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# Pathoanatomic changes in carcasses of broilers following complex use of preparations based on lactic acid and chlorine dioxide and probiotic supplements in the diet


V. L. Kovalenko*, V. G. Pavlunko*, O. M. Chechet**, O. B. Lozhkina***, V. V. Lisova****, M. E. Romanko*, N. V. Liniichuk*

** State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine*
***Odesa State Agrarian University, Odesa, Ukraine*
****Research of the Central State Testing Laboratory of the State Service for Food Safety and Consumer Protection in Kyiv Region and Kyiv, Kyiv, Ukraine*
*****National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine*



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*State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Donetska st., 30, Kyiv, 03151, Ukraine. Tel.: +38-067-756-86-44. E-mail kovalenkodoktor@gmail.com*

*Odesa State Agrarian University, Krasnova st., 3a, Odesa, 65049, Ukraine. Tel.: +38-098-493-02-99. E-mail: kiev-kiev12@ukr.net*

*National University of Life and Environmental Sciences of Ukraine, Heroyiv Oborony st., 5, Kyiv, 03041, Ukraine. Tel..: +38-098-411-18-45. E-mail lisova_vv@nubip.edu.ua*

*Research of the Central State Testing Laboratory of the State Service for Food Safety and Consumer Protection in Kyiv Region and Kyiv, Kyiv, Ukraine.*



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Chicken farming is one of the commonest and most available sources of dietary chicken meat in diet of most people, and methods of intensive raising of broiler chickens make this sphere increasingly profitable. A complex use of probiotic and biocide preparations in commercial breeding of broiler chickens is based on the principle of environmental cleanliness+health balance: Biocides (based, for example, on lactic acid or chloride dioxide) destroy pathogenic microorganisms in the environment (premises, equipment, water), preventing initial infection of the chickens and formation of biofilms, while probiotics (*Lactobacillus*, *Bacillus*, *Bifidobacterium*, etc.) colonize the intestines of chickens, competitively inhibiting pathogens (*Salmonella*, *E. coli*, *Clostridium*), thereby stimulating the immunity and improving feed metabolism. The broiler chickens raised according to the traditional raising method experienced a complex of subclinical conditions, such as protein myocardiodystrophy (71.3% of the chickens), protein steatosis (14.2% of the chickens), protein nephrotic syndrome (100.0% of the chickens), proventriculitis (81.3% of the chickens), catarrhal inflammation of the small intestine (76.2% of the chickens), early thymic involution (100.0% of the chickens), and involution of cloacal sac (100.0% of the chickens). On the other hand, no subclinical organ damages were observed in all the broiler chickens raised using a new probiotic complex that contains a probiotic based on *Bacillus subtilis*, *B. licheniformis*, *B. coagulans*, *Enterococcus faecium*, *Lactococcus lactis*, magnesium, chitosan, enzymes, grist of *Silybum*, given in a dose of 0.5 mg/kg of feed on days 1–7 and 22–27, and a probiotic based on *Bacillus subtilis*, *B. amyloliquefaciens*, and aluminosilicate, which was evenly sprayed in a room for poultry in a calculation of 10–30 g/m$^2$ on days 7 and 22. That is, this method allowed producing safe high-quality meat products without residual antibiotics, and also improving the quality of offal (heart, liver, stomach), which enter domestic and foreign markets. However, their gain in live mass did not statistically significantly differ from that in the broiler chickens raised using the traditional technique. When raising broiler chickens using a biocide based on chlorine dioxide in a dose of 1.0 mg/L of water and a biocide based on lactic acid, sprayed in a concentration of 0.5%, 0.3–0.5 L/m², with a three-hour exposure, we observed not only the absence of any subclinical lesions of their organs, but also a statistically significant (compared with the traditional technology of raising) increase in the live-mass gain in the broiler chickens, accounting for 18.3 ± 0.3%. Therefore, synergy of active compounds of both the groups allowed significantly reducing the use of antibiotics and decreasing mortality, while increasing the mass gains, and providing a high biosafety of the flock under intensive raising conditions. Moreover, the protective reactions of the body were successfully optimized, thereby enhancing the resistance to infections, as well as the productivity in industrial poultry farming.



*Keywords*: broiler chickens; macroscopic changes; protein myocardiodystrophy; steatotic liver disease; nephrotic syndrome; proventriculitis; disinfecting agents; lactic acid; chlorine dioxide; probiotics.


## Introduction

In the modern intensive poultry farming, prophylaxis of infectious diseases is based on complete elimination of pathogens at all development stages (Ponomarenko et al., 2021; Stegniy et al., 2023). The effectiveness of such prophylaxis is determined by the ability to disrupt the epizootic chain at all levels, from the environment to the internal barriers of the birds' bodies.

