

# Part 1. Veterinary medicine

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## INFLUENCE OF PROBIOTICS ON HISTOSTRUCTURE OF THE BURSA OF FABRICIUS IN BROILER CHICKENS

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**Summary.** The article deals with the data on the influence of probiotic preparation BPS-44 and 2% yeast *Saccharomyces cerevisiae* on the histostructure of tissues of the cloacal sac (bursa of Fabricius) of broiler chickens. The conducted histological researches have shown that in chickens of the control group at the end of the experiment in the cloacal sac were revealed intraepithelial microcystic cavities, but in the medullary substance of the lymph nodes, necrotic changes are recorded and glandular structures are formed, indicating an insufficient level of lymphopoiesis.

The use of these probiotic preparations to broilers from the experimental groups during the period of their growth in the ration caused a normalizing effect on the morphostructure of the cloacal sac and, in particular, on the action of 2% yeast *Saccharomyces cerevisiae*. It is evidenced by the absence of the formation of microcystic cavities in the epithelial layer of the cloacal sac (signs of slowing down the processes of age involution). In this case, the lymph nodes are numerical, the division into cortical and medullary substance is clear, they were densely populated with lymphoid elements, indicating the possibility of forming an adequate immune response in the poultry of this group.

**Keywords:** probiotics, broilers, histology, cloacal sac

**Introduction.** At the present stage of the development of poultry farming in Ukraine it is important to ensure high profitability of production. Simultaneously, the intensive use of poultry in conditions of high density and significant influence of anthropogenic and natural factors leads to a decrease in the organism's resistance, an increase in morbidity and mortality (Lysko, 2008; Belova Gabzalilova and Topuria, 2009; Bessarabov et al., 2006; Temiraev et al., 2007). Therefore, the problem of increasing the viability of poultry is highly relevant and would allow maintaining high productivity and profitability of poultry farming.

Due to this, search for new pharmacological agents that increase the resistance and productivity of poultry has a huge economic significance. In this regard, the use of preparations containing the natural intestinal microflora — probiotics — is worthy of attention. The most important lever that regulates these processes is the specific products of the physiological microflora of the gastrointestinal tract (Subbotin, 1999; Subbotin, 1994; Subbotin and Sidorov, 1991, 1998; Nozdrin, 2003; Shevchenko, 2002; Ivanova, 2002). The researchers proved the possibility of replacing antibiotics with probiotics capable of influencing the body at the systemic level and participating in the work of regulatory systems, increasing the nonspecific resistance and resistance of young animals to diseases (Ivanova, 2007).

Along with this, it should be noted that the immunobiological reactivity of the organism of young poultry depends on a large extent on the functioning of the immune system. It is known that the feature of the immune system of chicken-broilers in the condition of intensive fattening is that they are in the stage of formation and initial development, which determines the originality of the response to antigenic stimulation and stress factors of the external and internal environment of the macroorganism.

In the poultry, there are central and peripheral lymphoid organs of the immune system. The central lymphoid organs include: the thymus, the bursa of Fabricius and the bone marrow (Bajdevljatov and Baydevlyatova, 2016; Khenenou et al., 2012). The bursa of Fabricius belongs to the lympho-epithelial organs, whose involution develops according to some data after 35 days (Krasnikov et al., 2006; Ribatti, Crivellato and Vacca, 2006), or even at a later date — in 160 and even 220 days according to others (Zharova, 2008; Mazurkevich, 2000). Probably such a rush in terms of involution may be related, in particular, to active immunization of the poultry, because this organ is responsible for the formation of humoral immunity, all processes occurring in it, are directly related to immunomorphology.

In the scientific literature, the investigation of the influence of probiotic preparations on the morphological

structure of immunocompetent organs, and in particular the cloacal sac are fragmentary and require detailed study.

Due to this, the purpose of the presented work was to elucidate the influence of the preparation BPS-44, made on the basis of the production strain of *Bacillus subtilis* bacteria and 2.0% yeast *Saccharomyces cerevisiae* on the histostructure of tissue of broiler chickens cloacal sac.

