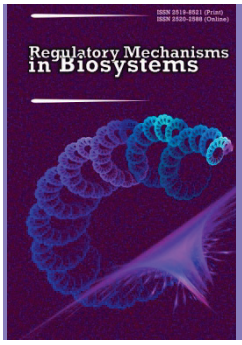

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Influence of protein-vitamin mineral supplements on the splenic morphometric parameters of quails

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Currently, a growing industrial poultry sphere in many countries is quail farming. Quails have a complex of unique economic-productive advantages, as compared with other poultry, – high body temperature, intensive metabolism, small size, fast maturation, and high egg productivity. In the study, we examined the specifics of microscopic structure of morphometry of the spleen of the control and experimental birds as characteristics sensitive to the action of supplements. The proposed methods are intended for studying effects of protein-vitamin mineral supplements on poultry farming. Therefore, for 21 days, with the main diet, the quails were given mineral supplements produced by the Multilife trade mark (crude protein, amino acids (lysine, threonine, methionine, cysteine), calcium, available phosphorus, sodium, chlorine, vitamins (A, D_3, E)), by the Missi trademark (protein, vitamins, minerals), and by the Standard Agro trademark (soybean press cake, vitamins A, D_3, E, B_1, B_2, B_6, B_{12}), multi-enzyme complex, salt, ground limestone, phosphate monocalcium, amino acids (lysine, threonine, methionine with cysteine). This is the first study of the ultrastructural organization of the spleen of Japanese quail (*Coturnix japonica*) fed with protein-vitamin mineral supplements. The tested supplements were beneficial for the productivity of the quails, as evidenced by increase in the body and spleen weight. Body weight of the quails increased by 5.8% in the group that was fed additionally with the Multilife supplement, and by 12.5% in the group that consumed the Missi supplement added to the fodder, and by 8.1% in the group that consumed the Standard Agro supplement with the diet. At the same time, the spleen weight increased by 7.2%, 16.0%, and 10.5%, respectively. However, the relative weight (0.1%) underwent no changes, which characterizes this parameter as permanent for age and species of animals. Analysis of the data of our morphological studies revealed that the splenic microscopic structure of the quails was characterized by presence of support-contractile apparatus (capsule and trabeculae) with the relative area of 4.6 ± 0.8% and single radial trabecula. The parenchyma was formed by white and red pulps without distinct boundaries. In the white pulp, there were designated lymphoid nodes with no light centers, and also periarterial lymphoid sheaths and ellipsoids. In the reticular stroma of the white pulp of the spleen, we found blood cells of various maturity, in particular, lymphocytes, which were clearly divided into large, average, and small; mononuclear and multinuclear macrophages, which contained hemosiderin and melanin, leukocytes, mostly eosinophilous. The ultrastructural organization of the spleen of the quails had the following peculiarities: nuclei of leukocytes contained large nuclei, endotheliocytes of the central artery of lymphoid follicle performed phagocyte function by accumulating autophagosomes in its cytoplasm. In the quails that had consumed the diet containing supplements, the macroscopic, microscopic, and ultramicroscopic structures underwent no changes. At the same time, there was seen a tendency towards increase in the relative area of white pulp and the support-contractile apparatus. The relative area of white pulp of the spleen increased by 0.5% in the group of quails that had received the diet with the Multilife supplement, by 1% in the group that had consumed the diet with the Missi supplement, and by 0.6% in the group that had consumed the diet with the Standard Agro supplement. The relative area of the red pulp in the spleen of the quails was observed to have the tendency towards decrease: by 0.4% in the group that received the Multilife supplement, by 1% in the group that consumed the Missi supplement, and by 0.5% in the group that consumed the Standard Agro supplement. The relative area of the support-contractile apparatus of the spleen increased by 0.4% in the group of quails that consumed the Multilife supplement, by 1% in the group that consumed the Missi supplement, and 0.5% in the group that consumed the Standard Agro supplement. Taking into account the data we obtained, we may conclude that our studies confirm the benefits of using modern protein-vitamin supplements in poultry farming for stimulation of growth and development. Especially valuable is the absence of negative effect on the morphofunctional parameters of the spleen, which we used as biomarkers.

Keywords: quail; organ morphology; anatomic parameters; morphofunctional condition; histological drug; feed supplement.