The key role in prophylaxis of infectious diseases of poultry is played by biocides that destroy pathogenic microorganisms in poultry premises, thereby ensuring bird safety. Those products include various chemical compounds (such as oxygen peroxide, peroxylactic and lactic acids, chlorine dioxide, etc.) that provide effective disinfection, are nontoxic to poultry, cause no serious corrosion on the equipment, and break down quickly. Rotation of biocides of different chemical classes prevents the microorganisms from developing a resistance to those preparations (Albero et al., 2018; Azirkina, 2020; Haque et al., 2024).

The most effective biocide preparations for prophylaxis of infectious diseases of poultry are those that eliminate bacteria, viruses, fungi, and spores through chemical oxidation or biological antagonism (Rutala & Weber, 2019; Tarka & Nitsch-Osuch, 2021; Wales & Davies, 2021). Modern disinfectants have a broad spectrum of action in a broad range of temperature and pH, maintain stability in the presence of organic loading, exert no teratogenic, carcinogenic, or allergic action, minimally corrode the equipment, and break down quickly. In Ukraine, the annual use of biocides in poultry farming exceeds 3,200 t, and the register of State Food Service includes 161 such products, 94% of which are specialized for poultry farms (Dyshlyuk & Orlova, 2017; Kovalenko et al., 2018; Chechet et al., 2022; Kupnevska et al., 2022).

However, an increase in the microbial resistance (plasmid-mediated resistance to antibiotics and chromosomal resistance to oxidizers) necessitates quarterly rotation of biocide compounds that belong to at least three chemical classes, otherwise 9–12 months later colonies of *Salmonella enteritidis* recover their numbers by 3–4 $\log_{10}$ (Albero et al., 2018; Azirkina, 2020; Haque et al., 2024).

Along with biocide products, a biological vector of prophylaxis of poultry infections is forming – probiotics. According to the definition of FAO/WHO (Hill et al., 2014), probiotics are live microorganisms that provide clinically confirmed efficacy when introduced at a level of $\geq 10^6$ CFU/g of feed or $\geq 10^8$ CFU/m³ of air. In COBB-500 broiler chickens, probiotics increase gain in live mass by 8–14%, improve feed conversion by 0.11–0.17 kg/kg, and reduce mortality by rate 38–46% (Reuben et al., 2021; Cai et al., 2024). Probiotics normalize microbiota, decontaminate premises, and remove unpleasant odors (Feye et al., 2020; Stefaniak et al., 2020). Therefore, the priority for the improvement of metabolism and productivity of poultry is polyfunctional supplements (Lyasota et al., 2020; Prylipko et al., 2021).

Probiotics are a biological alternative to antibiotics. They contain live microorganisms (*Bacillus subtilis*, *B. licheniformis*, and other bacteria of the genus) that improve gut microflora, create a biological barrier against pathogenic microorganisms, and boost immunity and metabolic activity in poultry. Due to such an action, probiotics improve feed conversion, increase body-mass gain, and reduce toxic loading and mortality (Hill et al., 2014; Feye et al., 2020; Reuben et al., 2021; Cai et al., 2024). Complex application of biocides and probiotics in poultry farming enhances the efficiency of prophylaxis, optimizes the microclimate in poultry houses, and increases the survival and productivity of the poultry (Chechet et al., 2022; Chechet, 2023; Stegniy et al., 2023).

Therefore, an integrated approach consisting of a quarterly rotation of biocide preparations and the use of probiotics with clinically confirmed effectiveness is an optimal strategy of veterinary-sanitary prophylaxis of infectious diseases in modern intensive poultry farming in Ukraine.

The objective was to conduct pathoanatomic analysis of slaughtered broiler chickens of the COBB-500 cross (aged 42 days) following the application of a complex of probiotics and bactericidal agents.

## Materials and methods

The experimental studies were performed in a vivarium of the State Scientific Research Institute of Laboratory Diagnostics and Veterinary-Sanitary Expertise. The studies were carried out according to the positions of The General Ethic Principles of Experiments on Animals, adopted by the First National Congress of Bioethics (Kyiv, 2001) and with adherence to the requirements of international principles of European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasburg, 1986), Rules of Conducting Work Using Experimental Animals, according to the Order of the Ministry of Healthcare as of November 1, 2000, No. 281 On Measures Regarding Further Improvement of Organizational Forms of Work Using Experimental Animals, and with adherence to the Law of Ukraine On Protection of Animals from Abuse (No. 3447-IV as of February 21, 2006, Kyiv) (Nichiporuk et al., 2022).

The birds of the control and experimental groups were kept in the institute's vivarium following the current sanitary rules of designing, furnishing, and operating experimental-biological clinics (vivariums) at a temperature of 18–20 °C and relative air humidity of 50–55%.