**Materials and methods.** The research was carried out on broiler chickens of the ROSS-308 cross, grown in one of the farms of the Lviv Region. The housing conditions of the chickens was standard, with free access to feed and water. Technological parameters of broiler rearing (temperature and light regime) were in accordance with the norms. Experiments were conducted in three groups of broiler chickens of 100 heads in each as shown in Table 1.

**Table 1** — Scheme of experiment

Groups	Name of the preparation	Scheme of preparation use	Age of the poultry, days
Control	No preparations were prescribed	—	4–43
Experimental 1	BPS-44	Three courses for 7 days in a row with 7 daily breaks	5–11 21–27 36–42
Experimental 2	<i>Saccharomyces cerevisiae</i> 2%	Constantly	4–43

The control group of broilers was fed the standard feed (SC) in accordance with the existing norms recommended for the ROSS-308 cross; the 1<sup>st</sup> experimental group in addition to the standard feed received a probiotic BPS-44 (registration certificate No. 2154-04-0254-06 dated 24 November 2006), made on the basis of the production strain of bacteria *Bacillus subtilis* ssp. *subtilis* 44-p, dose 0.21 g/kg, the 2<sup>nd</sup> experimental group — 2.0% of yeast *Saccharomyces cerevisiae*.

Vaccination of broilers was carried out in accordance with the scheme shown in Table 2.

**Table 2** — Vaccination scheme

Age, days	Vaccines	Method of input
11	BRONHIKAL® I SPF (Croatia)	watering
13	BIO-VAC La-Sota (Italy)	
15	GUMBOKAL IM FORTE SPF (Croatia)	

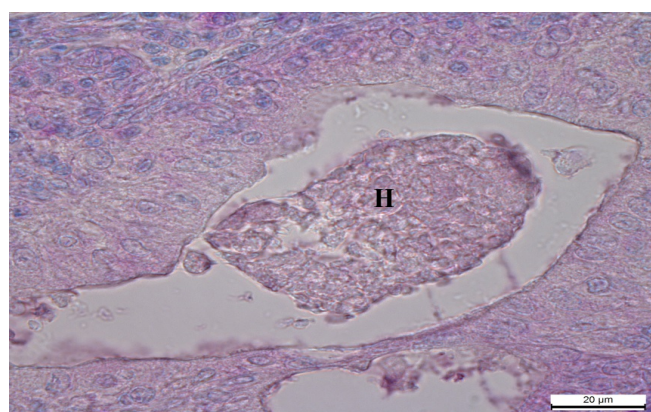
At the end of the experiment, which corresponded to the completion of the period of poultry breeding, was carried out the autopsy using the Shore method.

During the autopsy, pieces of the immune system organs, and in particular tissues of the cloacal sac for histological searches, were taken.

The material was fixed in 10.0% of neutral formalin solution and Buena liquid. The histological sections were made with the help of a microtome, were colored with hematoxylin-eosin, methyl-green and pyronin G according to Brach (Merkulov, 1969; Roskin and Levinson, 1957).

**Results.** Objective and correct method of morpho-physiological changes indications, occurring in the body under the actions of various environmental factors at the cellular-tissue level is the histological searches of the lymphoid tissue of the immune system.

For histological searches of chickens cloacal sac in the control group on many areas, the epithelial layer was infiltrated with lymphocytes, necrotic changes of epithelial cells were recorded, as a result of which, separate epithelial cells were desquamated into the lumen of the cloacal sac. Occasionally moderate proliferation of the epithelium was observed (Fig. 1).



**Figure 1.** Cloacal sac. Necrotic changes in the structural elements of the medullary substance of the lymph nodes. H — structural elements of the medullary substance of the lymph nodes. Methylene-green and pyronin for Brashe ×1000. Control group.

Microscopic cavities of various sizes were formed on many parts of the epithelial layer. The individual vessels of the mucous membrane itself were enlarged, filled with red blood cells and lymphocytes. Moderate infiltration of the loose connective tissue of the mucous membrane with plasma cells, lymphocytes, granulocytes, tissue basophils were recorded.

