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The microstructure of the liver in broiler chickens under the administration of a probiotic complex of *Bifidobacteria* and *Lactobacilli*

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ABSTRACT
The global consumption of broiler chicken meat is increasing annually, which involves the use of intensive technologies in poultry farming and the application of a significant number of additives aimed at preventing infectious diseases in livestock. One such means is the probiotic preparation TIMM-P, which includes *Bifidobacterium gallinarum, Lactobacillus rhamnosus, Lactobacillus plantarum,* and *Lactobacillus paracasei subsp. paracasei.* To determine the effectiveness of its use, two groups of one-day-old Cobb 500 broiler chickens, each comprising 50 birds, were formed. The probiotic TIMM-P was administered via drinking over 2 hours in the morning, before feeding, on days 1–5, 21–25, and 30–35 of the rearing period. The liver microstructure of one-day-old broiler chickens did not differ between the groups, more pronounced changes were recorded at an older age. On the 14th day of growth in broiler chickens that received the basic diet, the cytoplasm of hepatocytes was characterised by a reduced optical density and contained small lipid droplets. During the administration of the probiotic TIMM-P to the chickens in this period, the cytoplasm of hepatocytes had a uniform and intense colouration while maintaining the radiality of the tubular structure of the liver lobules. On the 28th day of growth in broiler chickens of the control group, the development of fatty liver dystrophy was observed. The administration of probiotics to broiler chickens was associated with the development of small-droplet fatty liver dystrophy. On the 42nd day of growth, the phenomena of apoptosis, necrosis of hepatocytes, and fatty liver dystrophy with sections of the portal tracts in a state of interstitial inflammation, with stasis in various sections of the venous bed were detected in the liver of broiler chickens of the control group. The use of probiotics in broiler chickens partially reduced the intensity of pathological changes in the liver; however, in some animals, destruction of the tubular structure within its lobules was detected, accompanied by perivascular infiltration by polymorphic cells. The use of probiotics in broiler chickens did not significantly affect the volume of hepatocytes and their nuclei. Still, it contributed to a tendency to reduce their nuclear-cytoplasmic ratio, which indicated a higher intensity of liver function regeneration. The results obtained from the study indicate a positive effect of the complex probiotic TIMM-P on the liver microstructure, which, considering the indicators of meat quality and safety, may justify its introduction into industrial-scale broiler chicken meat production.

Keywords: *Bifidobacterium gallinarum, Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus paracasei ssp. paracasei*, broiler chicken rearing

INTRODUCTION

Poultry farming is one of the fastest-growing livestock sectors, primarily due to modern meat production systems **[1]**, which utilize fast-growing poultry crosses **[2]**. Modern broiler chicken crosses are characterised by high growth intensity, which to some extent causes increased sensitivity to adverse environmental conditions, in particular high concentration of livestock in production areas, rapid spread of pathogenic and conditionally pathogenic agents of infectious diseases, intensive vaccination system and technological stresses, which in turn leads to a decrease in immunity and the occurrence of infectious pathology **[3]**. Against the backdrop of intensive broiler chicken meat production systems, the issue of preventing a significant number of infectious diseases has arisen, which cause economic losses **[4]** and also contribute to a substantial number of human toxicoinfections **[5]**. The solution to this problem has so far been based on the use of a wide range of antimicrobial agents, in particular antibiotics. At the same time, the introduction of antibiotics into animal feed was carried out in subtherapeutic doses, which in turn allowed for stimulating poultry productivity. This practice was widespread among many poultry producers.

The long-term use of antibiotics in broiler chicken farming has not only reduced their effectiveness against opportunistic and pathogenic microflora, but also caused adverse health effects for consumers and the entire ecosystem **[6]**. To mitigate the consequences of uncontrolled antibiotic use and adhere to the One Health concept, many countries worldwide, including the European Union, have banned or restricted their use as feed components. This necessitates the development of alternative means that can prevent dangerous infections and ensure the quality and safety of poultry products.

Among agents that can replace some functions of antibiotics, probiotics, prebiotics **[7]**, immunostimulants of various origins, herbal supplements **[8]**, and nanotechnology products **[9]**, **[10]** occupy an important place. From this list, probiotics are most common in poultry farming, in particular in meat farming, where they not only justify the economic efficiency of production, but also allow for the reduction the use of antibiotics **[11]**.

