AGRICULTURAL BIOLOGY,
2019, V. 54, № 2, pp. 269-279
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.5:591.3:57.044

doi: 10.15389/agrobiology.2019.2.269eng
doi: 10.15389/agrobiology.2019.2.269rus

HISTOBIOCHEMICAL ASPECTS OF THE EFFECT OF A COMBINATION OF SOME NATURAL METABOLITES ON GENERAL RESISTANCE IN EGG CHICKS

I.S. LUGOVAYA, T.O. AZARNOVA, I.I. KOCHISH, S.YU. ZAITSEV, M.S. NAIDENSKY, A.A. ANTIPOV

Skryabin Moscow State Academy of Veterinary Medicine and Biotechnology, 23, ul. Akademika K.I. Skryabina, Moscow, 109472 Russia, e-mail ine98@ya.ru (✉ corresponding author), azarena@list.ru, zoo-kafedra@yandex.ru, szaitsev@mail.ru, axis83@mail.ru

ORCID:
Lugovaya I.S. orcid.org/0000-0002-9925-7070
Azarnova T.O. orcid.org/0000-0001-8760-7603
Kochish I.I. orcid.org/0000-0001-8892-9858
Zaitsev S.Yu. orcid.org/0000-0003-1533-8680
Naidensky M.S. orcid.org/0000-0001-8565-1619
Antipov A.A. orcid.org/0000-0002-9667-7029

The authors declare no conflict of interests
Received January 22, 2018

Abstract

Industrial incubation of eggs accompanied by various stresses often leads to early embryonic death. For this reason, the ecologically safe methods to prevent negative stressful effects in embryogenesis are still important. The purpose of this work was to study the effect of natural metabolites on the histological structure of the Bursa fabricii and general resistance of chickens. Studies were performed on eggs of egg cross Shaver 2000 chickens (*Gallus gallus*) (Ptichnoe Enterprise, Moscow Province, 2011). 3-4 hours before the eggs were put into the incubator, their shells were treated with an aqueous solution of ethanolamine (colamine), succinic acid and serine (0.1; 0.1 and 0.2 %, respectively) using a spray gun, and on day 19 of incubation 0.1 % aqueous colamine solution was applied. The preparations were pre-dissolved in distilled water at 18-22 °C. The untreated eggs serve as control. Each batch included 544 eggs. The recorded indicators were hatching rates, hatchability and incubation losses, also blood samples were collected to determine the content of immunoglobulins, lysozyme activity and bactericidal activity. Bursa fabricii was collected for histological analysis ($n = 5$) from chickens aged one day. Transovarian treatment of eggs with a complex of biologically active substances containing ethanolamine, succinic acid and serine has positively influenced the viability of embryos and young hens due to leveling of oxidative stress reactions and stimulation of the central components of resistance, i.e. the immune response and non-specific defense of chicks aged one day. Ethanolamine turns into phosphatidylcholine and phosphatidylethanolamine, and serine is metabolized into phosphatidylserine, which are able to neutralize the destruction of cell membranes inevitably arising under free-radical oxidation. Succinic acid, an energy substrate and an antihypoxant, contributes to prevention of the lack of energy (the lack of ATP) in the body, which occurs during critical periods of chick embryogenesis. Applied biologically active substances can optimize the embryo's living conditions during incubation accompanied by technological stresses. The higher viability of chicks of the test group resulted in a decrease of all incubation loss categories with an increase in the chick hatching values and egg hatchability. The content of immunoglobulins responsible for the primary and secondary immune response also increased, which is a demonstration of the immunomodulatory action of the composition used. Immunoglobulin E level remained unchanged, which indicates the absence of hypersensitivity reactions. Ethanolamine, succinic acid and serine also promoted the activation of non-specific defense factors, i.e. blood bactericidal activity and lysozyme, which increased in the test group of chicks too. We also revealed that the composition of biologically active substances positively influence the histoarchitecture of the bursal sac in chicks of the test group, which was confirmed by our histological data. In this, signs of physiological intracellular hyperplasia, as well as the absence of involution processes in the organ have been noted. As the aftereffect of the composition use, the viability of young chicks during the first 60 days of growing increased due to the reduction in mortality. Thus, ethanolamine, succinic acid and serine can prevent oxidative stress and also have a positive effect on general resistance in egg chicks.