Introduction

To satisfy the growing demand for high-quality, high-nutritive and dietary food products, many countries of the world widely use quails (Kyryliv et al., 2017). Biological specifics of this bird, including fast growth rates, high flavour and food qualities of eggs and meat, contribute to the development of this sphere (Ibatullin et al., 2017). Production of quail eggs is cheaper than that of chickens, and growing quails is a highly profitable poultry sphere (Zherebov, 2011). Quails have fast metabolism, which promotes fast growth and development rates. Quails have been domesticated relatively recently and the parameters of their productivity depend heavily on the maintenance conditions. Therefore, development of

scientifically substantiated approaches to increasing their productivity is still underway, as well as searches for methods that would enhance the coefficient of using fodders, because the organism does not digest most of them (Volodkevych, 2013; Stojanovskyj et al., 2016). Quails are also used for scientific purposes as a model animal, for example, to determine the action of toxicants, in particular aerobic mould mycomycetes of the Deuteromycetes class (Orobchenko et al., 2019). The quail organism is sensitive to various factors, such as stress, and therefore study of their physiological condition in case of adaptation syndrome and search for means of increasing the survival of the population are today's goals (Dotsenko, 2017).

Nutrition is one of the key factors for normal growth and development of agricultural animals. Development of animals, poultry in particular, mainly depends on availability of feeds, which are the priority factors for maintenance, care, and diet (Fernández et al., 1995; Chen et al., 2016; Mylostyvyi et al., 2021; Sameliuk et al., 2022). One of the most promising spheres is the search for new feed additives and development of effective schemes of using biological compounds that stimulate growth, development, and productivity of animals, and also boost the level of non-specific resistance (Hussain et al., 2016; Bomko et al., 2018; Gutyj et al., 2019; Kosenko et al., 2021). Because of their broad spectrum and diversity, components of natural feed supplements of various origin enhance the metabolic processes, improve the immunity of animals, positively influence the digestive processes, etc. (Levytskyy, 2019; Slivinska et al., 2019; Martyshuk et al., 2020; Razanova et al., 2022). It has already been determined that inclusion of biologically active compounds in the diet is positive for the processes of hematopoiesis and body-weight gain (Garmata, 2018; Sobolev et al., 2020). In general, identification of the morphophysiological norm of an organism always remains one of the most important issues in biological, medical, and veterinary studies. Therefore, physiological-morphological studies of agricultural animals are performed on a large scale. They are needed for the control of reproduction of livestock and evaluation of influence of maintenance conditions, nutrition, prophylaxis of diseases, increase in productivity, etc, and for identifying the norm parameters for producing ecologically clean and safe products (Wu et al., 2018). In our study, we determined the effect of dietary supplements on the spleen of quails, since the spleen is a biomarker of influence of factors of various etiology (Dunaievska, 2016) on the organism and has been used in numerous studies (Casagrande et al., 2014; Mohapatra et al., 2014; Yakubu et al., 2015).

The objective of our study was identifying the influence of modern protein-vitamin mineral supplements that are advertized and proposed by manufacturers in poultry farming for stimulation of growth and development of poultry, specifically quails, by analyzing the specifics of microscopic structure and morphometry of the spleen in the control and experimental animals as characteristics sensitive to factors of various genesis.

The protein-vitamin mineral supplements (PVMS) were produced by different manufacturers. The PVMSs for quails manufactured by the Standard Agro contained soybean press cake, vitamins (A – 11,500 IU, D_3 – 52,500 IU, E – 525 mg, B_1 – 22 mg, B_2 – 70 mg, B_6 – 45 mg, B_{12} – 170 mg in 100 g), multienzymatic complex, salt, ground limestone, monocalcium phosphate, amino acids (lysine – 2.2%, threonine – 3.4%, metheonine with cysteine – 1.6%). The Multilife manufacturer provides the following content of its PVMS: crude protein – 20%, amino acids (lysine – 1.1%, threonine – 0.76%, metheonine with cysteine – 0.84%, methionine – 0.52%), calcium – 2.8%, available phosphorrus – 0.45%, sodium – 0.21%, chlorine – 0.24 %, vitamins (A – 15000 IU, D_3 – 4800 IU, E – 37.5 mg in 100 g). The protein-vitamin mineral supplement from the Missi manufacturer is used to prepare ready-to-eat mixed feed; this supplement satisfies the needs of quail for vitamins, minerals and proteins. Those complexes are used for enrichment of fodders, providing them with necessary vitamins, minerals, and amino acids.