Probiotic preparations are usually based on mono- or polycultures of microorganisms that belong to the healthy microbiome of the poultry digestive system and are introduced into feed mixtures or drinking water **[12]**. The most common microorganisms that are part of probiotic preparations include the genera *Lactobacillus*, *Bacillus*, *Bifidobacterium*, *Streptococcus, Enterococcus,* and *Lactococcus* spp., as well as *Saccharomyces* spp yeast. The use of probiotics contributes to the improvement of the organism's immune status, enhances growth performance, and supports the functional activity of the digestive system by maintaining the balance of beneficial intestinal microbiota, reducing inflammatory processes, and decreasing ammonia formation **[13]**. The main advantage of probiotics in the body of productive animals, in particular poultry, is the absence of harmful residues in the meat and eggs against the background of improved microbiological indicators. The systematic use of probiotics in poultry diets contributes to the normalization of digestion and nutrient absorption **[14]**. In addition, most probiotics affect the intestinal microbiome, neutralising *Salmonella* and other pathogenic microorganisms. In this regard, the liver-intestinal system plays a key role, where the processes of digestion, metabolism, immunity formation and toxin neutralisation are regulated **[15]**.

On this basis, a significant number of lactic acid bacteria are used not only in poultry feeding, but also as natural preservatives for fresh meat **[16]**, **[17]**, dried products **[18]** and meat products **[19]**, **[20]**, and **[21]**. The direct intake of probiotics into the body of consumers (as preparations) or indirectly through food products promotes the synthesis of short-chain fatty acids in the body, which contribute to the enhancement of neurogenesis, the development of microglia and the reduction of the synthesis of inflammatory mediators, maintaining the integrity of the blood-brain barrier, which has a positive effect on human health in both the short and long term **[22]**.

Probiotic supplements to poultry diets have a positive effect on growth and development and meat quality **[23]**. At the same time, the effectiveness of using poultry probiotics depends on the combination of different types of microorganisms, dose, method, and mode of use, which opens up new prospects for the poultry industry in achieving the optimal balance of quality/safety/biological usefulness, and product price. In this regard, the development of new probiotics that have species specificity and as natural components of a healthy poultry microbiome is promising **[24]**. However, there is a certain gap in understanding the mechanism of the influence of probiotics on the body of broiler chickens, particularly in terms of age, and their effect on the liver structure, which is both a vital organ for broiler chickens and a food product for humans after slaughter.

Based on this, the study aimed to investigate the liver microstructure of Cobb-500 broiler chickens during rearing when a complex probiotic, “TIMM-P”, derived from the healthy intestinal microbiota of chickens, was administered as part of the drinking water.

Scientific Hypothesis

The use of the complex probiotic preparation TIMM-P will enable the preservation of the liver's microstructure and function during the cultivation of broiler chickens using intensive technology.

Objectives

Primary objectives: to investigate age-related changes in the liver microstructure of Cobb 500 broiler chickens resulting from the use of a complex probiotic preparation. Auxiliary tasks: to perform morphometric analyses of individual structural components of the liver under the influence of a probiotic preparation in the dynamics of growing broiler chickens.

MATERIAL AND METHODS

Samples

Samples description: The study used liver samples from Cobb 500 broiler chickens. To determine the microstructure of the liver after using a probiotic complex of bifidobacteria and lactobacteria, 10 chickens from the control and experimental groups were slaughtered at the ages of 1, 14, 28, and 42 days. Before slaughter, the chickens were stunned with an electric current using a Le Reve poultry stunning device ("FAF", France).

Samples collection: For microscopic studies, the liver was separated from the thoracoabdominal cavity immediately after slaughter of chickens from the control and experimental groups (five birds each) on the 1st, 14th, 28th, and 42nd days of the study.

Sample preparation: For histological studies, liver pieces up to 1 cm^3 in size were selected, fixed in a 10-12% solution of neutral formalin, and transported to the laboratory in sealed containers for histological studies.

Number of samples analysed: A total of 20 livers of broiler chickens from the control and 20 livers of broiler chickens from the experimental groups were used.

Chemicals

Neutral formalin (Chemlaborreaktiv LLC, Ukraine).
Ethyl alcohol (Chemlaborreaktiv LLC, Ukraine).
Hematoxylin (Diapath, Italy, 2020).
Eosin (Leica Geosystems, Germany, 2020).
Paraffin (Chemlaborreaktiv LLC, Ukraine).

