestinal intraepithelium and lamina propria in chickens.*Vet. Immunol. Immunopathol*., 1992, 31: 347-360.
50. Vervelde L., Jeurissen S.H. Postnatal development of intra-epithelial leukocytes in the chicken digestive tract: phenotypical characterization in situ.*Cell Tiss. Res*., 1993, 274: 295-301.
51. Olah I., Nagy N., Magyar A., Palya V. Esophageal tonsil: a novel gut-associated lymphoid organ. *Poult. Sci*., 2003, 82: 767-770.
52. Matsumoto R., Hashimoto Y. Distribution and developmental change of lymphoid tissues in the chicken proventriculus. *J. Vet. Med. Sci*., 2000, 62: 161-167.
53. Kajiwara E., Shigeta A., Horiuchi H., Matsuda H., Furusawa S. Development of Peyer's patch and cecal tonsil in gut-associated lymphoid tissues in the chicken embryo. *J. Vet. Med. Sci*., 2003, 65: 607-614.
54. Befus A.D., Johnston N., Leslie G.A., Bienenstock J. Gut associated lymphoid tissue in the chicken. I. Morphology, ontogeny, and some functional characteristics of Peyer's patches. *J. Immunol*., 1980, 125: 2626-2632.
55. Olah I., Glick B., Taylor R.L. Jr. Meckel's diverticulum. II. A novel lymphoepithelial organ in the chicken. *Anat. Rec*., 1984, 208: 253-263.
56. Nagy N., Olah I. Pyloric tonsil as a novel gut-associated lymphoepithelial organ of the chicken. *J. Anat.,* 2007,211: 407-411.
57. Fisinin V.I., Surai P.F. *Zhivotnovodstvo segodnya*, 2011, 9: 40-47.
58. Lillehoj H.S., Trout J.M. Avian gut-associated lymphoid tissues and intestinal immune responses to *Eimeria* parasites. *Clinic. Microbiol. Rev*., 1996, 9: 349-360.
59. Yasuda M., Tanaka S., Arakawa H., Taura Y., Yokomizo Y., Ekino S. A comparative study of gut-associated lymphoid tissue in calf and chicken.*Anatom. Rec*., 2002, 266: 207-217.
60. Liebler-Tenorio E.M., Pabst R. MALT structure and function in farm animals.*Vet. Res*., 2006, 37: 257-280.
6. Garboczi D.N., Ghosh P., Utz U., Fan Q.R., Biddison W.E., Wiley D.C. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. *Nature*, 1996, 384: 134-141.
62. Garboczi D.N., Biddison W.E. Shapes of MHC restriction. *Immunity*, 1999, 10: 1-7.
63. Parham P. Pictures of MHC restriction. *Nature,* 1996, 384: 109-110.
64. Garcia K.C., Teyton L., Wilson I.A. Structural basis of T cell recognition.*Annu. Rev. Immunol*., 1999, 17: 369-397.
65. Morita C.T., Beckman E.M., Bukowski J.F., Tanaka Y., Band H., Bloom B.R., Golan D.E., Brenner M.B. Direct presentation of nonpeptide prenyl pyrophosphate antigens to human gd T cells. *Immunity*, 1995, 3: 495-507.
66. Morita C.T., Tanaka Y., Bloom B.R., Brenner M.B. Direct presentation of non-peptide prenyl pyrophosphate antigens to human gd T cells. *Res. Immunol*., 1996, 147: 347-353.
67. Morita C.T., Lee H.K., Leslie D.S., Tanaka Y., Bukowski J.F., Marker- Hermann E. Recognition of nonpeptide prenyl pyrophosphate antigens by human gd T cells. *Microbes Infect*., 1999, 1: 175-186.
68. Morita C.T., Mariuzza R.A., Brenner M.B. Antigen recognition by human gd T cells: pattern recognition by the adaptive immune system. *Springer Semin. Immunopathol*., 2000, 22: 191-217.