Materials and methods

During our studies, we followed the main rules of the laboratory practice of the GLP (1981), the General Ethical Principles of the Experiments on Animals, adopted by the First National Congress of Bioethics (Kyiv, 2001). All the experimental part of the study was performed according to the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), the Rules of Conducting Work using Experimental Animals, adopted by the Order of the Ministry of Healthcare No. 281 as of November, 1 2000, On the Measures on Further Improvement of Organization Forms of Work using Experimental Animals and the corresponding law of Ukraine On the Protection of Animals from Abuse (No. 3447-IV as of 2/21/2006, Kyiv).

In the conditions of the Mykolai farm in Zhytomyr Oblast during 2017–2023, we studied the influence of the protein-vitamin mineral supplements manufactured by the domestic producers of various trademarks: Multilife (group 1), Missi (group 2), Standart Agro (group 3) on the Japanese quails (*Coturnix japonica* Temminck & Schlegel, 1849), which were selected according to the analogue principle. The experiment lasted for 21 days – from the age of forty days of the quails to sixty days of age. Throughout the experiment, the first (control) group received the main diet, balanced by nutrients, and quails of the experimental groups received the diet with the protein-vitamin mineral supplements in the doses recommended by the manufacturers.

The anatomic necropsy was carried out by complete evisceration after the birds had been slaughtered (Nichiporuk et al., 2022). We quickly performed the anatomical dissection of the birds' spleen. The spleen was taken from birds of both sexes. There were 27 quails in each group. The birds were weighed with 0.01 g accuracy on the PS 1000/C/2 laboratory scales (Radwag manufacturer, Poland), and the spleen was weighed on up-to-0.1-mg-accuracy analytic scales. Relative weight of the spleen was measured as the ratio of its absolute weight to the weight of birds, calculated as a percentage.

For the histological studies, the spleen was fixated in 10–12% cooled aqueous solution of neutral formalin. For the further studies, the fixated material was rinsed in tap water for 24–48 h. Later, it was dehydrated in ethyl alcohol of ascending concentrations – 40%, 70%, 96%, 100%, and was embedded in paraffin according to the scheme proposed in the manual (Horalskyi et al., 2019). The paraffin sections were prepared on a MC-2 sledge microtome. The thickness of the sections did not exceed 4–10 μm. To characterize the morphology of the cells and tissues, we used hematoxylin staining of the histosections with the Erlich's hematoxylin (Diapath, Italy, 2020) and eosin (Leica Geosystems, Germany, 2020), according to the Brachet and Van Gieson method.

Morphometric studies were undertaken for an objective evaluation of structural-functional condition of the birds' spleen at the cellular, tissue, and organ levels, identification of interrelations and inter-dependencies of quantitative characteristics of structures of the organ. At the same time, splenic microstructures were measured with a scale of ocular-microtome and Goryaev morphometric grid (quadratic grid) of a Micros MC-50 microscope (Australia) and a MBS-10 microscope with the standard tube length. For a stereometric analysis of the histostructures, we used a stereological method of local volumetric analysis. In all the birds, at one increase, we counted the squares occupied by the studied histostructure, and by comparing its area occupied on the section surface in relation to the general area, we determined the percentage ratio of a needed parameter.

The morphometric studies were carried out using the Master of Morphology software with the accuracy of up to 0.1 μm. The thickness of the connective-tissue capsules, diameter of the lymphoid nodes and their structural units, length and width of the trabeculae, and the thickness of the vessel walls were measured with a MOV-1-15 ocular micrometer (15 measurements for each histosection, 3 preparation from each animal) (Horalskyi et al., 2019).

The microphotography of the histological preparations was performed using a CAM V-200 videocamera (Inter Med, PRC, 2017), installed in the Micros MC-50 microscope (Australia, 2012) and connected to a personal computer. Based on the data we obtained, we identified important anatomical indicators of morphofunctional condition of the spleen: relative areas of white pulp, red pulp, lymphoid nodes, periarterial lymphoid sheaths, the support-contractile apparatus (capsule and trabeculae), the vessels – %; thickness of capsules and trabeculae, diameter of the lymphoid nodes and vessels – μm.