Animals, Plants and Biological Materials

For the study, 100 heads of cross-Cobb-500 one-day-old broiler chickens were selected. According to the principle of analogues, one control group and one experimental group of 50 heads each were formed.

Instruments

Sledge microtome MS-2 (StandardPribor LLC, Ukraine, 2015).
Micros MC-50 microscope (InterMed, PRC, 2017).

Laboratory Methods

Liver slices were embedded in paraffin **[25]**. Following fixation and rinsing, the samples were passed through a graded series of ethanol solutions (40º, 60º, 70º, 80º, 96º, and 100º) and xylene, and subsequently embedded in paraffin. Subsequently, histological sections were made from paraffin blocks on a sledge microtome MS-2, their thickness did not exceed 8 – 10 µm.

To study morphology at the tissue and cellular levels and conduct histo- and cytometric studies after deparaffinisation, histological sections were stained with hematoxylin and eosin **[25]**. Microscopy of sections and histometric studies of structural elements of tissues were performed using a Micros MC-50 microscope. The volume of hepatocytes and their nuclei was determined using the formula (1):

$$V = \pi/6 * A * B^2 \qquad (1)$$

Where: V – volume of hepatocyte (nucleus),
π – 3.14,
A – length of the hepatocyte (nucleus),
B – width of the hepatocyte (nucleus).
The nuclear-cytoplasmic ratio was determined using the following formula:
NCR = Nuclear volume/(cell volume – nuclear volume)

Description of the Experiment

Study flow: A total of 100 Cobb 500 broiler chickens were reared from one day old to 42 days of age. Throughout the experiment, the birds were fed a complete feed balanced in terms of nutritional and biologically active components (Table 1).

Table 1 Study design.

Groups	n	Feeding conditions
Control	50	Basic diet
Experimental	50	Basic diet + probiotic "TIMM-P" according to the course of application

Chickens were given tap water without restriction. Broiler chickens in the experimental group were given the probiotic "TIMM-P" as part of their drinking water, which included 5 highly active strains of microorganisms isolated from healthy poultry, namely, *Bifidobacterium gallinarum, Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus paracasei ssp. paracasei.* The preparation "TIMM-P" was a dry, free-flowing powder, without foreign inclusions, from cream to light brown in colour, easily soluble in water. In this probiotic, the number of lactic acid bacteria is not less than 10^9 CFU/g, and the number of bifidobacteria is not less than 10^9 CFU/g.

The broiler chickens in the experimental group were administered the probiotic "TIMM-P" via drinking for two hours in the morning, prior to the first feeding, on days 1–5, 21–25, and 30–35 of the rearing period **[26]**.

Quality Assurance

Number of repeated analyses: Each analysis was conducted using 20 samples.

Number of experiment replication: 1

Reference materials: -

Calibration: Each instrument was calibrated before each experiment, and calibration checks were performed regularly to maintain measurement accuracy. Each instrument was calibrated before each experiment, and calibration checks were performed periodically to maintain measurement accuracy.

Laboratory accreditation: The experiments were conducted based on the Ukrainian Laboratory of Quality and Safety of Agricultural Products, which is managed through the implementation of a management system built (since 2007) followingthe requirements of DSTU EN ISO/IEC 17025:2019 ((EN ISO/IEC 17025:2017, IDT; ISO/IEC) 17025:2017, IDT) and confirmed by the Accreditation Certificate of the National Accreditation Agency of Ukraine.

Data Access

The data supporting the findings of this study are not publicly available.

Statistical Analysis

Statistical software Microsoft Excel 2016.

Digital data processing was performed by variational statistical methods using one-way analysis of variance. In the experiment, the data of morphometric analysis of the liver of broiler chickens in terms of age were compared within the group and between the experimental variants. The data in the tables are presented as x±SD (mean±standard deviation). The difference between the variants was considered significant at $P<0.05$ using the Tukey test.

RESULTS AND DISCUSSION

The liver of broiler chickens is subjected to significant metabolic and physiological stress from the moment of hatching. A key feature of metabolic processes in birds is the significant role they play in the synthesis and metabolism of lipids, which primarily occur in the liver, unlike in mammals, where lipogenesis also takes place in adipose tissue **[27]**.