Keywords: chicks, embryogenesis, antioxidants, lipoperoxidation, active immunity, bursal sac, chick hatching, ethanolamine, succinic acid, serine

High production in poultry, including the hatching rate, is achievable only at adequate, timely formation of antioxidant systems and resistance in birds [1]. Uneven heating of eggs in the batch, egg candling, the absence of air ionization, bioacoustics, possible failures in the operation of incubators and other technological stresses prevent the full formation of embryos and lead to mass early embryo death or suppressed, weak young chicks [2-4]. Many papers prove that the biological indicators are affected by the content of gases [5-7], temperature [8, 9], and humidity in the incubator [10].

A developing and growing body is much more sensitive to the effects of environmental factors in comparison with adults because its antioxidant, immune and other systems, as well as non-specific protection, are still underdeveloped and unable to resist the effects of stress factors to the full [11-15]. The development of embryos includes critical periods; therefore, young chicks obtained in inadequate production conditions often have a low potential of resistance, as well as viability and productivity [16].

The peripheral immune organs of chicks (spleen and lymphoid diverticulum) develop morphofunctionally throughout postembryogenesis, and, by day 42, this process is not completed. By the hatching time, only the central immune organs, thymus and bursal sac, are formed morphologically and functionally [17]. Since the bursal sac plays a key role in the formation of poultry immunity, the study of its morphological and functional characteristics in connection with the search for effective methods to prevent the effects of stress arising during industrial incubation of eggs is considered one of the most important tasks [18]. The stem cells of the bone marrow form the population of clones of B-lymphocytes in the bursal sac. Then, they leave bursa and colonize thymus-independent zones of peripheral immune organs and structures, where under the influence of antigens they are differentiated and turned into plasma cells that synthesize antibodies [19].

Extreme stress states of various origins are characterized by premature involution and atrophy of the bursal sac, which can be considered as the morphological equivalent of the secondary immunodeficiency [20]. According to Turitsyna [20], histostructure modifications in the bursal sac may also be connected with an antigen-induced immune response to the conducted vaccinations and evolution of infections (while changes in the number of immunoglobulins in chicks' blood serum are also observed). The changes in the size and shape of a bursa, a decrease in the number of lymph follicles, thinning of the cortical layer, cirrhosis, and abundant infiltration of the organ by macrophages and granulocytes are possible in the case of immunization and infections [20].

Immunomorphological studies are informative to evaluate immune function [21]. This determines the both practical and fundamental interest in studying the histostructure of immune-competent organs that are directly involved in immunological response, in particular, the bursal sac.

Technological factors have a significant impact on the bursal sac histostructure. According to Travnikova [22], a complex of pathological processes is observed among chicks in conditions of the high cage density. Leukocytes, pseudo-eosinophils, macrophages, and piocytes are accumulated in the bursal lumens. The same infiltrating cells are found in the bursal subepithelial layer, as in the lumen [22]. Various fodder boosters promoting immune activation, which positively affects the histologic pattern of immune-competent organs [23]. Thus, when using probiotic products, the mass of immune protection organs increases due to physiological hyperplasia [24]. The defects of bursal sac microstructure might have been neutralized in the embryogenesis already. For example, the introduction of the immunomodulator (methisoprinol) inside the egg promotes positive changes in the histological structure of this organ [25].

Note, the morphological, histological, and biochemical indicators of the bursal sac state were studied during late ontogeny mainly, and a comprehensive study of the organ in early postembryogenesis, i.e., immediately after the hatching, is a matter of special interest.

The composition of biologically active substances (BASs) studied in the previous paper had a positive effect on the number of blood cells, some of which were producers of nonspecific protection factors of the body, for example, a lysozyme [26]. To investigate the functioning of the immune system deeper, it is necessary to assess not only the histological state of the bursal sac when using the developed composition but also to determine the content of immunoglobulins in the blood of chicks, which will allow detecting the presence or absence of an immunomodulatory effect.

The role of the immunoglobulin M (IgM) in immune responses is short-term and represents the body's primary response to any pathogen. IgY of chickens is largely similar to that of mammals in structure and function, both being determined by the same immunoturbidimetric method, which allows the latter to be used to assess the immunity of poultry [27]. IgA is the main form of antibodies in the body's secretions [28]. It should be noted that birds do not have immunoglobulin D because the genes encoding it are absent in their organisms [29].

It is also known that among all species of birds chickens have the strongest humoral response [30]. It has been reported that even chickens that had not been immunized have natural antibodies [31]. It was concluded that they can play an important role in both activation and regulation of specific humoral immune responses of poultry [32]. The half-life period of immunoglobulins at day-old chicks ranges within 3 days [33].