For electronic-microscope study of the spleen, the materials from the animals were gathered right after the dissection of the thoracoabdominal

cavity. The length of the selected pieces was no more than 1 mm. The material was transferred using a Pasteur pipette and fixated in 2.5% solution of glutaraldehyde aldehide using a phosphate buffer with postfixation in 1% solution of osmium tetroxide according to Caulfield. Then, the material was dehydrated in alcohols of ascending concentrations (70%, 80%, 90%, 100%) and acetone, were embedded in a mixture of epon-araldite, according to the generally accepted technique (Horalskyi et al., 2019). The ultrathin sections were made on a Reihart ultramicrotome (Austria, 2010), were contrasted in 2% solution of uranyl acetate and lead citrate, and examined on a PEM-125K microscope (Selmi, Russia) at 4–20 times increases. The morphometric studies were conducted on a semiautomatic device for graphic-study analyzer using the Organela software (Ukraine, 2015).

The statistical analysis of the data included identification of distribution pattern according to the Shapiro-Wilk criterion ($P < 0.05$). After determining the fact of a normal distribution, we used the Tukey test ($P < 0.05$) in the SPSS statistical pack. Correlation between the parameters was analyzed using Spearman's criterion ($P < 0.05$). We determined the mean value (x) and standard error (SE).

Results

In quails of the control group, according to studies of the organs, the spleen measured 8.1 ± 0.03 mm in length, 7.7 ± 0.3 mm in width, and 5.5 ± 0.4 mm in height. Weight of a quail accounted for 190.6 ± 4.8 g. Absolute weight of the spleen equaled 200 ± 0.7 mg, relative weight – 0.10 ± 0.004%. Microscopic structure of the spleen of control-group quails was formed by the stroma and parenchyma. The stroma was formed by the capsule and trabeculae, which together comprised the spleen support-contractile apparatus. The spleen was surrounded by a serous membrane, which grew together with the capsule. It contained thin collagen fibers and a mesothelium layer. The capsule consisted of two layers: external and internal. In the connective tissue of the capsule, there dominated elastic fibers. Soft collagen fibers and fibroblasts and fibrocytes were also present there. The spleen capsule of the quails was evenly developed and had a thickening on the entire perimeter. The maximal value was seen in the splenic hilum, reaching the thickness of 20.4 µm. On the visceral surface of the spleen, the thickness of the capsule was the thinnest – 8.6 µm. At the same time, the mean value of thickness of the spleen capsule of the quails accounted for 16.5 ± 5.3 µm.

By visual examination of the histological preparations, which had been stained with hematoxylin and eosin and, especially using the Van Gieson method, we identified that the trabecular apparatus of the spleen of control-group quails was developed poorly. We found vascular, radial and connective trabeculae. Connective trabeculae were located in the red pulp unevenly and singly, were small and often connected with the vascular trabeculae. The radial trabeculae occurred rarely. Vascular trabeculae were mostly oval and rounded, and their network was developed better. According to the morphometric studies, the trabeculae were 96.2 ± 21.5 µm in length and 41.7 ± 9.3 µm in width. A common feature of all trabeculae was the poor development of myocyte bundles. Relative area of the support-contractile apparatus of the spleen of the quails was 4.6 ± 0.8%.

Parenchyma of the spleen of the Japanese quails was formed by white and red pulp without distinct boundaries between them (Fig. 1a). In the white pulp, lymphoid nodes and periarteriolar lymphoid sheaths were distinguished, which were often located near each other and distributed unevenly, and also ellipsoids were seen (Fig. 2b). By shape, the lymphoid nodes were mainly rounded, and also oval and elongated occurred. Analysis of the histological preparations, stained using Brasher's method, revealed blood cells of various maturity in the reticular tissue of the spleen white pulp, in particular, lymphocytes that were distinctly divided into large, average-sized, and small; mononuclear and multinuclear macrophages, which contained hemosiderin and melanin; leukocytes, mostly eosinophilous (Fig. 2). In quails of the experimental group, the histostructure and cellular composition of the spleen underwent no changes (Fig. 1).