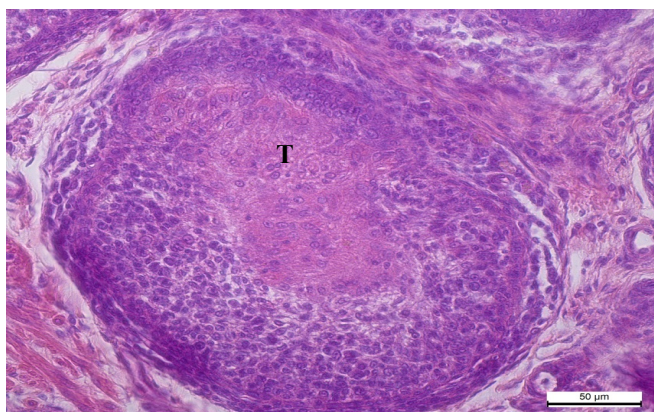
Cellular composition of lymphoid nodes was represented mainly by B-lymphocytes, as well as T-lymphocytes, lymphoblasts, proliferocytes, plasmacytes, macrophages and granulocytes. Among lymphocytes there were small, medium and large.

The medullary substance of most lymphoid nodules in the control poultry group contained a small amount of cellular elements (Fig. 2).

In certain lymphoid nodules, marked necrotic changes were developing, especially in the medullary substance. As a result, the central part of some lymphoid nodes were

filled with necrotic detritus. In the center of individual lymphoid nodes glandular structures were formed, which is a sign of inhibition of lymphopoiesis. The proliferation of lymphoblasts in lymphoid nodes was not significant. Lymphatic vessels of the stroma are expanded, lymphatic vessels were full. There was also an enlargement of the blood vessels that were full of red blood cells and single granulocytes. Occasionally there were perivascular edemas.

In some capillaries, red blood cells were in several rows, it is noted that they were glued, indicating the development of a stasis. Necrotic changes in individual endothelial cells were observed. In some vessels, slightly expressed proliferative processes of the endothelium were recorded. In adventitia, there is proliferation of pericytes. As well, there were perivascular infiltrations from plasma cells and lymphocytes.

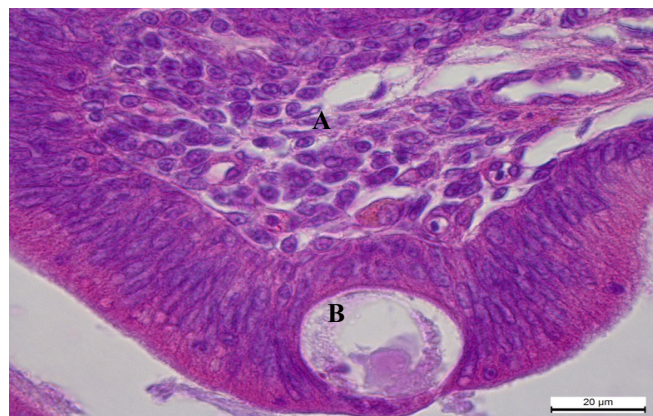


**Figure 2.** Cloacal sac. T —necrotic detritus in the central part of the lymphoid nodule  $\times 400$ . Control group.

Separate vessels of the muscle membrane were enlarged, filled with red blood cells and isolated granulocytes. Intramuscular connective tissue was somewhat swollen. The serous membrane was formed by a basic plate containing a slightly swollen loose connective tissue covered with a mesothelium.