The broiler chickens of the cross COBB-500 aged five days were divided into three groups of 21 individuals each. The chickens of all groups were fed the same standard commercially produced feeds according to the standard scheme and the norms for broiler chickens, which before the age of 15 days were fed with the Starter mixed feed, and starting from the age of 15 days with the Grover mixed feed. The control-group chickens were raised according to the standard method with addition of antibiotics and vitamins to the feed according to the traditional feeding scheme. The broiler chickens had free access to water.

The chickens of Group 1, together with feed, received the probiotic Biomagn (*Bacillus subtilis*, *B. licheniformis*, *B. coagulans*, *Enterococcus faecium*, *Lactococcus lactis*, magnesium, chitosan, enzymes, grist of *Silybum*) in a dose of 0.5 mg/kg of feed on days 1–7 and 22–27. In addition, in the premises where they were kept, the probiotic preparation Biozapin (*Bacillus subtilis*, *B. amyloliquefaciens*, aluminosilicate) was evenly sprayed in a calculation of 10–30 g/m² on days 7 and 22.

The chickens of Group 2, together with water, consumed the biocide preparation Diolaid (the main active compound is chlorine dioxide) in a dose of 1.0 mg/L of water. Additionally, the room where they were held was disinfected once a week, using the biocide preparation Biolaid (the main active compound is lactic acid) in a concentration of 0.5%, 0.3–0.5 L/m², with three-hour exposure time (Table 1).

Slaughter of all the broiler chickens was performed at the age of 42 days, that is the entire experiment lasted for 37 days. After slaughter, their pathoanatomic necropsy and evaluation of the state of the organs and tissues were performed according to RI.DNDILDVSE 7.2-7-01 Performing Pathoanatomic Necropsy of All Species of Animals and Poultry and Identification of Causes of Their Death (Chechet, 2023).

**Table 1**
Scheme of the drug application

| Drug | Group 1, n = 21 | Group 2, n = 21 | Control, n = 21 |
|---|---|---|---|
| Biomagn | days 1–7; 22–27 | days 1–7; 22–27 | – |
| Biozapin | days 7; 22 | days 7; 22 | – |
| Diolaid | – | throughout the experiment | – |
| Biolaid | – | once a week | – |
| Antibiotics | – | – | days 5–10 |
| Vitamins | – | – | 5–10 |

The results of the experiment were statistically analyzed in the Statistica 10 software (StatSoft Inc., USA). We calculated standard deviation (SD) and means (x). The differences between the groups were calculated with the Tukey Test, where the differences were considered significant at $P < 0.05$.

## Results

At the beginning of the experiment, mean body mass was the highest in the Control chickens, the lowest in the Group 2 chickens, and intermediate in the Group 1 chickens (Table 2). At the end of the experiment, mean body mass was the highest in the chickens of Group 2, the lowest in the Control chickens, and the chickens of Group 1 exhibited an intermediate value. Accordingly, the mean gain of body mass at the end of the experiment was observed in the chickens of the Group 2, and the lowest in the chickens of Control, while the chickens of the Group 1 had an intermediate value.

**Table 2**
Parameters of changes in body mass
of the broiler chickens during the experiment (n = 63)

| Parameters | Groups of broiler chickens | | |
|---|---|---|---|
| | Group 1 | Group 2 | Control |
| Body mass at the beginning of the experiment, kg | 0.13 ± 0.04 | 0.12 ± 0.04 | 0.14 ± 0.01 |
| Body mass at the end of the experiment, kg | 2.73 ± 0.43 | 2.81 ± 0.10* | 2.38 ± 0.24 |
| Gain in body mass at the end of the experiment, kg | 2.59 ± 0.43 | 2.68 ± 0.10 | 2.23 ± 0.24 |
| Percentage of the body mass at the end of the experiment, compared with the control group, % | 116 ± 19 | 120 ± 14 | 100 |

*Note*: $P < 0.05$.

However, as we see in Table 2, individual parameters of body mass (2.73 ± 0.43 kg) and individual parameters of gain of body mass

(2.59 ± 0.43 kg) of the broiler chickens of Group 1 at the end of the experiment were overlapped with individual parameters of body mass (2.38 ± 0.24 kg) and individual parameters of gain of body mass (2.23 ± 0.24 kg) in Control at the end of the experiment. Therefore, in general, the difference in body mass between the two groups of the broiler chickens at the end of the experiment was not statistically significant (P > 0.05). Such data indicate that intake of the probiotic Biomagn by the broiler chickens along with the probiotic preparation Biozapin had varying effects on the individuals, but was characterized by an upward tendency in their body mass compared with the traditional raising method, because the body-mass gain at the end of the experiment in the broiler chickens of Group 1 was 116 ± 19%, compared with the control group.