According to the analysis of morphometric studies, the diameter of periarterial lymphoid sheaths of the spleen of the quails was 21.3 ± 6.7 µm, and such of lymphoid nodes measured 41.5 ± 9.1 µm. In the lymphoid nodes, light centers were absent, there differentiated the periarterial zone of 10.9 ± 1.6 µm diameter. The white pulp of the spleen accounted for 13.5 ± 2.1% of the relative area of the organ, and the lymphoid node occupied the greater part of white pulp – 8.2 ± 1.4%, and smaller part was occupied by periarterial lymphoid sheaths (5.3 ± 1.8%), and the ratio of lymphoid nodes to periarterial lymphoid nodes equaled 1:0.6.

The red splenic pulp in the quails accounted for 81.9 ± 1.2% of its general weight. It contained a large amount of erythrocytes, macrophages and blood vessels. The length of the vessels equaled 28.3 ± 18.6 µm, width was 17.8 ± 14.7 µm, their diameter was 12.4 ± 3.6 µm, and the thickness of vascular wall was 4.0 ± 1.1 µm.

Study of the ultrastructural organization of the quail spleen revealed that almost all leukocytes of nuclei contained large nucleoli (Fig. 3a), the largest of them in plasmatic cells. Such peculiarities were seen in both the experimental and control groups. The internal wall of the pulp artery was formed by elongated endotheliocytes, to the basal membrane of which, one-two layers of smooth muscle cells were closely adjacent, with thin layers of fibrous tissue in between. Single endotheliocytes of the central artery of lymphoid nodes performed a phagocyte function and accumulated autophagosomes in their cytoplasm. In the splenic red pulp in all the groups of quails, there dominated venous sinuses, lumens of which were filled with formed blood elements and plasma with a small amount of lymphocytes and monocytes (Fig. 3). As the electronic-microscopical studies revealed, the structure of spleen in quails of the experimental groups was similar to such of the control group. At the same time, in some cases, we observed somewhat more lymphocytes per unit area of the white pulp than in birds of the control group (Fig. 3b).

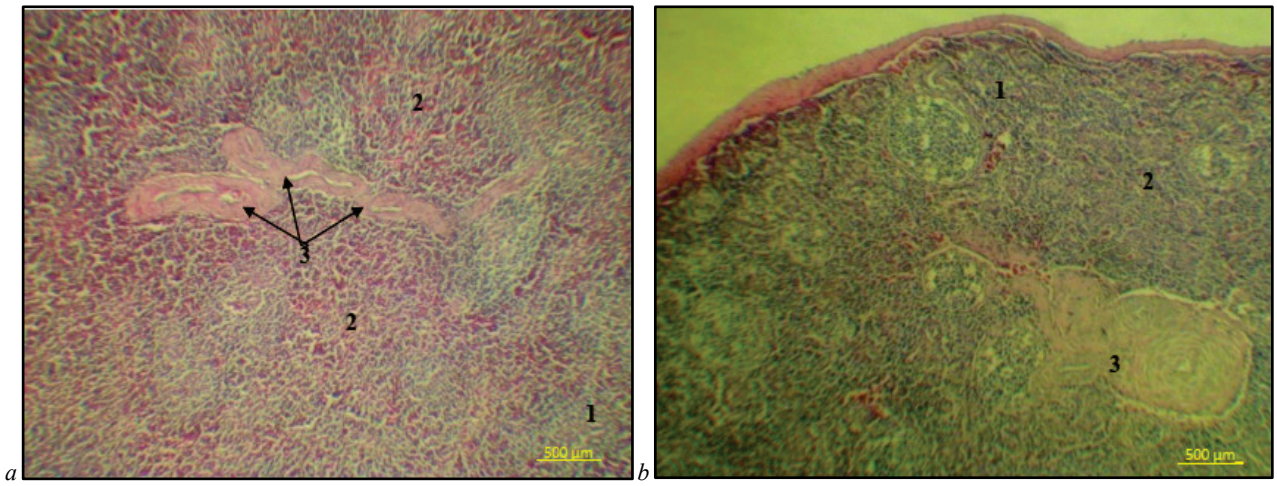

Fig. 1. Splenic pulp of the quails: *a* – sixty-day-aged control group: *1* – white pulp, *2* – red pulp, *3* – vessels (hematoxylin and eosin); *b* – experimental group 1 that received the Multilife protein-vitamin mineral supplement, 21st day of the experiment: *1* – white pulp, *2* – red pulp, *3* – vascular trabecula (hematoxylin and eosin)