69. Harrington L.E., Hatton R.D., Mangan P.R., Turner H., Murphy T.L., Murphy K.M., Weaver C.T. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol*., 2005, 6: 1123-1132.
70. Ivanov I.I., McKenzie B.S., Zhou L., Tadokoro C.E., Lepelley A., Lafaille J.J., Cua D.J., Littman D.R. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell*, 2006, 126: 1121-1133.
71. Weaver C.T., Hatton R.D., Mangan P.R., Harrington L.E. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu. Rev. Immunol*., 2007, 25: 821-852.
72. Bucy R.P., Chen C.L., Cihak J., Losch U., Cooper M.D. Avian T cells expressing gamma delta receptors localize in the splenic sinusoids and the intestinal epithelium. *J. Immunol.*, 1988, 141: 2200-2205.
73. Lillehoj H.S. Avian gut-associated immune system: implication in coccidial vaccine development. *Poult. Sci*., 1993, 72: 1306-1311.
74. Dunon D., Courtois D., Vainio O., Six A., Chen C.H., Cooper M.D., Dangy J.P., Imhof B.A. Ontogeny of the immune system: gamma/delta and alpha/beta T cells migrate from thymus to the periphery in alternating waves. *J. Exp. Med*., 1997, 186: 977-988.
75. Dunon D., Cooper M.D., Imhof B.A. Thymic origin of embryonic intestinal gamma/delta T cells. *J. Exp. Med*., 1993, 177: 257-263.
76. Suzuki K., Oida T., Hamada H., Hitotsumatsu O., Watanabe M., Hibi T., Yamamoto H., Kubota E., Kaminogawa S., Ishikawa H. Gut cryptopatches: direct

evidence of extrathymic anatomical sites for intestinal T lymphopoiesis.*Immunity,* 2000, 13: 691-702.
77. Arstila T.P., Toivanen P., Lassila O. Helper activity of CD4+ alpha beta T cells is required for the avian gamma delta T cell response. *Eur. J. Immunol*., 1993, 23: 2034-2037.
78. Kasahara Y., Chen C.H., Cooper M.D. Growth requirements for avian gamma delta T cells include exogenous cytokines, receptor ligation and in vivo priming.*Eur. J. Immunol*., 1993, 23: 2230-2236.
79. Berin M.C., McKay D.M., Perdue M.H. Immune-epithelial interactions in host defense. *Am. J. Trop. Med. Hyg*., 1999, 60: 16-25.
80. Berin M.C., Yang P.C., Ciok L., Waserman S., Perdue M.H. Role for IL-4 in macromolecular transport across human intestinal epithelium. *Am. J. Physiol*., 1999, 276: C1046-1052.
81. Mehr R., Edelman H., Sehgal D.R.M. Analysis of mutational lineage trees from sites of primary and secondary Ig gene diversification in rabbits and chickens. *J. Immunol*., 2004, 172: 4790-4796.
82. Clench M.H. The avian cecum: uptade and motility review. *J. Exp. Zool*., 1999, 283: 441-447.
83. Brummermann M., Braun E.J. Effect of salt and water balance on colonic motility of white leghorn roosters. *Am. J. Physiol*., 1995, 268: R690-698.
84. Lai H.C., Duke G.E. Colonic motility in domestic turkeys. *Am. J. Dig. Dis*., 1978, 23: 673-681.
85. Friedman A., Bar Shira E., Sklan D. Ontogeny of gut associated immune competence in the chick. *World's Poultry Sci. J*., 2003, 59: 209-219.
86. Yamamoto H., Watanabe H., Mikami T. Distribution of immunoglobulin and secretory component containing cells in chickens. *Am. J. Vet. Res*., 1977, 38: 1227-1230.
87. Mansikka A., Veromaa T., Vainio O., Toivanen P. B-cell differentiation in the chicken: expression of immunoglobulin genes in the bursal and peripheral lymphocytes. *Scan. J. Immunol*., 1989, 29: 325-331.