Therefore, it is interesting to determine the quantitative changes in natural antibodies under the influence of various compounds during embryogenesis to identify their potential immunomodulatory effect. A method to stimulate the chicks' embryonic development, increasing non-specific resistance, which includes transovarian treatment of eggs with a composition of natural metabolites, consisting of colamine, succinic acid, and serine, has been developed for this study. It is well known that abnormal intensification of free radical reactions leads to excessive activation of lipid peroxidation (LP) and damages of bilipid membranes. Colamine in animals and poultry is easily converted into phosphatidylethanolamine and phosphatidylcholine; serine is rarely used for the synthesis of ethanolamine [34] and primarily serves as a component of phosphatidylserine or a donor of the carbon skeleton in biochemical processes. The effectiveness of these elements in the composition is practically confirmed in a series of experiments [35]. Since the synthesis processes are energy-consuming, succinic acid is added to the composition as the main intermediate of the tricarboxylic acid cycle and the substrate of biological oxidation, the natural pool of which is quickly wasted under any medium and severe stresses. Succinic acid supports ATP synthesis both in the Krebs cycle and in the mitochondrial respiratory chain, compensating a decrease in the amount of ATP, typical for the stressed state. It is important to note that not only succinate but also colamine (with the help of choline) in the body are able to prevent disturbances in the mitochondrial respiratory chain function, preventing energy losses and excessive synthesis of reactive oxygen species [34, 36].

The immune system of birds is the most vulnerable to stress and the first to suffer the consequences of excessive generation of free radical particles. The use of antioxidant preparations prevents the formation of destructive changes in the bilipid membrane skeleton [37]. The proposed composition of metabolites, which has a significant antioxidant effect [35], is able to prevent the excessive generation of free radicals effectively and reduce the intensity of lipid peroxidation in chicks' bodies, while maintaining the structural integrity of internals, including the bursal sac.

The peculiarities of changes in the specific and non-specific resistance (the contents of immunoglobulins, lysozyme, and the bactericidal activity of blood serum) and histological architectonics of the bursal sac when using the proposed composition of the metabolites are revealed in the present paper for the first time.

The work objective was to study the effects of natural metabolites on the histological structure of the bursal sac and the total resistance of the obtained chicks.

Techniques. Studies (Ptichnoe Enterprise, Moscow Province, 2011) were performed on hatching eggs of the egg cross Shaver 2000 (Hendrix Genetics Company, Holland) chickens (*Gallus gallus*). The optimal composition of natural metabolites and the treatment multiplicity were selected earlier [26, 37]. The experimental batch of eggs was treated twice. 3-4 hours before the eggs were put into the incubator, their shells were treated with an aqueous solution of colamine, succinic acid and serine (0.1; 0.1 and 0.2%, respectively) using a spray gun, and on day 19 of incubation, 0.1% aqueous colamine solution was applied. The preparations were previously dissolved in distilled water at 18-22 °C. A batch of eggs, which was not subjected to treatment, was used as a control ("dry" control), as it was proved that, in this case, the results do not differ from the "wet control" [38].

Each batch consisted of 544 eggs. The recorded indicators were hatching rates, hatchability, and incubation losses. To determine the safety of chicks, 100 animal units were grown from each group under standard conditions. In whole blood taken from day-old chicks after decapitation, the immunoglobulin content was determined by the immunoturbidimetric method, in serum by lysozyme activity nephelometrically, and bactericidal activity (BASC) by the photoelectric nephelometric method [39].

Samples of bursal sac tissue were taken from one-day chicks for histological analysis (n = 5). The organ samples were embedded in paraffin and the histological sections with a thickness of 5-7 microns were prepared on a microtome MPS-2 (OAO HZ Tochmedpribor, Russia) according to standard techniques [40] followed by staining with Meyer's hemalum and eosin. Microscopy of histological sections was performed using a biological microscope ScienOpBP-52 (ScopeTec, China) with the magnification of ×7, ×10 eyepieces and ×4, ×10 and ×40 lenses. The photos were shot with a digital camera-eyepiece for a microscope DCM800 (ScopeTec, China, 8000 pixels, USB 2.0). Morphometry was performed using an eyepiece micrometer using the program ImageJ (National Institutes of Health, USA) with a set of modules for medical morphometry. Measurements were carried out at a total increase of ×70 in 30 consecutive fields equal to the area of the ocular grid.

The data were processed statistically in the Microsoft Excel program. The mean values (M) and standard errors of means (±SEM) were calculated. The significance of differences was assessed according to Student's t-criterion.