At the same time, individual parameters of body mass (2.81 ± 0.10 kg) and individual parameters of body-mass gain (2.68 ± 0.10 kg) of the broiler chickens of Group 2 at the end of the experiment did not overlap with individual values of the body mass (2.38 ± 0.24 kg) and body-mass gain (2.23 ± 0.24 kg) in Control at the end of the experiment (Table 2). Therefore, in general, the difference in body mass between these two groups of broiler chickens at the end of the experiment was statistically significant (P < 0.05). Such data indicate that pairing the probiotics Biomagn and Biozapin and pairing the biocide preparations Diolain and biocide Biolaid had the same positive effect on all the experimental broiler chickens, leading to a statistically significant increase in their body mass compared with the traditional raising method. At the same time, the body-mass gain at the end of the experiment in the broiler chickens of Group 2, compared with the control, measured 120 ± 14%.

Survival of the birds in all groups was 100%.

The pathoanatomic necropsy of the slaughtered control-group COBB-500 broiler chickens aged 42 days revealed no macroscopically noticeable changes on their skin and skin derivatives. The skeletal musculature of all the individuals was well-developed, the muscles had a uniform pale-pink color, were moist and elastic, with a distinct fibrous structure on the section (Fig. 1). The skeleton corresponded to the age standards: Both tubular and flat bones were covered by glossy periosteum, were of regular form, with no macroscopic changes. The subcutaneous adipose tissue was developed completely. The fatty depots (subcutaneous, thoracoabdominal, and subepicardial adipose tissues) contained light-yellow, soft, moist fat with no visible changes.

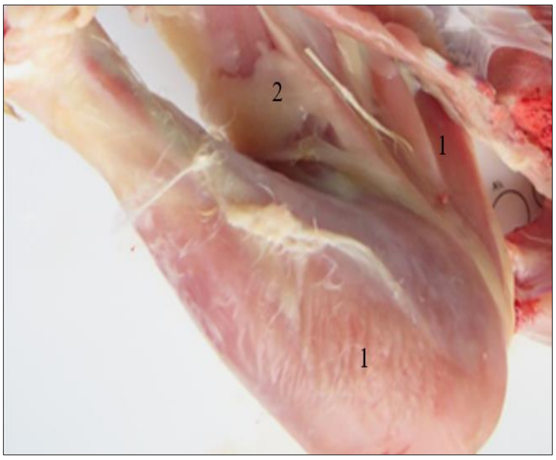


**Fig. 1.** Fragment of the carcass of the control broiler chicken:
*1* – limb muscles; *2* – subcutaneous adipose tissue

The arrangement of the organs of the thoracoabdominal cavity was regular. All the internal organs (heart, liver, spleen, stomach, intestines, kidneys, lungs, and reproductive organs) had physiological development corresponding to the age of 42 days (Fig. 2).

The thymus in all the Control broiler chickens was soft, elastic, pale-pink, with a nacre tint, had a somewhat wrinkled capsule and a notable lobular structure. The lobules varied in size (from 3–4 mm to 7–8 mm), and were evenly surrounded by the adipose tissue. On the section, the cortex and medulla were clearly differentiated. No macroscopic changes were observed.


**Fig. 2.** The organs of the thoracoabdominal cavity
of the control broiler chicken: *1* – heart; *2* – liver; *3* – intestines

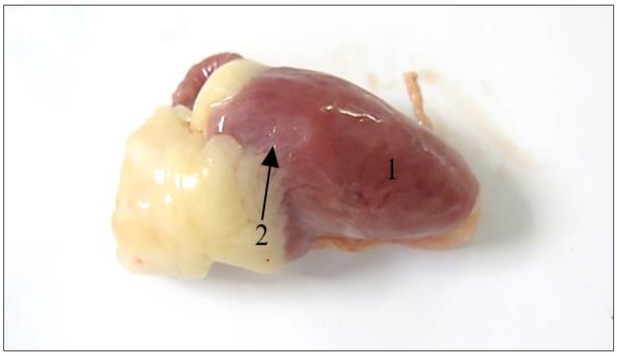


**Fig. 3.** The heart of the control broiler chicken:
*1* – pink-red region; *2* – grayish region

The heart had a classic cone-like shape. The pericardium was thin, smooth, glossy, and translucent. Under the epicardium, yellowish fatty inclusions localized. The coronary vessels were noticeably filled with blood. The lumen of the atrium contained small postmortem blood coagulants. The heart valves had no macroscopically visible changes. In 71% of the broiler chickens of the control group, the myocardium had a varying color: Regions of saturated pink-red color alternated with brighter sites of grayish tint, ranging in size and shape, which is a sign of protein myocardiodystrophy (Fig. 3).