88. Sorvari R., Sorvari T.E. Bursal fabricii as a peripheral lymphoid organ. Transport of various materials from the anal lips to the bursal lymphoid follicles with reference to its immunological importance. *Immunology*, 1978, 32: 499-505.
89. Porter E.M., Bevins C.L., Ghosh D., Ganz T. The multifaceted Paneth cell.*Cell. Mol. Life Sci.*, 2002, 59: 156-170.
90. Nile C.J., Townes C.L., Michailidis G., Hirst B.H., Hall J. Identification of chicken lysozyme g2 and its expression in the intestine. *Cell. Mol. Life* Sci., 2004, 61: 2760-2766.
91. Gobel T.W., Kaspers B., Stangassinger M. NK and T cells constitute two major, functionally distinct intestinal epithelial lymphocyte subsets in the chicken. *Int. Immunol*., 2001, 13: 757-762.
92. Lehrer R.I., Ganz T. Endogenous vertebrate antibiotics. Defensins, protegrins, and other cysteine-rich antimicrobial peptides. *Ann. NY Acad. Sci*., 1996, 797: 228-239.
93. Lehrer R.I., Ganz T. Defensins of vertebrate animals. *Curr. Opin. Immunol*., 2002, 14: 96-102.
94. Bezuidenhout A.J., Van Aswegen G. A light microscopic and immunocytochemical study of the gastrointestinal tract of the ostrich (*Struthio camelus* L.). *Onderstepoort J. Vet. Res*., 1990, 57: 37-48.
95. Brockus C.W., Jackwood M.W., Harmon B.G. Characterization of beta-defensin prepropeptide mRNA from chicken and turkey bone marrow. *Animal Gen*., 1998, 29: 283-289.
96. Lillehoj H.S., Min W., Dalloul R.A. Recent progress on the cytokine regulation of intestinal immune responses to *Eimeria*. *Poult. Sci*., 2004, 83: 611-623.
97. Burns R.B., Maxwell M.H. Ultrastructure of Peyer's patches in the domestic fowl and turkey. *J. Anat*., 1986, 147: 235-243.
98. Casteleyn C., Doom M., Lambrechts E., Van den Broeck W., Simoens P., Cornillie P. Locations of gut-associated lymphoid tissue in the 3-month-old chicken: a review. *Avian. Pathol*., 2010, 39: 143-150.
99. Gallego M., Del Cacho E., Zapata A., Bascuas J.A. Ultrastructural identification of the splenic follicular dendritic cells in the chicken. *Anat. Rec*., 1995, 242: 220-224.
100. Jeurissen S.H., Wagenaar F., Janse E.M. Further characterization of M cells in gut-associated lymphoid tissues of the chicken. *Poult. Sci*., 1999, 78: 965-729.
101. Kitagawa H., Imagawa T., Uehara M. The apical caecal diverticulum of the chicken identified as a lymphoid organ. *J. Anat*., 1996, 189: 667-672.
102. Kitagawa H., Hiratsuka Y., Imagawa T., Uehara M. Distribution of lymphoid tissue in the caecal mucosa of chickens. *J. Anat*., 1998, 192(Pt 2): 293-298.
103. Del Cacho E., Gallego M., Sanz A., Zapata A. Characterization of distal lymphoid nodules in the chicken caecum. *Anat. Rec*., 1993, 237: 512-517.
104. Friedman A., Elad O., Cohen I., Bar Shira E. The gut associated lymphoid system in the post-hatch chick: dynamics of maternal IgA. *Israel J. Vet. Med*., 2012, 67: 75-81.
105. Masteller E.L., Thompson C.B. B cell development in the chicken. *Poult. Sci*., 1994, 73: 998-1011.
106. Masteller E.L., Pharr G.T., Funk P.E., Thompson C.B. Avian B cell development. *Int. Rev. Immunol*., 1997, 15: 185-206.
107. Bar-Shira E., Sklan D., Friedman A. Establishment of immune competence in the avian GALT during the immediate post-hatch period. *Develop. Comp. Immunol*., 2003, 27: 147-57.