Results. Penetrance of eggshells for biologically active substances was based scientifically by Karmoliev et al. [41]. The use of natural metabolites led to a decrease in the abnormal intensity of free-radical reactions, as well as the processes of lipid peroxidation among young chicks of the experimental group and, consequently, to the optimization of metabolic processes [42]. It determined a significant increase in embryonic viability, reflected in the reduction of incubation losses at a significant increase in hatching by 9.38% ($p \leq 0.001$) (in control and experience, 75.0 and for 84.38% respectively) and hatchability by

9.26% ($p \leq 0.001$) (81.27 and 90.53%).

It should be noted that the high viability of the individuals of the experimental group remained for a long period of post-embryonic development. While breeding the group of 100 young chicks during 60 days, covering the main critical periods of post-embryonic development, the death loss in the experimental group decreased by 1.2 times with an increase in viability by 1% (in control and experience, 94 and 95% respectively).

These positive effects were observed against the background of increased active immunity, i.e., immunoglobulin levels, recorded in the experimental group, were optimal for the studied individuals. Indicators of specific body protection of day-old chicks in the experimental group were higher than in the control (Table 1).

1. Blood immunoglobulins (g/ml) **in cross Shaver 2000 day-old chicks (*Gallus gallus*) under egg treatment with the composition of metabolites** (Ptichnoe Enterprise, Moscow Province, 2011)

Immunoglobulin	Group	
	control (n = 5)	test (n = 5)
IgM	0.300±0.007	0.330±0.008*
IgG (IgY)	3.000±0.100	3.300±0.070
IgA	0.250±0.009	0.260±0.007
IgE	1.1000±0.01	1.100±0.020

N o t e. See the description of groups in the section Techniques.
* Differences form control are statistically significant at $p < 0.05$.

The content of IgM, responsible for the primary immune response, in chicks from the experimental group, was significantly higher (10%, $p < 0.05$), while the content of IgG (IgY), which determines the secondary immune response, also increased by 10% (see Table 1). The IgA content increased slightly (by 4%), while the content of IgE remained unchanged. These data indicate the immunomodulatory effect of the used composition against the background of oxidative stress during incubation. Manifestations of hypersensitivity were absent, as evidenced by the equal IgE indicators in the control and experiment. The immunomodulatory synergistic effect of the studied BAS was also indicated by an increase in the content of non-specific protection factors — BASC (3.8%) and lysozyme (4.8%).

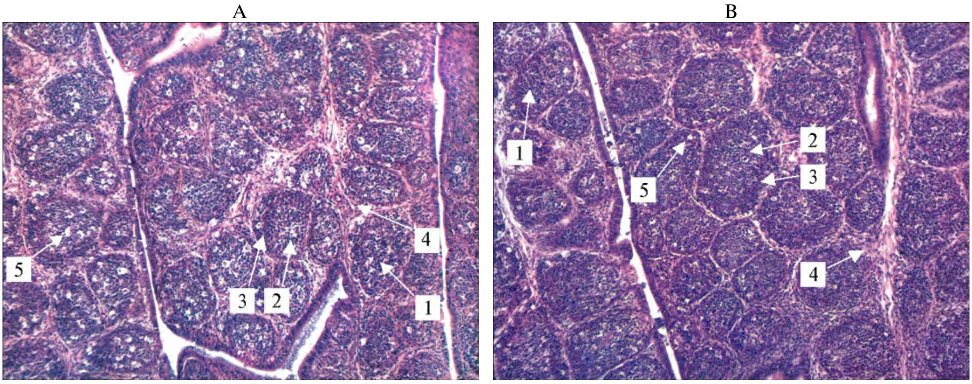
High body reactivity of chicks from the experimental group was a consequence of the positive effects of natural metabolites on the histologic architectonics of the bursal sac (Table 2, Fig.).

2. Morphometric bursal sac indicators in day-old cross Shaver 2000 chicks (*Gallus gallus*) under egg treatment with the composition of metabolites (Ptichnoe Enterprise, Moscow Province, 2011)

Indicator	Control (n = 5)	Test (n = 5)
Bulk density, %:		
stroma	35.00±1.22	23.60±1.08*
follicles cortical zone	30.00±1.00	59.00±2.35**
follicles medullar zone	35.00±1.38	18.00±0.89**
Area, $\times 10^6$ rm^2:		
follicle	7291.138±440.780	9360.420±798.910
cortical zone	3680.440±349.870	7818.922±602.680**
brain zone	3703.168±387.320	1374.432±83.240**
Cortical brain index	99.39	568.88

N o t e. See the description of groups in the section "Techniques."
*, ** Differences form control are statistically significant at $p < 0.05$ and $p < 0.01$, respectively.