The lungs had a distinct anatomical shape, soft consistency, were elastic, of uniform pink-red color. On the section, the pattern of the parabronchial complexes was distinct; the blood vessels contained a moderate amount of blood. The Galen's lung float test confirmed the normal filling of the organ with air: Pieces of the lungs were freely floating on the water surface. Macroscopic changes were absent.

The spleen had rounded-oval shape, was glossy and elastic, had a capsule, was of dark-brownish color, and of somewhat flaccid consistency. On the section, the pulp was dark-red, notably granular, and the cut surface of the pulp was minimal (Fig. 4).

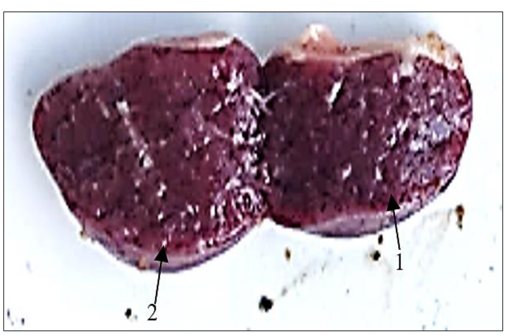


**Fig. 4.** The spleen of the control broiler chicken on the section:
*1* – red pulp; *2* – white pulp

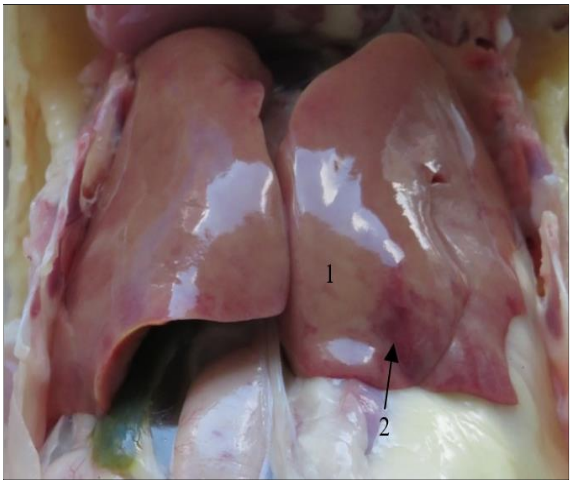


**Fig. 5.** The liver of the control broiler chicken:
*1* – region of a clayey color; *2* – bluish region

The liver in most of the broiler chickens had no macroscopic changes. Nevertheless, 14.2% of the broiler chickens presented with signs of protein steatosis: tension of the capsule, rounded margins of the organ, flaccidity of the parenchyma, nonhomogeneous color on the outer surface (regions of clayey color alternated with regions of bluish color), and a pale and nonhomogeneous parenchyma on the section (Fig. 5). The bladder contained homogeneous yellow-green bile of somewhat liquid consistency. The patency of the bile ducts was preserved.

The proventriculus in most (81%) of the Control broiler chickens was in the state of proventriculitis: The mucous membrane was swollen, with loci of hyperemia, covered by a thick layer of pale mucus, and the surface was uneven. The glands of this section of the stomach were enlarged, and a viscous gray-white mass secreted upon pressing. No content was present. The gizzard was rounded, with elastic consistency, and filled with feed masses. The cuticle was easily removable, the mucous membrane beneath it was pale-pink and moist. The mucous membrane was dark-red, whereas the serous membrane was dark-pink.

The small intestine of most (76%) of the Control broiler chickens was catarrhally inflamed: The serous membrane was gray-pink, pale, swollen, with single point-like hemorrhages, covered by a thin layer of mucus. The lumen contained a foam-like yellowish-orange viscous fluid with admixtures of mucus and undigested fodder (Fig. 6). In the large intestine, no macroscopic changes were observed.


**Fig. 6.** The small intestine of the control broiler chicken:
*1* – yellowish-orange viscous fluid with admixtures of mucus;
*2* – point-like hemorrhage

The kidneys of all the control broiler chickens were enlarged: The capsule was tensed, the surface was lumpy, and the parenchyma on the section was protruding. Their consistency was somewhat densified, and the coloration was not uniform, ranging from light-gray to dark-brown, indicating the presence of protein nephrotic syndrome (Fig. 7).

The cloacal sac in all the control broiler chickens was involuted: Its volume was reduced, the shape was oval, the consistency was loose, and the mucous membrane was swollen and pale (Fig. 8).