108. Lawrence E.C., Arnaud-Battandier F., Grayson J., Koski I.R., Dooley N.J., Muchmore A.V., Blaese R.M. Ontogeny of humoral immune function in normal chickens: a comparison of immunoglobulin-secreting cells in bone marrow, spleen, lungs and intestine. *Clin. Exp. Immunol*., 1981, 43: 450-457.
109. Bar-Shira E., Sklan D., Friedman A. Impaired immune responses in broiler hatchling hindgut following delayed access to feed. *Vet. Immunol. Immunopathol*., 2005, 105: 33-45.
110. Malkinson M. The transmission of passive immunity to *Escherichia coli* from mother to young in the domestic fowl (*Gallus domesticus*). *Immunology*, 1965, 9: 311-317.
111. Sahin O., Zhang Q., Meitzler J.C., Harr B.S., Morishita T.Y., Mohan R. Prevalence, antigenic specificity, and bactericidal activity of poultry anti-*Campylobacter* maternal antibodies. *Appl. Environ. Microbiol*., 2001, 67: 3951-3957.
112. Popiel I., Turnbull P.C. Passage of *Salmonella enteritidis* and *Salmonella thompson* through chick ileocecal mucosa. *Infect. Immun*., 1985, 47: 786-792.
113. Henderson S.C., Bounous D.I., Lee M.D. Early events in the pathogenesis of avian salmonellosis. *Infect. Immun*., 1999, 67: 3580-3586.
114. Bar-Shira E., Friedman A. Development and adaptations of innate immunity in the gastrointestinal tract of the newly hatched chick. *Dev. Comp. Immunol.*, 2006*,* 30: 930-941.
115. Staeheli P., Puehler F., Schneider K., Gobel T.W., Kaspers B. Cytokines of birds: conserved functions — a largely different look. *J. Interferon Cyt. Res*., 2001, 21: 993-1010.
116. Kaiser P., Poh T.Y., Rothwell L., Avery S., Balu S., Pathania U.S., Hughes S., Goodchild M., Morrell S., Watson M., Bumstead N., Kaufman J., Young J.R. A genomic analysis of chicken cytokines and chemokines. *J. Interferon Cytokine Res*., 2005, 25: 467-484.
117. Bird S., Zou J., Wang T., Munday B., Cunningham C., Secombes C.J. Evolution of interleukin-1[beta]. *Cyt. Growth Factor Rev*., 2002, 13: 483-502.
118. Ogle C.K., Mao J.X., Wu J.Z., Ogle J.D., Alexander J.W. The production of tumor necrosis factor, interleukin-1, interleukin-6, and prostaglandin E2 by isolated enterocytes and gut macrophages: effect of lipopolysaccharide and thermal injury. *J. Burn Care Rehab*., 1994, 15: 470-477.
119. Radema S.A., Van Deventer S.J., Cerami A. Interleukin 1 beta is expressed predominantly by enterocytes in experimental colitis. *Gastroenterol*., 1991, 100: 1180-1186.
120. Wigley P., Kaiser P. Avian cytokines in health and disease. *Brazilian J.Poult. Sci*., 2003, 5: 1-14.
121. Coloe P.J., Bagust T.J., Ireland L. Development of the normal gastrointestinal microflora of specific pathogen-free chickens. *J. Hyg*., 1984, 92: 79-87.
122. Barnes E.M., Impey C.S., Cooper D.M. Manipulation of the crop and intestinal flora of the newly hatched chick. *Am. J. Clin. Nutr*., 1980, 33: 2426-2433.
123. Sick C., Schneider K., Staeheli P., Weining K.C. Novel chicken CXC and CC chemokines. *Cytokine*, 2000, 12: 181-186.
124. Kaiser P., Hughes S., Bumstead N. The chicken 9E3/CEF4 CXC chemokine is the avian orthologue of IL8 and maps to chicken chromosome 4 syntenic with genes flanking the mammalian chemokine cluster. *Immunogenetics*, 1999, 49: 673-684.