According to the results of histological studies, microscopically, the liver lobes of one-day-old broiler chickens in both the control and experimental groups were covered with a capsule on the outside and exhibited a lobular structure. In their center or eccentrically, there was a central vein (Figure 1). Each lobule is built of parenchyma and interlobular connective tissue, which in chickens of this age group is poorly developed and therefore almost not detected on histopreparations stained with hematoxylin and eosin (Figure 1, 2), which is characteristic of the typical structure of the organ for animals of the Aves class. At the same time, in some areas of the histopreparation, between the liver lobules, barely noticeable thin layers of loose connective tissue were still differentiated, which was somewhat better manifested in the areas of the portal tracts - at the contact boundary of two or three lobules, where the hepatic triads - arteries, veins and bile duct - were located in the connective tissue (Figure 2).

Figure 1 Fragment of the microscopic structure of the liver of one-day-old broiler chickens of the control group: a – liver capsule; b – liver parenchyma; c – central vein; d – liver lobule. Hematoxylin and eosin.

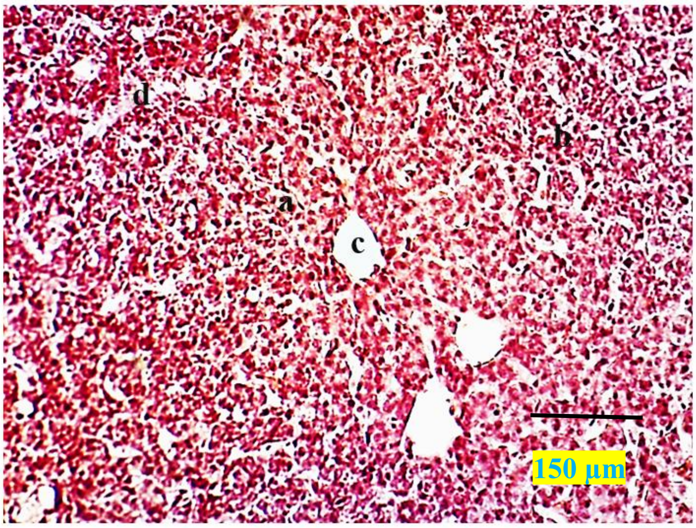

Figure 2 Fragment of the microscopic structure of the liver of one-day-old broiler chickens of the experimental group: a – liver lobule; b – liver parenchyma; c – central vein; d – interlobular connective tissue. Hematoxylin and eosin.

According to the results of morphometric studies of the liver parenchyma of broiler chickens of the control and experimental groups, the average diameter of the hepatic tubules on their cross-section did not significantly differ (Table 1). Hepatocytes of broiler chickens were small in size and had a multifaceted (rounded) shape. According to cytometry, the average volume of hepatocytes and their nuclei in broiler chickens of the control group did not significantly differ from those of the experimental group. The nuclear-cytoplasmic ratio of hepatocytes of the liver of chickens of the control group was also within the limits characteristic of chickens of the experimental group (Table 1).

The histoarchitectonics of the liver in 14-day-old Cobb 500 broiler chickens of the control group, compared to those at one day of age, exhibited a similar microscopic structure, albeit with certain distinctive features. Thus, the process of liver morphogenesis in chickens of this age group is not completely completed, as indicated by the histoarchitectonics of the radial arrangement of hepatic tubules (Figure 3).

Table 1 Morphometric indicators of the liver of broiler chickens under the influence of a complex probiotic preparation.

Age of broiler chickens, days	Group	Indicator			
		diameter of hepatic tubules, μm	hepatocyte volume, μm^3	volume of hepatocyte nuclei, μm^3	NCR
1	control	24.13 ±0.38[a]	749.97 ±6.39[a]	217.33 ±8.25[a]	0.418 ±0.022[a]
	experiment	25.27 ±0.41[a]	756.03 ±7.61[a]	219.03 ±6.34[a]	0.417 ±0.019[a]
14	control	28.77 ±0.48[b]	839.97 ±14.29[b]	244.97 ±5.54[b]	0.421 ±0.018[ab]
	experiment	29.03 ±0.69[b]	862.03 ±11.68[c]	251.07 ±7.06[b]	0.420 ±0.018[ab]
28	control	34.07 ±0.54[c]	964.77 ±3.55[d]	286.03 ±7.43[c]	0.432 ±0.020[ab]
	experiment	35.93 ±0.62[c]	988.10 ±12.82[e]	292.37 ±7.89[c]	0.429 ±0.017[ab]
42	control	37.07 ±0.54[d]	1075.33 ±16.61[f]	327.17 ±6.37[d]	0.444 ±0.014[b]
	experiment	37.83 ±0.48[d]	1092.17 ±17.93[f]	331.10 ±6.25[d]	0.440 ±0.011[ab]