As can be seen from the data of Table 2, the volume density of the bursal sac stroma of chicks from treated eggs is by 11.4% significantly less, and the bulk density of the brain zone of the follicles is by 29% more than in the control. The density of the brain zone of follicles significantly decreased (by 17%); the area of the cortical zone increased and the brain zone decreased (by 2.12 and 2.69 times, compared with the control, respectively). As a result, there was an increase in the cortical brain index (CBI), which reflects the functional activity of the organ: the higher the index is, the better the organ functions [43]. In the authors' study in the experimental group, CMI exceeded the control indicators by 5.72 times. The data obtained are consistent with the micromorphological results of histological examination of the organ (see Fig.).



The histological structure of the bursal sac in day-old cross Shaver 2000 chicks (*Gallus Gallus*) of the control (A) **and test** (B) **groups:** 1 — lymphatic node, 2 — medullary part, 3 — cortical part, 4 — stroma connective-tissue elements, 5 — small cystic cavities (hematoxylin and eosin staining; microscope ScienOpBP-52, ScopeTec, China; eyepiece ×10, lens ×10).

Among the chicks of the control group, a significant involutional process was observed in the bursal sac. The capsule of the organ was thickened; the trabecular pattern was sharply expressed by increasing the connective-tissue elements of the stroma (fibrocytes, fibroblasts, and intercellular fibrous structures). The lumen of the bursal sac is fitted with a plural-row cylindrical epithelium with no signs of damage. Directly behind the epithelial layer in the depth of the mucous membrane, there were multiple closed lymphoid follicles mainly of medium and small size, having a rounded, oval or trapezoidal shape and an indistinct division into cortical and brain zones. Lymph knots were adjacent to each other and located in the folds in one or two rows. The cortical zone was thinned, had the form of a narrow strip lying on the periphery of the follicles on the border with the organ stroma, and was represented by a small accumulation of small lymphocytes. The lighter brain zone consisted of rarely located large lymphocytes and separate plasma cells, as well as a large number of macrophages and granulocytes. The apoptotic cells were often found among the lymphocytes. In the follicles, the numerous small cystic cavities were clearly expressed. At the same time, the organ showed no significant signs of hemodynamic disturbances.

In the chicks of the control group, the bursal sac capsule was not thickened; the trabecular pattern was poorly expressed due to the weak development of the connective-tissue elements of the stroma. The lumen of the bursal sac was fitted with a plural-row cylindrical epithelium with no signs of damage. Directly behind the epithelial layer in the depth of the mucous membrane, there were multiple closed lymphoid follicles mainly of big and medium size, having a rounded, oval or trapezoidal shape. Lymph knots were adjacent to each other and located in the folds in three or four rows. The cortical zone occupied a large area of the follicle, spreading from the center to the periphery, and was represented mainly by a relatively dense cluster of small lymphocytes. The centrally located brain zone was lighter, relatively poorly developed, included large lymphocytes, plasma cells, as well as a small number of macrophages and individual granulocytes. In the follicles, rare individual places of the polycystic process were observed, apoptotic bodies were rare.

The histological studies reveled a number of positive changes in test chicks compared to control. So, young chicks of the control group had signs of a decrease in the functional activity of the bursal sac. They were characterized by an increase in the proportion of connective tissue in the organ, a small size of lymphoid follicles with the development of the processes of delimitation in the cortical and medullar layer. At the same time, the formation of microcystic cavities and an increase in the number of macrophages in the brain layer of the follicles clearly indicated the death of lymphocytes. The absence of a significant inflammation in the organ gave a reason to assume that the process of lymphocyte death was due to apoptosis, not necrosis, which allows us to consider non-infectious factors as the cause of this process [43]. It is also confirmed by the increase in the number of apoptotic cells, which were found in lymphoid follicles during the histological examination of the bursal sac.

The literature data [44, 45] show that the influence of non-specific environmental factors (imperfection of egg incubation, feeding, and layer housing) on the formation of organs among chicks during embryonic development is quite high. As a result of such adverse effects, the process of formation of immunocompetent organs is disrupted, which is manifested by a decrease in non-specific resistance and immunological reactivity.