125. Martins-Green M. The chicken chemotactic and angiogenic factor (cCAF), a CXC chemokine. *Int. J. Biochem. Cell Biol*., 2001, 33: 427-432.
126. Petrenko O., Ischenko I., Enrietto P.J. Isolation of a cDNA encoding a novel chicken chemokine homologous to mammalian macrophage inflammatory protein-1[beta]. *Gene*, 1995, 160: 305-306.
127. Kogut M.H. Dynamics of a protective avian inflammatory response: the role of an IL-8-like cytokine in the recruitment of heterophils to the site of organ invasion by *Salmonella enteridis*. *Comp. Immunol. Microbiol. Infect. Dis*., 2002, 25: 159-172.
128. Kogut M.H., Rothwell L., Kaiser P. Priming by recombinant chicken IL-2 induces selective expression of IL-8 and IL-18 mRNA in chicken heterophils during receptor-mediated phagocytosis of opsonized and nonopsonized*Salmonella enterica* serovar enteridis. *Mol. Immunol*., 2003, 40: 603-610.
129. Crhanova M., Hradecka H., Faldynova M., Matulova M., Havlickova H., Sisak F., Rychlik I. Immune response of chicken gut to natural colonization by gut microflora and to *Salmonella enterica* serovar *enteritidis* infection. *Infect. Immun*., 2011, 79: 2755-2763.
130. Lan J.G., Cruickshank S.M., Singh J.C., Farrar M., Lodge J.P., Felsburg P.J., Carding S.R. Different cytokine response of primary colonic epithelial cells to

commensal bacteria. *World J. Gastroenterol*., 2005, 11: 3375-3384.
131. Hooper L.V., Midtvedt T., Gordon J.I. How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu. Rev. Nutr*., 2002, 22: 283-307.
132. Hooper L.V., Wong M.H., Thelin A., Hansson L., Falk P.G., Gordon J.I. Molecular analysis of commensal host—microbial relationships in the intestine.*Science*, 2001, 291: 881-884.
133. Mirinics Z.K., Calafat J., Udby L., Lovelock J., Kjeldsen L., Rothermund K., Sisodia S.S., Borregaard N., Corey S.J. Identification of the presenilins in hematopoietic cells with localization of presenilin 1 to neutrophil and platelet granules. *Blood Cells Mol. Dis*., 2002, 28: 28-38.
134. Fraering P.C., Ye W., Strub J.M., Dolios G., LaVoie M.J., Ostaszewski B.L., Van Dorsselaer A., Wang R., Selkoe D.J., Wolfe M.S. Purification and characterization of the human gamma-secretase complex. *Biochemistry*, 2004, 43: 9774-9789.
135. Fisinin V.I., Surai P.F. *Ptitsevodstvo*, 2012, 3: 9-12.
136. Fisinin V.I., Surai P.F. *Ptitsevodstvo*, 2013 (v pechati).
137. Surai P.F., Fotina T.I. *Suchasna Veterinarna Meditsina*, 2012, 6: 14-19.
138. Surai K.P., Surai P.F., Speake B.K., Sparks N.H.C. Antioxidant-prooxidant balance in the intestine: Food for thought. 1. Prooxidants. *Nutritional Genomics & Functional Foods*, 2003, 1: 51-70.
139. Surai K.P., Surai P.F., Speake B.K., Sparks N.H.C. Antioxidant-prooxidant balance in the intestine: Food for thought. 1. Antioxidants. *Current Topics in Nutraceutical Research*, 2004, 2: 27-46.
140. Surai P.F. *Selenium in nutrition and health*. Nottingham, UK, 2006.
141. Kettunen H., Peuranen S., Tiihonen K. Betaine aids in the osmoregulation of duodenal epithelium of broiler chicks and affects the movement of water across the small intestinal epithelium in vitro. *Comp. Biochem. Physiol. A: Mol.Integr. Physiol*., 2001, 129: 595-603.