Note: Different superscript letters indicate values that were significantly different in the same column of the table ($p \leq 0.05$) according to the results of comparison using the Tukey test. x±SD, n=5.

By microscopic analysis of the parenchyma of the liver, the cytoplasm of hepatocytes was characterized by a decrease in optical density, which manifested as a significant decrease in basophilia in the cytoplasm in the coloured histopathological preparations of hematoxylin and eosin. In the cytoplasm of hepatocytes, individual droplets of lipid inclusions of small size were detected, as a result of which the cytoplasm of such hepatocytes acquired a foamy, heterogeneous colour. At the same time, the microscopic structure of the liver parenchyma was characterised by a more pronounced radial arrangement of hepatic tubules formed by peptocytes. At the same time, almost the same diameter of hemocapillaries was observed.

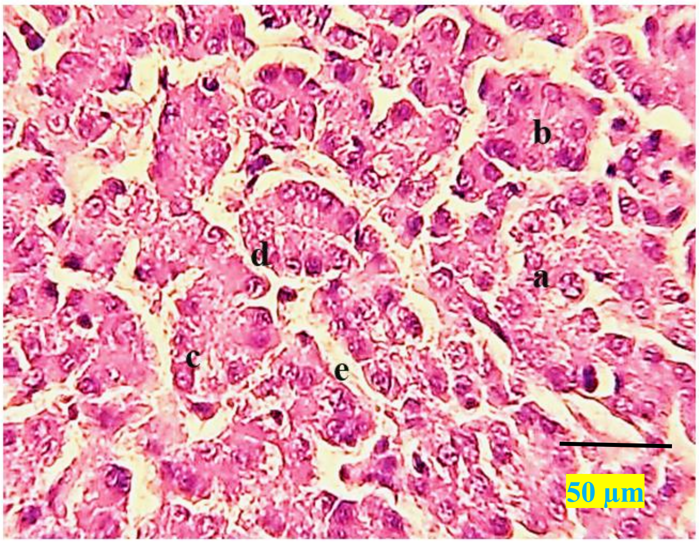

Figure 3 Fragment of the microscopic structure of a liver lobule of 14-day-old broiler chickens of the control group: a – liver parenchyma; b – hepatic tubules; c – hepatocytes; d – droplets of fat inclusions; e – sinusoidal capillaries. Hematoxylin and eosin.

In broiler chickens of the experimental group of 14 days of age, compared with chickens of the control group of the same age, no significant differences were observed in the microscopic structure of the liver. The lobules of the liver parenchyma were formed by hepatocytes and interlobular connective tissue, which was detected only in individual places, at the interface of contacts of two or three lobules in the form of thin layers of loose connective tissue, which was clearly differentiated in the areas of the portal tracts.

In most broiler chickens of the experimental group at this age, the cytoplasm of hepatocytes was uniformly and intensely stained compared to the control group (Figure 4). Simultaneously with the expansion of the lumen of the central veins of the liver lobules, the density of the location of the hepatic tubules decreased due to the increase in the lumen of the sinusoidal capillaries, which branched in the radial direction between the hepatic tubules and flowed into its central vein.

Thus, the results of the studies indicate that the histoarchitectonics of the liver of broiler chickens fed probiotics was characterized by a more mature structure, as evidenced by the uniformity of cytoplasmic staining of hepatocytes, the formation of a network of wide blood capillaries of approximately the same diameter. Such features of the histoarchitectonics of the liver lobules in chickens of the experimental group that we discovered indicated the activity of the hemomicrocirculation of the liver in them, compared with chickens of the control group and indicated the positive effect of the probiotic complex of bifido- and lactobacteria on the digestive system, which is consistent with the results obtained by other scientists **[28]**.