Chicks have several stages of the bursal sac development: growth (up to 2 weeks of age), maturity (up to 5-8 weeks), early involution (up to 9-15 weeks), late involution (up to 25-30 weeks), and rudimentary (after 30 weeks) until complete disappearance [46]. Taking into account that one-day chicks were used in the experiment, it can be affirmed that the processes of atrophy of the bursal sac, or the so-called accidental involution of the organ started early. At the same time, the rapid decrease in its mass and volume, primarily under the influence of glucocorticosteroids in various stressful situations, was observed. In these studies, the mass of the bursal sac in the experiment was 0.084 ± 0.002 g vs. 0.069 ± 0.001 g in the control, with the 21.7% difference ($p < 0.001$) between the test and control.

The impact of stress factors on the embryo, and then in the postembryonic period on the chick leads to the formation of secondary immunodeficiency, expressed by the inferiority of the morphological structure of immunocompetent organs [46].

Histological examination of bursal sac in the test group showed signs of a moderately developed physiological hyperplasia of the organ in the absence of the processes of involution. Hyperplasia was expressed in an increase in the size of the lymphoid follicles of the organ by increasing the number of lymphocytes while maintaining the shape and structural elements that make up the follicles. The increase in the size of the follicles was primarily due to an increase in the cortical layer, while the brain layer was characterized by the small size and rather rare processes of apoptosis among lymphocytes. Together, these data indicate the full development of the organ, typical for chicks in this age period.

Thus, the treatment of chicken eggs with the composition of natural metabolites which contributes to a more complete timely formation of all the structures of bursal sac reduces embryonic death, positively influences the overall resistance of chicks, and determines higher quality and safety of young birds. The physiological intracellular hyperplasia occurs in the bursal sac of day-old chicks, while the involutive processes are not fixed. In other words, the complex of natural metabolites optimizes the histoarchitectonics of the bursal sac.

REFERENCES

1. Vasil'ev S.S., Korneva G.V. *Uchenye zapiski Kazanskoi gosudarstvennoi akademii veterinarnoi meditsiny im. N.E. Baumana*, 2010, 201: 182-186 (in Russ.).
2. Zakarial H., Al-Anezi M.A. Effect of ascorbic acid and cooling during egg incubation on hatch-