**Fig. 7.** The kidneys of the control broiler chicken
(indicated with arrows)

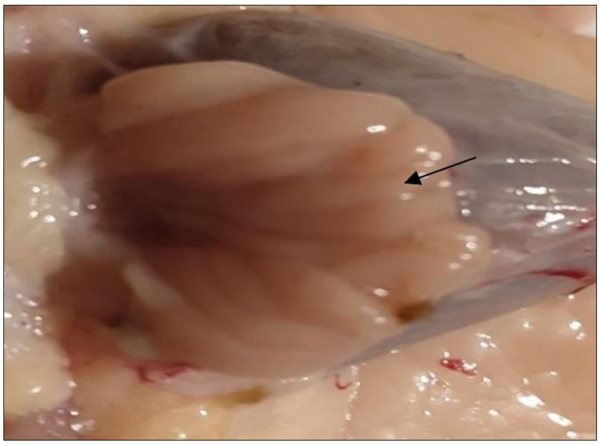

**Fig. 8.** The cloacal sac of the control broiler chicken:
pale, swollen mucous membrane (indicated with arrows)

During the pathoanatomic examination of the slaughtered 42 days-old COBB-500 broiler chickens of the experimental groups 1 and 2, we observed a similar macroscopic appearance of all the organs and tissues in both the groups. Their skin and skin derivatives, similarly to the broiler chickens of control, exhibited no macroscopically noticeable changes. The skeletal musculature of all the individuals was well-developed, the muscles had a uniform pale-pink color, were moist and elastic, with a distinct fibrous structure on the section. The subcutaneous adipose tissue was completely formed. Fatty depots (subcutaneous, thoracoabdominal, and subepicardial adipose tissues) contained light-yellow, soft, moist fat with no visible changes. The skeleton corresponded to the age standards: Both tubular and flat bones were covered with glossy periosteum, were of regular form, with no macroscopical changes.

The thymus was elastic, pale-pink, with a nacre tint, and an expressed lobular structure. Unlike the broiler chickens of the control group, the organ's capsule was not wrinkled, and all the lobules had approximately the same size (6-8 mm), and each lobule on the section had clearly differentiated cortex and medulla. No adipose tissue was macroscopically seen between the lobules. As in the Control broiler chickens, no macroscopic changes were observed.

The arrangement of the organs of the thoracoabdominal cavity was anatomically regular. All the internal organs (heart, liver, spleen, stomach, intestines, kidneys, lungs, and reproductive organs) had a physiological level of development corresponding to the age of 42 days and exhibited no macroscopic pathoanatomic changes (Fig. 9).

The heart (similarly to the control broiler chickens) had a classical cone-like shape. No macroscopically noticeable changes were observed in the pericardium, epicardium, endocardium, and the heart valves. Under the epicardium, yellowish fatty depositions localized. The myocardium, in contrast to the Control broiler chickens, was elastic, of quite uniform pink-red color (Fig. 10).

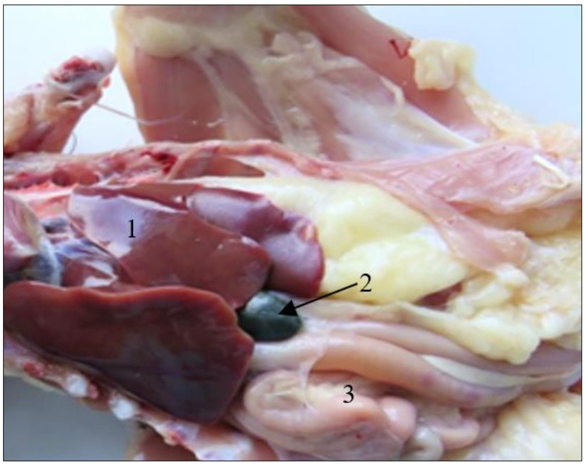


**Fig. 9.** The organs of the thoracoabdominal cavity of the group 1 broiler chicken: *1* – liver; *2* – bladder; *3* – intestines

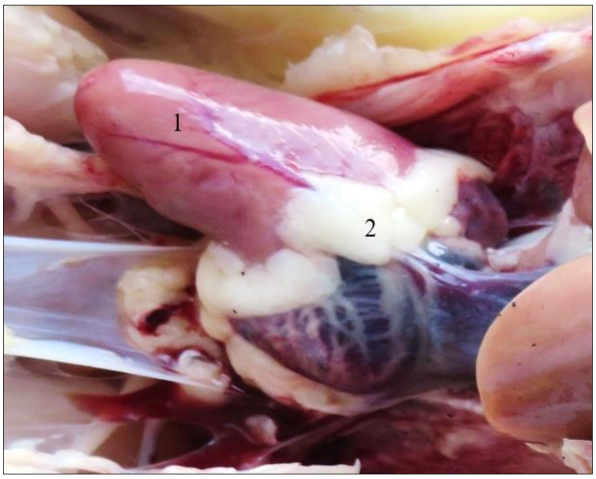


**Fig. 10.** The heart of the group 2 broiler chicken: *1* – myocardium; *2* – fatty inclusions

The lungs, similarly to the control broiler chickens, had a typical anatomic shape, soft consistency, elasticity, and uniform pink-red color. On the section, the pattern of the parabronchial complexes was distinct; the blood vessels contained a moderate amount of blood. The Galen's lung float test confirmed the normal filling of the organ with air: Pieces of the lungs freely floated on the water surface. No macroscopic changes were observed.