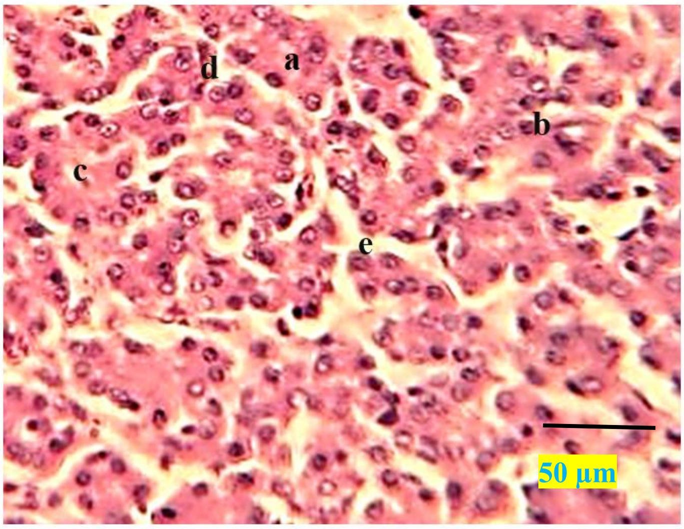

Figure 4 Fragment of the microscopic structure of the liver of 14-day-old broiler chickens of the experimental group: a – fragment of a liver lobule; b – liver parenchyma; c – hepatic tubules; d – hepatocytes; e – sinusoidal capillaries. Hematoxylin and eosin.

According to the morphometric results in 14-day-old broiler chickens from the control and experimental groups, the average diameter of hepatic tubules increased by 1.19 times and 1.15 times, respectively, compared to one-day-old chickens. At the same time, the average diameter of hepatic tubules in the experimental group of this age did not differ significantly from that of the control group (Table 1).

According to the cytometry results of hepatocytes, the average volumes in broiler chickens of the control and experimental groups increased compared to those in one-day-old chickens. Similar changes, in the direction of increase, were observed when determining the volumes of hepatocyte nuclei (Table 1). At the same time, the indicators of NCR of hepatocytes in chickens of this age group did not change compared to one-day-old chickens.

In broiler chickens of the control group of 28-day-old age, compared with 14-day-old age, the histoarchitectonics of the liver was characterised by certain features. When staining histopreparations with hematoxylin and eosin, the cytoplasm of hepatocytes poorly perceived dyes, as a result of which it had a light colour. The nuclei of hepatocytes were rounded and were located in the cytoplasm of cells in the centre, or eccentrically. In the cytoplasm of hepatocytes, fine granularity was detected, which indicated the accumulation of a protein component in it. In the cytoplasm of individual cells, large drops of fatty inclusions were detected (Figure 5), which indicated the beginning of the development of fatty liver dystrophy. Such changes in the cytoarchitectonics of hepatocytes that we detected stated a violation of protein and lipid metabolism in the body and were a sign of the development of liver pathology in broiler chickens of this age group.

The feeding factor played a key role in this study, as the adequate supply of nutrients and energy to broiler chickens depended on liver function and the body's ability to maintain a healthy digestive microbiome **[29]**, **[30]**.

The radial arrangement of the hepatic tubules of the liver lobules in the control chickens was weakly expressed (Figure 6). In most cases, hemocirculatory disorders were detected, which were manifested by local dilatation and hyperemia of sinusoidal capillaries. A slight perivascular infiltration by polymorphic cells of the central veins of the liver lobules was observed (Figure 6), and in the areas of the portal tract. Their blood vessels were dilated and filled with blood.

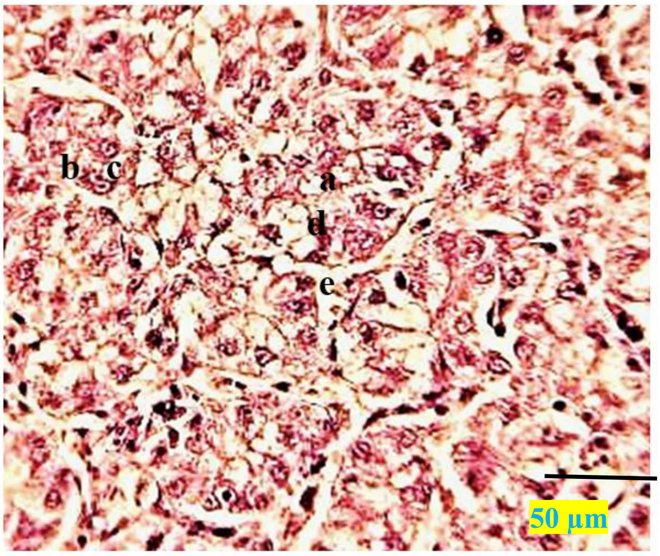

Figure 5 Fragment of the microscopic structure of the liver lobule of 28-day-old broiler chickens of the control group: a – hepatic tubules; b – hepatocytes; c – hepatocyte nuclei; d – fatty inclusions; e – sinusoidal capillaries. Hematoxylin and eosin.