ability, culling, mortality, and the body weights of broiler chickens. *Poultry Science*, 75, 1996: 1204-1209 (doi: 10.3382/ps.0751204).
3. Zulkifli I., Siegel P. Is there a positive side to stress? *World's Poultry Science Journal*, 1995, 51(1): 63-76 (doi: 10.1079/WPS19950006).
4. Reijrink I.A.M., Meijerhof R., Kemp B., Graat E.A.M., van den Brand H. Influence of prestorage incubation on embryonic development, hatchability, and chick quality. *Poultry Science*, 2009, 88: 2649-2660 (doi: 10.3382/ps.2012-02816).
5. Everaert N., Kamers B., Witters A., De Smit L., Debonne M., Decuypere E., Bruggeman V. Effect of four percent carbon dioxide during the second half of incubation on embryonic development, hatching parameters, and posthatch growth. *Poultry Science*, 2007, 86: 1372-1379 (doi: 10.1093/ps/86.7.1372).
6. Everaert N., De Smit L., Debonne M., Witters A., Kamers B., Decuypere E., Bruggeman V. Changes in acid-base balance and related physiological responses as a result of external hypercapnia during the second half of incubation in the chicken embryo. *Poultry Science*, 2008, 87: 362-367 (doi: 10.3382/ps.2007-00345).
7. Molenaar R., van den Anker I., Meijerhof R., Kemp B., van den Brand H. Effect of eggshell temperature and oxygen concentration during incubation on the developmental and physiological status of broiler hatchlings in the perinatal period. *Poultry Science*, 2011, 90: 1257-1266 (doi: 10.3382/ps.2010-00684).
8. Shim M.Y., Pesti G.M. Effects of incubation temperature on the bone development of broilers. *Poultry Science*, 2011, 90: 1867-1877 (doi: 10.3382/ps.2010-01242).
9. Willemsen H., Li Y., Willems E., Franssens L., Wang Y., Decuypere E., Everaert N. Intermittent thermal manipulations of broiler embryos during late incubation and their immediate effect on the embryonic development and hatching process. *Poultry Science*, 90(6), 2011: 1302-1312 (doi: 10.3382/ps.2011-01390).
10. Van der Pol C.W., van Roovert-Reijrink I.A.M., Maatjens C.M., van den Brand H., Molenaar R. Effect of relative humidity during incubation at a set eggshell temperature and brooding temperature posthatch on embryonic mortality and chick quality. *Poultry Science*, 2013, 92: 2145-2155 (doi: 10.3382/ps.2013-03006).
11. Wang F.C., Fang C.G., Zhang Y.H., Wang H.B., Han Y.C. Effects of compound chinese herbal immune synergist on immunity of egg-laying chicken. *Acta Veterinaria et Zoot echnica Sinica*, 2006, 37(2): 187-192.
12. Akbarian A., Michiels J., Degroote J., Majdeddin M., Golian A., De Smet S. Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. *J. Anim. Sci. Biotechno.*, 2016, 7(37): 1-14 (doi: 10.1186/s40104-016-0097-5).
13. Aughey E. *Comparative veterinary histology with clinical correlates*. Manson Publishing, The Veterinary Press, 2010.
14. Betteridge D.J. What is oxidative stress? *Metabolism*, 2000, 49(2): 3-8 (doi: 10.1016/S0026-0495(00)80077-3).
15. Sies H. Oxidative stress: from basic research to clinical application. *Amer. J. Med.*, 1991, 91: 315-385 (doi: 10.1016/0002-9343(91)90281-2).
16. Shcherbatov V.I., Smirnova L.I., Shcherbatov O.V. *Inkubatsiya yaits sel'skokhozyaistvennoi ptitsy: monografiya* [Incubation of poultry eggs: monograph]. Krasnodar, 2015 (in Russ.).
17. Seleznev S.B., Ovsishcher L.L. *Vestnik Rossiiskogo universiteta druzhby narodov. Seriya: Agronomiya i zhivotnovodstvo,* 2007, 1-2: 48-53 (in Russ.).
18. Smerdova M.D., Vakhrusheva T.I. *Vestnik Krasnoyarskogo gosudarstvennogo agrarnogo universiteta,* 2010, 12(51): 106-110 (in Russ.).
19. Yakimenko L.L., Yakimenko V.P. *Uchenye zapiski uchrezhdeniya obrazovaniya «Vitebskaya ordena «Znak pocheta» gosudarstvennaya akademiya veterinarnoi meditsiny»*, 2011, 47(1): 321-323 (in Russ.).
20. Turitsyna E.G. *Mezhdunarodnyi vestnik veterinarii*, 2010, 1: 44-48 (in Russ.).
21. Sandul P.A., Luppova I.M., Sandul A.V. *Uchenye zapiski uchrezhdeniya obrazovaniya «Vitebskaya ordena «Znak pocheta» gosudarstvennaya akademiya veterinarnoi meditsiny»*, 2011, 47(1): 293-296 (in Russ.).
22. Travnikova N.A., Drozdova L.I. V sbornike: *Molodezh' i nauka* [In: Youth and science]. Moscow, 2000: 126-128 (in Russ.).
23. Mullakaev A.O., Shukanov A.A. *Vestnik Chuvashskogo gosudarstvennogo pedagogicheskogo universiteta im. I.Ya. Yakovleva*, 2012, 4(76): 120-123 (in Russ.).
24. Skovorodin E.N., Deblik A.G., Malikova A.R., Izhbulatova D.A. *Niva Povolzh'ya,* 2006, 1(1): 45-48 (in Russ.).
25. Tykałowski B., Stenzel T., Lewczuk B., Andrzejewski M., Koncicki A. Effect of methisoprinol administered in ovo on the histological structure of the bursa of Fabricius in turkeys. *Bulletin — Veterinary Institute in Pulawy*, 2009, 53(3): 471-476.
26. Yartseva I.S., Azarnova T.O., Indyukhova Ye. N., Kochish I. I., Naydenskiy M.S. Antioxidant Properties of Ethanolamine, Succinic Acid and Serine when Treating Hatching Eggs. *Journal of*