142. Kettunen H., Tiihonen K., Peuranen S., Saarinen M.T., Remus J.C. Dietary betaine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidia-infected broiler chicks.*Comp. Biochem. Physiol. A: Mol. Integr. Physiol*., 2001, 130: 759-769.
143. Klasing K.C., Adler K.L., Remus J.C., Calvert C.C. Dietary betaine increases intraepithelial lymphocytes in the duodenum of coccidia-infected chicks and increases functional properties of phagocytes. *J. Nutrit*., 2002, 132: 2274-2282.
144. Ji C., Shinohara M., Vance D., Than T.A., Ookhtens M., Chan C., Kaplowitz N. Effect of transgenic extrahepatic expression of betaine-homocysteine methyltransferase on alcohol or homocysteine-induced fatty ver. *Alcohol. Clin. Exp. Res*., 2008, 32: 1049-1058.
145. Ji C., Kaplowitz N. Betaine decreases hyperhomocysteinemia, endoplasmic reticulum stress, and liver injury in alcohol-fed mice. *Gastroenterology*, 2003, 124: 1488-1499.
146. Samara K., Liu C., Soldevila-Pico C., Nelson D.R., Abdelmalek M.F. Betaine resolves severe alcohol-induced hepatitis and steatosis following liver transplantation. *Dig. Dis. Sci*., 2006, 51: 1226-1229.
147. Kharbanda K.K., Mailliard M.E., Baldwin C.R., Sorrell M.F., Tuma D.J. Accumulation of proteins bearing atypical isoaspartyl residues in livers of alcohol-fed rats is prevented by betaine administration: effects on protein-L-isoaspartyl methyltransferase activity. *J. Hepatol*., 2007, 46: 1119-1125.
148. Shi Q.-Z., Wang L.-W., Zhang W., Gong Z.-J. Betaine inhibits Toll-like receptor 4 expression in rats with ethanol-induced liver injury. *World J. Gastroenterol*., 2010, 16: 897-903.
149. Fortin G. L-Carnitine and intestinal inflammation. *Vitam. Horm*., 2011, 86: 353-366.
150. Izgüt-Uysal V.N., Agaç A., Karadogan I., Derin N. Effects of L-carnitine on neutrophil functions in aged rats. *Mech. Ageing. Dev*., 2003, 124: 341-347.
151. Izgüt-Uysal V.N., Agaç A., Karadogan I., Derin N. Peritoneal macrophages function modulation by L-carnitine in aging rats. *Aging. Clin. Exp. Res*., 2004, 16: 337-341.
152. Derin N., Agaç A., Bayram Z., Asar M., Izgüt-Uysal V.N. Effects of L-carnitine on neutrophil-mediated ischemia-reperfusion injury in rat stomach. *Cell Biochem. Funct*., 2006, 24: 437-442.
153. Shekhawat P.S., Srinivas S.R., Matern D., Bennett M.J., Boriack R., George V., Xu H., Prasad P.D., Roon P., Ganapathy V. Spontaneous development of intestinal and colonic atrophy and inflammation in the carnitine-deficient jvs (OCTN2(−/−)) mice. *Mol. Genet. Metab*., 2007, 92: 315-324.
154. Thangasamy T., Jeyakumar P., Sittadjody S., Joyee A.G., Chinnakannu P. L-carnitine mediates protection against DNA damage in lymphocytes of aged rats. *Biogerontology*, 2009, 10: 163-172.
155. Thangasamy T., Subathra M., Sittadjody S., Jeyakumar P., Joyee A.G., Mendoza E., Chinnakkanu P. Role of L-carnitine in the modulation of immune response in aged rats. *Clin. Chim. Acta*, 2008, 389: 19-24.
156. Deng K., Wong C.W., Nolan J.V. Long-term effects of early-life dietary L-carnitine on lymphoid organs and immune responses in Leghorn-type chickens.*J. Anim. Physiol. Anim. Nutr. (Berl.)*, 2006, 90: 81-86.