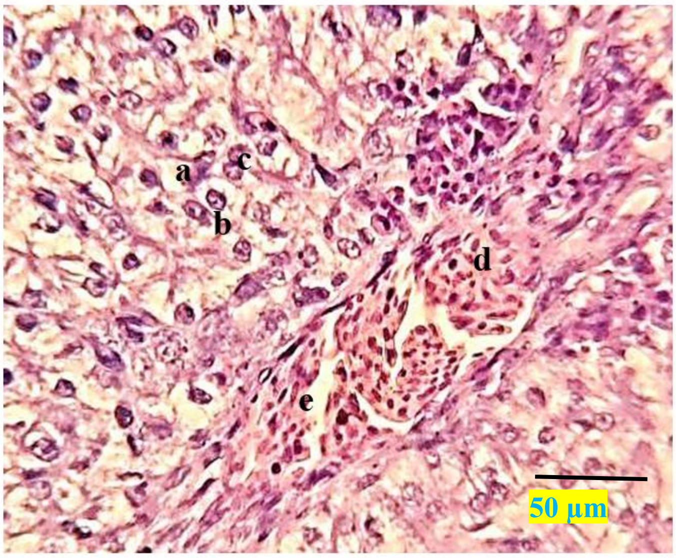

Figure 6 Fragment of the microscopic structure of the liver lobe of 28-day-old broiler chickens of the control group: a – hepatic tubules; b – hepatocytes; c – hepatocyte nuclei; d – central vein; e – blood cells; e – perivascular infiltration by polymorphic cells. Hematoxylin and eosin.

In the experimental group of 28-day-old chickens, compared with the control, the microscopic structure of the liver was largely preserved. This may be due to the ability of probiotics to control the number of pathogenic and opportunistic microorganisms, such as *Salmonella* and Campylobacter **[31]**, by synthesizing bacteriocins and maintaining a balance of pro-inflammatory and anti-inflammatory responses. in the intestines of broiler chickens, which improves feed conversion and stimulates the immune response **[32]**. In addition, probiotics can increase the efficiency of feed digestion by producing their own phytases, lipases, amylases and proteases, as well as by stimulating the secretory activity of the poultry intestine. An important factor is also the ability of probiotics to synthesise a number of vitamins, exopolysaccharides and antioxidants in the intestine **[33]**.

As for the histoarchitectonics of the liver of chickens of the experimental group, it had a characteristic structure of a formed tubular gland. A thin layer of mesothelial cells forms the liver capsule. Upon histological examination, a typical structure of the liver lobules is visualised. Between the hepatic tubules, a network of wide hemocapillaries is clearly visible, the cavities of which are dilated. The cytoplasm of such hepatocytes was light in colour and contained small transparent intracytoplasmic vacuoles, which indicates the development of small-droplet fatty liver dystrophy (Figure 7). Thus, despite the positive effects, probiotics cannot completely eliminate the negative factors inherent in the intensive broiler chicken meat production technology **[34]**.