The spleen had the same macroscopic appearance as in the control broiler chickens.

The liver, in contrast to the control broiler chickens, had no macroscopic changes. The volume of the organ was not enlarged, as evidenced by its sharp margins, a slightly tensed capsule, and no protrusion of the parenchyma on the section. The color of the liver on the surface and on the section was uniform, brown, and the surface of the section was glossy and moist (Fig. 11). The bladder was moderately filled with homogeneous yellow-green bile, and the patency of the bile ducts maintained.

The proventriculus, by contrast to the control broilers, presented with no macroscopic changes. In the gizzard, similarly to the control broilers, no macroscopic changes were observed (Fig. 12).

The small and large intestines exhibited no macroscopic changes. Their serous membrane was gray-pink (Fig. 13), and the mucous membrane was pale-pink, covered by a thin layer of translucent gray-white mucus, with distinct folds. The kidneys and cloacal sac demonstrated no visible changes.

In general, the results we obtained indicate that the replacement of growth stimulators in poultry farming with a symbiotic-biocide complex prevented subclinical pathologies in 14.2% of the broilers, leading to elevated growth rates and increased yield of category A meat.

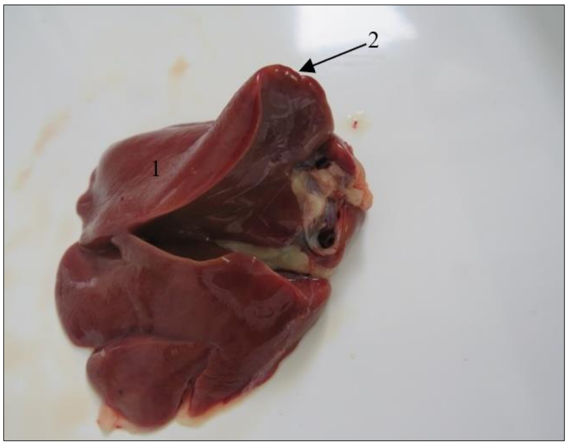


**Fig. 11.** The liver of the group 1 broiler chicken: *1* – even color of the organ; *2* – sharp margin of the organ

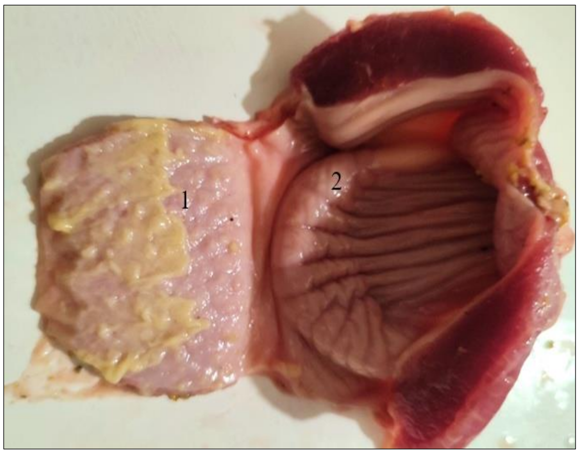


**Fig. 12.** The stomach of the group 1 broiler chicken: *1* – proventriculus; *2* – gizzard


**Fig. 13.** The intestines of the group 2 broiler chicken: *1* – small intestine; *2* – large intestine

## Discussion

The results of our study indicate that the body mass in the Control broilers at the end of the experiment measured 2.23 ± 0.24 kg. In the group 1 broilers, the body mass at the end of the experiment accounted for 2.73 ± 0.43 kg, but was not statistically significantly different from that of control. In the group 2 broilers, the body mass at the end of the experiment measured 2.81 ± 0.10 kg and statistically significantly differed from that of the control broilers ($P < 0.05$). At the same time, the gain in the live mass of the group 2 broilers was 18.3 ± 0.3 % higher, compared with the control broilers.

In the control broilers, the gain in the body mass during the experiment accounted for 2.23 ± 0.24 kg. In the group 1 broilers, the gain in the body mass at the end of the experiment equaled 2.59 ± 0.43, that is, was 116 ± 19 % higher than in control. Meanwhile, in the group 2 broilers, the gain in the body mass at the end of the experiment measured 2.68 ± 0.10, i.e. 120 ± 14% higher compared with control. The survival of the population in all the groups of broilers was 100%.