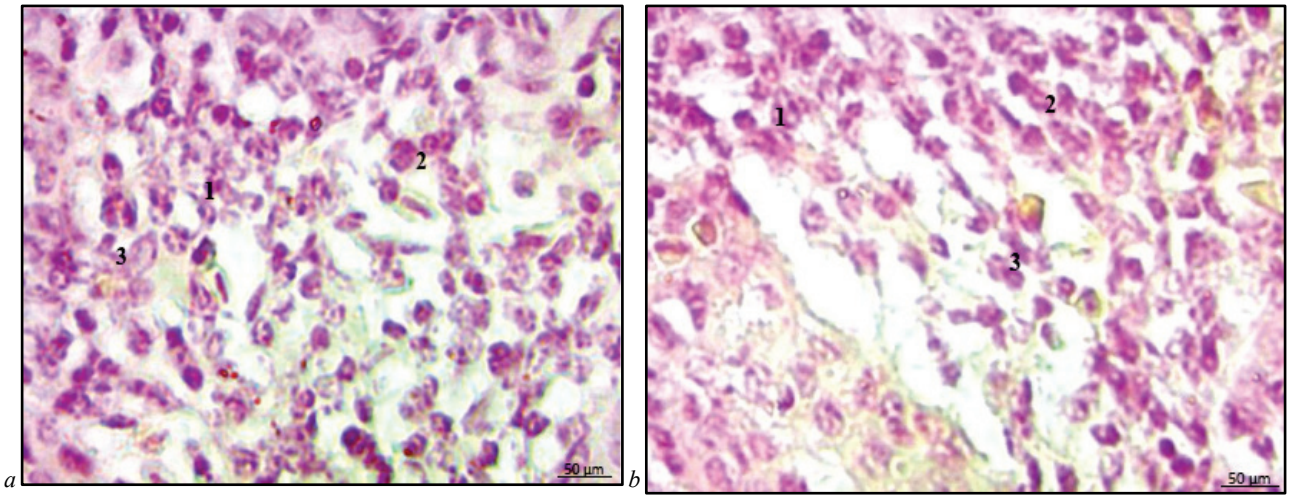

Fig. 2. Splenic pulp of the quails: *a* – sixty-day-old control group: *1* – lymphocytes, *2* – macrophages, *3* – eosinophilous leukocytes (hematoxylin and eosin); *b* – experimental group 1 that received the Missi protein-vitamin mineral supplement, 21st day of the experiment: *1* – lymphocytes, *2* – macrophages, *3* – eosinophilous leukocytes (hematoxylin and eosin)