157. Buyse J., Swennen Q., Niewold T.A., Klasing K.C., Janssens G.P., Baumgartner M., Goddeeris B.M. Dietary L-carnitine supplementation enhances the lipopolysaccharide-induced acute phase protein response in broiler chickens.*Vet. Immunol. Immunopathol*., 2007, 118: 154-159.
158. Celik L., Oztürkcan O. Effects of dietary supplemental L-carnitine and ascorbic acid on performance, carcass composition and plasma L-carnitine concentration of broiler chicks reared under different temperature. *Arch. Tierernahr*., 2003, 57: 27-38.
159. Celik L., Oztürkcan O., Inal T.C., Canacankatan N., Kayrin L. Effects of L-carnitine and niacin supplied by drinking water on fattening performance, carcass quality and plasma L-carnitine concentration of broiler chicks. *Arch.Tierernahr*., 2003, 57: 127-136.
160. Kheirkhah A.R., Rahimi S., Torshizi M.A.K., Malekmohamadi H. Effect of different levels of L-carnitine supplementation in broiler breeders and their progeny's diets on performance, blood factors, carcass characteristics and immune system of broilers. *J. Vet. Res*., 2009, 64: 283-289.
161. Mucida D., Park Y., Cheroutre H. From the diet to the nucleus: vitamin A and TGF-b join efforts at the mucosal interface of the intestine. *Semin. Immunol*., 2009, 21: 14-21.
162. Iwata M., Hirakiyama A., Eshima Y., Kagechika H., Kato C., Song S.Y. Retinoic acid imprints gut-homing specificity on T cells. *Immunity*, 2004, 21: 527-538.
163. Cassani B., Villablanca E.J., De Calisto J., Wang S., Mora J.R. Vitamin A and immune regulation: role of retinoic acid in gut-associated dendritic cell education, immune protection and tolerance. *Mol. Aspects Med*., 2012, 33: 63-76.
164. Feng T., Cong Y., Qin H., Benveniste E.N., Elson C.O. Generation of mucosal dendritic cells from bone marrow reveals a critical role of retinoic acid.*J. Immunol*., 2010, 185: 5915-5925.
165. Yamaguchi T., Hirota K., Nagahama K., Ohkawa K., Takahashi T., Nomura T., Sakaguchi S. Control of immune responses by antigen-specific regulatory T cells expressing the folate receptor. *Immunity*, 2007, 27: 145-159.
166. Kunisawa J., Hashimoto E., Ishikawa I., Kiyono H. A pivotal role of vitamin B9 in the maintenance of regulatory T cells in vitro and in vivo. *PLoS One*, 2012, 7(2), E32094.
167. Lallès J.P., Bosi P., Janczyk P., Koopmans S.J., Torrallardona D. Impact of bioactive substances on the gastrointestinal tract and performance of weaned piglets: a review. *Animal*, 2009, 3: 1625-1643.
168. Fisinin V.I., Surai P.F. *Ptitsevodstvo*, 2012, 2: 11-15.
169. Surai P.F., Dvorska J.E. Effects of mycotoxins on antioxidant status and immunity. In: *The Mycotoxins Blue Book* /D.E. Diaz (ed.). Nottingham University Press, 2005: 93-137.
170. Fisinin V.I., Surai P.F. *Zhivotnovodstvo Rossii*,2012, 5: 11-14.
171. Fisinin V.I., Surai P.F. *Zhivotnovodstvo Rossii*,2012, 6: 3-5.
172. Fisinin V.I., Surai P.F. *Kombikorma*, 2012, 5: 59-60.
173. Fisinin V.I., Surai P.F. *Kombikorma*, 2012, 5: 59-60.
174. Fisinin V.I., Surai P.F. *Ptitsa i ptitseprodukty,* 2012, 3: 38-41.
175. Fisinin V.I., Surai P.F. *Ptitsa i ptitseprodukty*, 2012, 4: 36-39.
176. Surai P.F., Fisinin V.I. *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2012, 4: 3-13.