Biological & Scientific Opinion, 2013, 1(3): 154-156 (doi: 10.7897/2321-6328.01304).
27. Ratcliffe M.J.H. Allotype suppression in the chicken. V. Abnormal isotype ratios of chronically suppressed IgM and IgG allotypes. *Cell. Immunol.*, 1984, 83: 208-214.
28. Mansikka A. Chicken IgA H chains. Implications concerning the evolution of H chain genes. *J. Immunol.*, 1992, 149(3): 855-861.
29. Man'ko V.M., Devrishov D.A. *Veterinarnaya immunologiya. Fundamental'nye osnovy* [Veterinary immunology. Fundamentals]. Moscow, 2011 (in Russ.).
30. Higgins D.A. Comparative immunology of avian species. In: *Poultry immunology*. Carfax Publishing Company, Abingdon, UK, 1996.
31. Parmentier H.K., Lammers A., Hoekman J.J., Vries Reilingh G. De, Zaanen I.T., Savelkoul H.F. Different levels of natural antibodies in chickens divergently selected for specific antibody responses. *Dev. Comp. Immunol., 2004, 28(1):* 39-49 (doi: 10.1016/S0145-305X(03)00087-9).
32. Lammers A., Klomp M.E.V., Nieuwland M.G.B., Savelkoul H.F.J., Parmentier H.K. Adoptive transfer of natural antibodies to non-immunized chickens affects subsequent antigen-specific and cellular immune responses. *Dev. Comp. Immunol.*, 2004, 28(1): 51-60 (doi: 10.1016/S0145-305X(03)00102-2).
33. Patterson R., Youngner J.S., Weigle W.O., Dixon F.J. The metabolism of serum proteins in the hen and chick and secretion of serum proteins by the ovary of the hen. *J. Gen. Physiol.*, 1962, 45: 501-513.
34. Marri R., Grenner D., Meie P. *Biokhimiya cheloveka* [Human biochemistry]. Moscow, 2009 (in Russ.).
35. Azarnova T.O., Kochish I.I., Naidenskii M.S., Zaitsev S.Yu., Yartseva I.S. *Prikladnaya analiticheskaya khimiya*, 2012, 1(7): 46-49 (in Russ.).
36. Fisinin V.I., Surai P. *Ptitsevodstvo*, 2013, 5: 4-10 (in Russ.).
37. Lugovaya I.S. *Ptitsa i ptitseprodukty*, 2017, 2: 56-59 (in Russ.).
38. Azarnova T.O., Naidenskii M.S., Khobot'eva D.S., Sharikova E.A. *Ptitsefabrika,* 2006, 4: 47-48 (in Russ.).
39. Merkulov G.A. *Kurs patologicheskoi tekhniki* [Course of pathological technique]. Leningrad, 1969 (in Russ.).
40. Lelevich S.V., Kurstak I.A., Grinevich N.I., Vorob'ev V.V. *Osnovy klinicheskoi biokhimii. Posobie dlya studentov mediko-diagnosticheskogo fakul'teta* [Basics of clinical biochemistry. Handbook for students of medical and diagnostic faculty]. Grodno, 2013 (in Russ).
41. Karmoliev R.Kh., Ruchii O.S., Karmoliev R.R., Kochish I.I., Karmoliev R.Kh., Nesterov V.V. *Sposob kolichestvennogo opredeleniya proniknoveniya suktsinata margantsa tetragidrata v inkubatsionnye yaitsa kur. FGOU VPO "Moskovskaya gosudarstvennaya akademiya veterinarnoi meditsiny i biotekhnologii im. K.I. Skryabina" (RF). № 2008140854/12. Zayavl. 15.10.2008. Opubl. 27.02.2010. Byul. № 6* [Method of quantitative determination of manganese succinate tetrahydrate penetration in chickens hatching eggs. Skryabin Moscow State Academy of Veterinary Medicine and Biotechnology. No. 2008140854/12. Appl. 15.10.2008. Publ. 27.02.2010. Bul. No. 6] (in Russ.).
42. Azarnova T.O., Yartseva I.S., Indyukhova Ye.N., Naydenskiy M.S., Zaitsev S.Yu. Effects of the nanostructured complex of biologically active compounds on the free-radical processes and the liver state of the chicken cross Shaver 2000. *Journal of Nanomaterials & Molecular Nanotechnology*, 2013, 2(5): 1-3 (doi: 10.4172/2324-8777.1000123).
43. Smerdova M.D., Borodulina I.V. *Agrarnyi vestnik Urala*, 2009, 3(57): 80-82 (in Russ.).
44. Surai P.F., Borodai V.P. *Suchasne ptakhivnitstvo*, 2010, 7: 31-36 (in Russ.).
45. Turitsyna E.G. *Materialy regional'noi nauchno-prakticheskoi konferentsii «Agrarnaya nauka na rubezhe vekov»* [Proc. Conf. «Agrarian science at the turn of the century»]. Krasnoyarsk, 2007, 2: 240-243 (in Russ.).
46. Vrakin V.F., Sidorova M.V. *Morfologiya sel'skokhozyaistvennykh zhivotnykh* [Morphology of farm animals]. Moscow, 2000 (in Russ.).