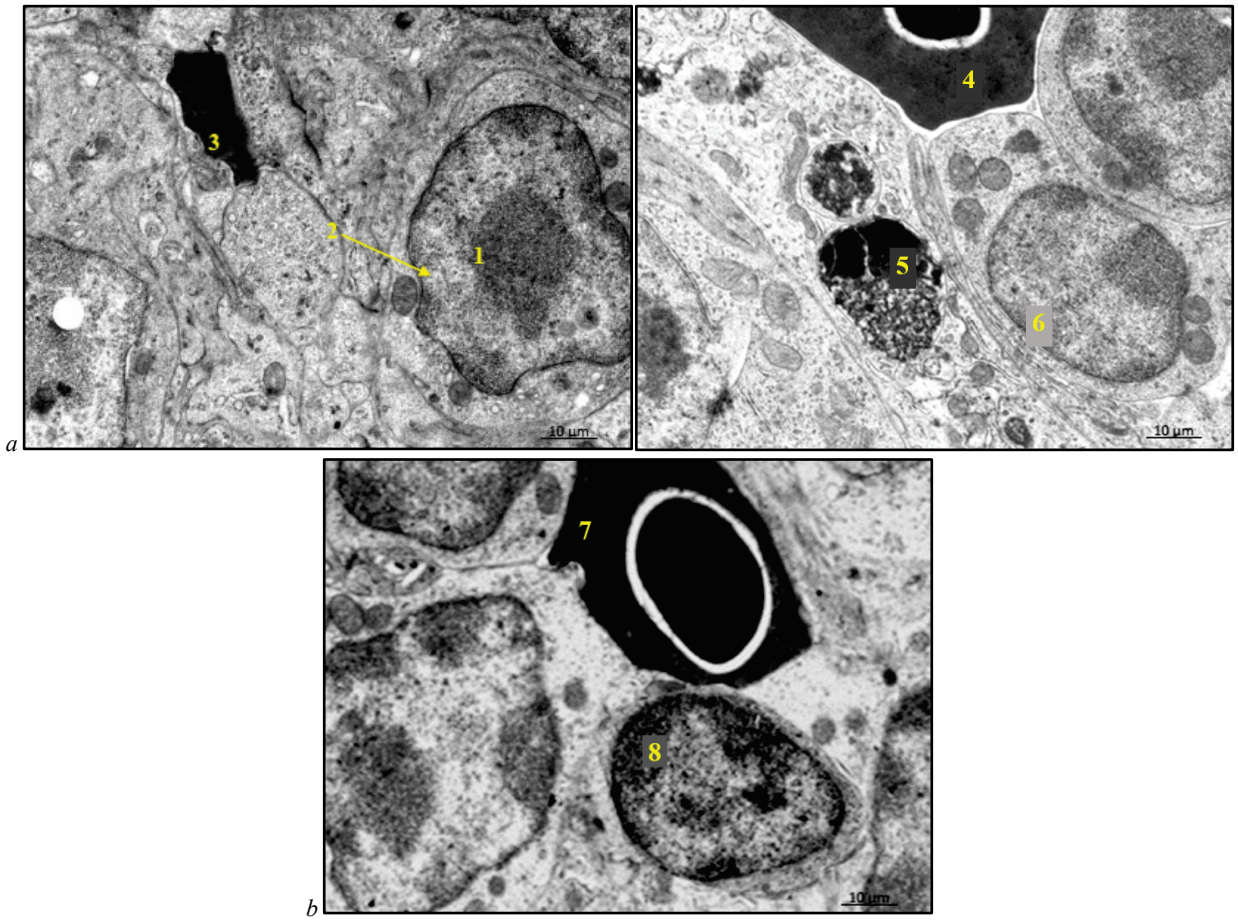

Fig. 3. Ultramicroscopic structure of the spleen of quails of the control group (*a*) and quails of experimental group 2 that received the Missi protein-vitamin mineral supplement (*b*), 21st day of the experiment: *1* – nucleolus, *2* – lymphocyte, *3* – erythrocyte in the lumen of venous sinus, *4* – lumen of venous sinus, *5* – residual bodies in endotheliocyte, *6* – lymphocyte, *7* – lumen of the venous sinus, *8* – lymphocyte

The studied protein-vitamin mineral supplements had a positive effect on the productivity of the quails, as evidenced by increase in weight of the body and the spleen. However, relative weight underwent practically no changes, characterizing this parameter as permanent for age and species of animals (Table 1).

At the same time, body weight of the quails increased by 5.8% in the group that had received the Multilife-supplemented diet, by 12.5% in the group that had consumed the Missi-supplemented diet, and by 8.1% in the group that had consumed the Standard Agro-supplemented diet. At the same time, weight of the spleen increased by 7.2%, 16.0% and 10.5%, respectively (Table 1). Subject to protein-vitamin mineral supplements, the histostructure in the experimental groups remained unaltered. In the splenic structure, the pulp and support-contractile apparatus differentiated distinctly. We observed a tendency towards increase in relative area of the pulp and the support-contractile apparatus in the experimental groups of the quails (Table 1). Therefore, maximal increase in relative area of the white pulp and relative area of the support-contractile apparatus was seen in the groups of birds that received the Missi supplement (0.7% and 1.0%, respectively), relative area of the red pulp underwent insignificant decrease within 1.7%. It has to be noted that this group was observed to have the most intensively growing parameters. We saw no changes in the ultramicroscopic structure of the quail spleen.