Those data verify the earlier provided results indicating that in the experimental-group broilers, subjected to the complex of either Biomagn (in fodder) and Biozapin or Diolaid and Biolaid, the body mass accounted for 0.054 kg already on day 10, being 15% higher than in the control (Chechet, 2023). On day 20, the live mass of the broiler chickens in the experimental group that consumed Biomagn exceeded the control by 9.7% and 12.9%, respectively (Cui et al., 2004; Chechet et al., 2022). As demonstrated earlier, the symbiotic Biomagn paired with the probiotic Biozapin and the biocide complex consisting of Diolaid and Biolaid display a synergic action toward digestion, absorption, and immune reaction, significantly optimizing the metabolic processes in the broilers of the cross COBB-500, having increased the feed conversion by 9.4% (Chechet et al., 2022 Motola et al., 2023).

In a large number of the control broiler chickens, raised according to the traditional method, the pathoanatomic necropsy revealed protein myocardiodystrophy (71.0% of the chickens), protein steatosis (14.2% of the chickens), protein nephrotic syndrome (100.0% of the chickens), proventriculitis (81.0% of the chickens), catarrhal inflammation of the small intestine (76.0% of the chickens), early thymic involution (100.0% of the chickens), and cloacal sac involution (100.0% of the chickens).

Earlier, it was determined that protein myocardiodystrophy in broiler chickens, occurring during traditional raising, is microscopically characterized by vacuolization of sarcoplasm of the cardiac muscular fibers of the heart and delineation of the microfibrils, resulting from malfunctioned absorption of selenium and α-tocopherol due to proventriculitis and catarrhal inflammation of the small intestine (Chechet et al., 2022; Trocino et al., 2022). Also, deficiency of antioxidants (selenium and α-tocopherol) was observed to trigger lipid peroxidation in cardiomyocytes, which can develop into myopathy (Trocino et al., 2022).

Our studies also revealed 14.2% of the control broilers having macroscopically noticeable protein steatosis. At the same time, the earlier conducted histological studies (Chechet, 2023) revealed 62% of the control-group broilers having granular hepatic dystrophy in the liver. This indicates that in 47.8% of the control-group broilers, hepatic lesion was not intense enough to be seen macroscopically.

It has to be noted that 100% of the control broilers were observed to have protein nephrotic syndrome. According to the modern knowledge (Soderland et al., 2010; Perazella, 2018), such changes are due to toxic loading on the kidneys. Earlier, a niche for *Clostridium* spp. and *E. coli* was seen to emerge in the intestines of the control-group broiler chickens (Chechet, 2023). Such a mixed subclinical infection caused proventriculitis and catarrhal inflammation of the small intestine.

The modern data suggest that the involution of thymus and cloacal sac, which we established earlier, creates an immunosuppressive background against which a catarrhal inflammation of the small intestine can become chronic (Cui et al., 2004).

At the same time, in both experimental groups of the broiler chickens, protected with the new biocide-probiotic complex, all the examined organs had no macroscopic changes. This is consistent with the data from other authors, reporting that *Bacillus* spp. probiotics enhance the activity of glutathione peroxidase by 27%, whereas chlorine dioxide reduces the bacterial loading on the premises by 2.4 $\log_{10}$ (Kupnevska et al., 2022; Shulyak et al., 2022). In addition, we demonstrated that the use of a complex of probiotic cultures instead of antibiotics in feeding broiler chickens fulfils a protective function, promoting the development of birds' natural gut microbiota, which reliably counters infectious agents that cause diarrhea and respiratory syndrome. This ensures high-quality biosafe meat products with no residuals of antibiotics, as well as improved quality of offal (heart, liver, stomach), which enter the domestic and foreign markets (Chechet, 2023).

## Conclusions

The broiler chickens raised traditionally experience a complex of subclinical lesions, including protein myocardiodystrophy, protein steatosis, protein nephrotic syndrom, proventriculitis, catarrhal inflammation of the small intestine, early thymic involution, and involution of cloacal sac. Meanwhile, following the application of the new probiotic complex that includes the probiotics Biomagn (*Bacillus subtilis*, *B. licheniformis*, *B. coagulans*, *Enterococcus faecium*, *Lactococcus lactis*, magnesium, chitosan, enzymes, grist of *Silybum*) and Biozapin (*Bacillus subtilis*, *Bacillus amyloliquefaciens*, aluminosilicate), we observed no subclinical lesions of the organs in all the broiler chickens.

Following the use of the pair of probiotics Biomagn and Biozapin and the pair of biocides Diolaid (the main active agent is chlorine dioxide) and Biolaid (the main active agent is lactic acid), the chickens experienced no subclinical lesions of the organs. Moreover, we observed a statistically significant (compared with the traditional raising method) increase in the gain of live mass in the broiler chickens, measuring 18.3 ± 0.3%.

The perspectives of further research include studies of microstructural changes in the internal organs of the slaughtered broiler chickens following the complex use of disinfecting and probiotic agents.

The authors declare no conflict of interests.

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