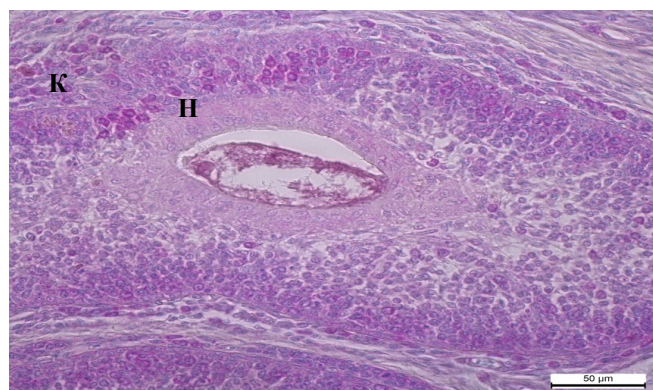
The mucous membrane of the cloacal sac was lined with a simple columnar epithelium, which in some areas went into a simple multi-row, contained a smooth groove (Fig. 3).

Somewhere epithelial proliferation was observed. Necrotic changes of epithelial cells were also registered, as a result of which epithelial cells are desquamated into the lumen of the cloacal sac. Microcystic cavities were formed on many parts of the epithelial layer, some of which contained a moderate amount of weakly basophilic content. Infiltration of the epithelial layer by lymphocytes was noted. Moderate infiltration of the mucous membrane by the cells of the lymphoid series, including plasmocytes, was observed. In the connective tissue of the mucous membrane and submucosal membrane, tissue basophils, as well as plasmocytes, single lymphocytes and granulocytes were visualized.



**Figure 3.** Cloacal sac. Intraepithelial cyst. Infiltration of the submucosal lymphocytes. A — infiltration of the submucosal membrane lymphocytes. B — intraepithelial cyst. Hematoxylin-eosin  $\times 1000$ . Control group.

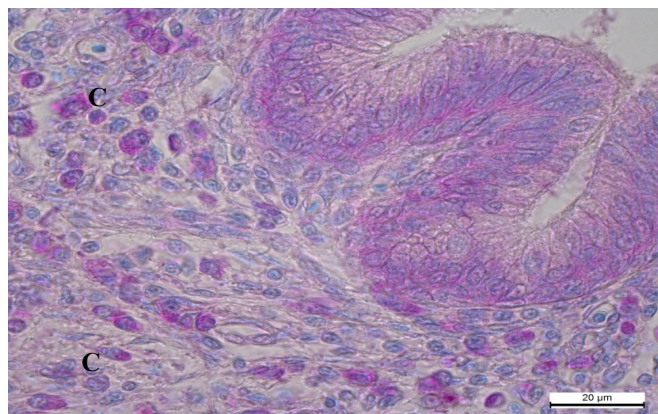
Lymphoid nodules were found in the organism of the broilers of the group fed probiotic preparation based on *Bacillus subtilis* ssp *subtilis* in the folds of the cloacal mucous membrane (Fig. 4). The division of most lymphoid nodes into cortical and medullary substances was preserved. There was a depletion of the medullary substance of most lymphoid nodes by cellular elements. In addition, in some lymphoid nodes, necrotic changes in the structural elements of the medullary substance were developed. As a result, the central part of some lymph nodes was filled with necrotic masses. Sometimes glandular structures were formed in the medullary substance, indicating the inhibition of lymphopoiesis. The proliferation of lymphoblasts in lymphoid nodes was expressed slightly. The number of cellular elements in the cortical substance was moderate. Occasionally there were necrotized lymphocytes. The number of macrophages that phagocyte the remains of necrotic cells increased.



**Figure 4.** Necrotic changes in the structural elements of the medullary substance of the lymph nodes. Moderate amount of B-lymphocytes in the cortical substance of the lymph nodes. H — necrotic changes of structural elements of the medullary substance, K — B-lymphocytes in the cortical substance of the lymph nodes. Methylene-green and pyronin for Brashe  $\times 400$ . Experimental group 1.

There was perivascular edema. In adventitia, there was proliferation of pericytes, which indicates the course of processes of physiological regeneration in tissues and the formation of new capillaries. There were available perivascular infiltrations from plasma cells, lymphocytes and single tissue basophils. Connective tissue around the hyperemic vessels were slightly swollen.