Table 1
Changes in weight and relative parameters of the spleen of the quails subject to protein-vitamin mineral supplements (x ± SD, n = 27)

Parameter	Control group	Group that received Multilife-supplemented diet	Group that received Missi-supplemented diet	Group that received Standard-Agro-supplemented diet
Absolute weight of spleen, mg	210.7 ± 6.9	225.8 ± 6.7	244.5 ± 7.4	232.9 ± 5.1
Relative weight of spleen, %	0.101 ± 0.004	0.102 ± 0.003	0.104 ± 0.005	0.103 ± 0.002
Relative area of white pulp, %	13.5 ± 2.1	14.0 ± 3.0	14.2 ± 3.2	14.1 ± 2.7
Relative area of support-contractile apparatus, %	4.6 ± 0.8	5.0 ± 0.8	5.6 ± 0.7	5.1 ± 0.9
Relative area of red pulp, %	81.9 ± 1.2	81.0 ± 1.7	80.2 ± 2.1	80.8 ± 1.3

Note: we found no significant difference between the groups; statistical analysis was performed using the Tukey test.

Discussion

In our studies, we used birds of both sexes. According to the results of a previous study, weight of the spleen had sexual dimorphism (Ojedapo & Amao, 2014). However, we found no significant difference between the absolute and relative weight of the spleen of males and females, which allowed us to identify the mean values of other parameters. In particular, relative weight is an important criterion of morphofunctional condition of the organ. Similarly to our experiment, the experimental groups in those studies (Ojedapo & Amao, 2014) were formed in 1:1 sex ratio. According to the results of our studies, in the composition of the splenic white pulp of the quails, we distinguished lymphoid nodes and periarterial lymphoid sheaths. According to our studies, the lymphoid nodes of the spleen had no light centers, and the periarterial zone was around the central artery of the node. Those data are consistent with the reports of other scientists, who determined that the spleen is fixated by a connective-tissue capsule of uneven thickness and is composed of unevenly distributed collagen, reticular, and elastic fibers with a small amount of muscle cells; trabeculae of the connective tissue were poorly developed; the splenic parenchyma was composed for indistinctly designated white pulp and red pulp in the network of reticular cells and reticular fibers (Kadam et al., 2019). In the white pulp, we also designated periarterial lymphatic tissue and ellipsoids (Ojedapo & Amao, 2014). Researchers pay great attention to effects of feeds and supplements on animals not only in order to increase profitability, but also to provide the population with ecological products. It has been determined that inclusion of Primix Bionorm K and Biovir biologically active supplements to the diets of animals increased their body weight by 7.5% and exerted positive effect on hematopoiesis (Senapati et al., 2015; Garmata, 2018; Flores-Santina et al., 2018). Usage of Proenzyme feed supplement in quail nutrition had a positive effect on the productivity, slaughter parameters, and weight of the internal organs. Therefore, weight of the spleen in the 56 day-old quails that had consumed Proenzyme in the dose of 0.1% of fodder weight increased from 0.114 ± 0.009 g to 0.143 ± 0.020 g (Baluh, 2016), which correlates with our studies.

Conclusion

Usage of Multilife, Missi, and Standard Agro protein-mineral vitamin supplements in feeding the Japanese quails positively influenced their organism, which was seen in increase in their body weight: the most effective was the Missi supplement, causing the highest increment in absolute body weight (12.5%), compared with the control. Less notable effects were exerted by the Standard Agro protein-vitamin mineral supplement (8.1%) and the Multilife supplement (only 5.8%).

Increase in body weight of the birds, which had consumed the supplements, promoted the tendency towards increase in the absolute weight of the spleen by 15.1 mg in the first; by 33.8 mg in the second, and by 22.2 mg in the third group. At the same time, relative weight of the spleen in all groups of the animals accounted for 0.11%, which is a stable parameter for this breed and age category of quails.

Micro- and ultramicrostructure of the spleen of the experimental groups corresponded to such of the control. However, the histometrical study revealed some morphometric peculiarities: 0.5% increase in the area of the splenic white pulp in quails of the first, 0.7% in the second, and 0.6% in the third group, compared with the control, indicating positive effect of protein-mineral vitamin supplements Multilife, Missi, Standard Agro on the morphogenesis processes in the spleen.

It would be promising to study the action of protein-vitamin supplements towards other organs and during their complex intake.

The presented study was conducted in adherence to the plans of scientific research of the Polissia National University and is a fragment of the research topic The Development, Morphology, and Histochemistry of the Organs of Animals in the Normal Condition and During Pathology (State Registration Number 0113U000900) by the Department of Normal and Pathological Morphology, Hygiene, and Expertise of the Faculty of Veterinary Medicine.

Authors declare no conflict of interests.

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