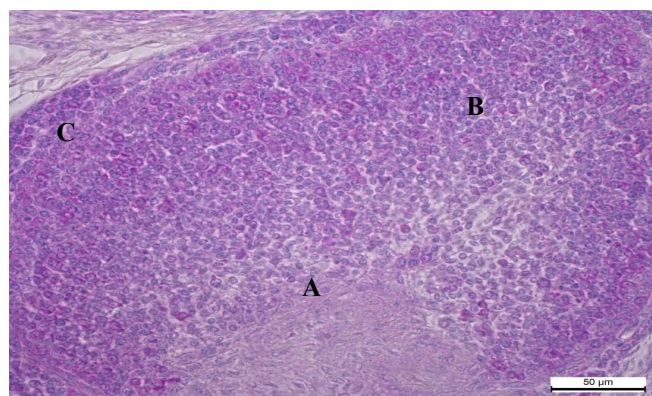
When carrying out the histological characteristics of the cloacal sac of chickens- broiler under the action of 2.0% yeast *Saccharomyces cerevisiae* it was found that the mucous membrane of the cloacal sac was uneven, within one fold the inequalities of the epithelial layer were intensified (Fig. 5). There was a proliferation of the epithelium, and in some areas moderate hypersecretion of mucus. Necrotic changes in epithelial cells were rare. Microscopic cavities in the epithelial layer did not occur. Moderate infiltration of the mucous membrane by the cells of the lymphoid series was observed. Plasma cells predominated in the specified cell infiltrates.



**Figure 5.** Cloacal sac. Significant amount of plasma cells in the connective tissue of the mucous membrane. C — plasma cells in the connective tissue of the mucous membrane. Methylene-green and pyronin for Brashe  $\times 1000$ . Experimental group 1.

Lymphoid nodes were located in the folds of the cloacal mucous membrane, and in most lymphoid nodes the division into medullary and cortical substance was distinct (Fig. 6).

The cellular composition of the lymphoid nodules remained unchanged for the introduction of 2.0% yeast *Saccharomyces cerevisiae* with the predominance of B-lymphocytes in the structure of the tissue. The proliferation of lymphoblasts in lymphoid nodes was well expressed. The number of cellular elements in the cortical substance was significant. Only in certain lymphoid nodes in the medullary substance, and to a lesser extent, in the cortical substance, there were single necrotized lymphocytes.



**Figure 6.** Cloacal sac. Clear division of lymph nodes into cortical and medullary substances. Moderate amount of cellular elements in the cortical substance of the lymph nodes. A — medullary substances, B — B-lymphocytes, C — cortical substance. Hematoxylin-eosin  $\times 400$ . Experimental group 2.

There was an enlargement of the blood vessels that were full of red blood cells and single granulocytes. There were also perivascular infiltrates that consisted mainly of plasma cells and lymphocytes. The individual vessels of the muscle were slightly enlarged, containing red blood cells and single granulocytes. No pronounced perivascular edema was detected. The subserous basis, which is also formed by a loose connective tissue was poorly expressed.

**Conclusions.** As a result of the histological search of the central organ of the immune system of broiler chickens it was found that in the poultry of the control group, which were fed with standard combined feed in the cloacal sac intraepithelial microvascular cavities were developed, and in the medullary substance of the lymph nodes necrotic changes were recorded and glandular structures were formed, indicating an insufficient level of lymphopoiesis.

In the poultry of the experimental group 1, which were fed with the probiotic BPS-44 in the epithelial layer of the cloacal sac, isolated single microcystic cavities were detected, and necrotic changes in the medullary substance of the lymph nodes were recorded.

The most optimal morphological state of the cloacal sac was found in the chickens of the second experimental group, which were fed with 2% yeast *Saccharomyces cerevisiae* in the composition of the mixed fodder. In particular, we did not register the formation of microscopic cavities in the epithelial layer of the cloacal sac, lymph nodes were numerical, the division into cortical and medullary substance was clear, they were densely populated with lymphoid elements, which indicate the possibility of forming of complete immune response in the poultry of this group.

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