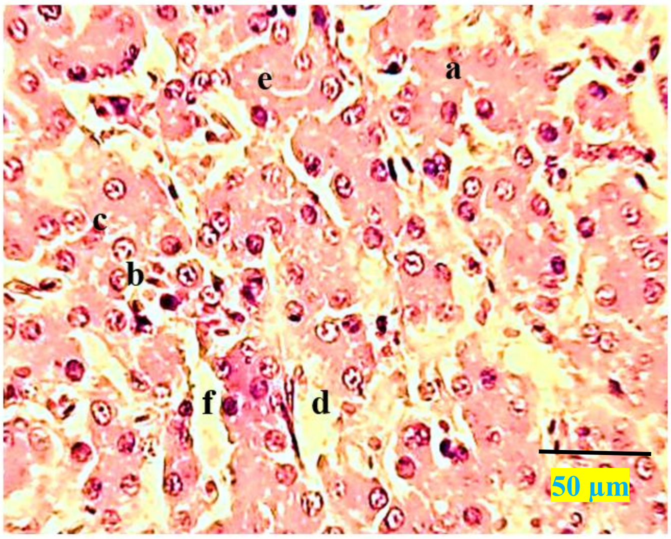

Figure 7 Fragment of the microscopic structure of the liver lobule of 28-day-old broiler chickens of the experimental group: a – liver parenchyma; b – dyscomplexation of hepatocytes of hepatic tubules; c – hepatocytes; d – hepatocyte nuclei; e – fatty inclusions; f – sinusoidal hemocapillaries. Hematoxylin and eosin.

A feature of broiler chicken farming is the use of high-energy feed mixtures, which are designed for rapid growth and muscle mass gain, involving a high content of carbohydrates, which can stimulate lipogenesis in the liver. This, in turn, is a trigger for the occurrence of pathological conditions, in particular fatty liver dystrophy **[35]**. Although there is another point of view, which indicates that high-energy feed rations do not always have an adverse effect on the microstructure of the chicken liver **[36]**.

In some broiler chickens of the experimental group, partial and, in some cases, even total dyscomplexation of hepatocytes, characterized by the radial arrangement of hepatic tubules, was detected in the liver lobules. In such cases, in the areas of the triads, expansion and filling of the bile ducts with bile were observed (Figure 8).

In places in the lumen of dilated central veins, formed blood elements were detected and focal perivascular infiltration of polymorphic cells around the central veins of the liver lobules was observed. One of the reasons for the detection of such pathological changes in liver cells of clinically healthy broiler chickens may be the intensive vaccination regimen **[37]**.

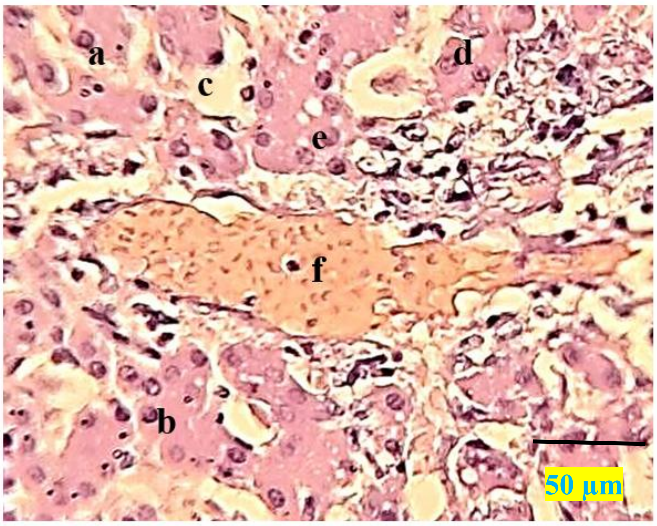

Figure 8 Fragment of the microscopic structure of the liver lobe of 28-day-old broiler chickens of the experimental group: a – liver parenchyma; b – hepatocytes; c – sinusoidal hemocapillaries; d – hepatocyte nuclei; e – fatty inclusions; f – bile duct. Hematoxylin and eosin.

According to the results of morphometric studies, the average diameter of hepatic tubules in 28-day-old broiler chickens was found to be 1.19 times greater than in 14-day-old chickens in control animals, and 1.24 times greater in experimental animals. At the same time, the average diameter of the liver tubules of the chickens of the experimental group did not differ from the control (Table 1). The average volume of hepatocytes and their nuclei in 28-day-old broiler chickens compared with 14-day-old chickens increased in both the control and experimental groups. According to these results, the indicators of the NCR of liver hepatocytes in chickens of this age group, compared with 14-day-old chickens, maintained a tendency to increase (Table 1).

In broiler chickens of the control group of 42 days of age, the histoarchitectonics of the liver is disrupted. The radial arrangement of the hepatic tubules of the liver lobules is absent (Figure 9). The presence of fine granularity was observed in the cytoplasm of hepatocytes, indicating the development of protein granular dystrophy in these cells and perivascular accumulations of polymorphic cells in the areas surrounding the central veins of the liver lobules (Figure 10).

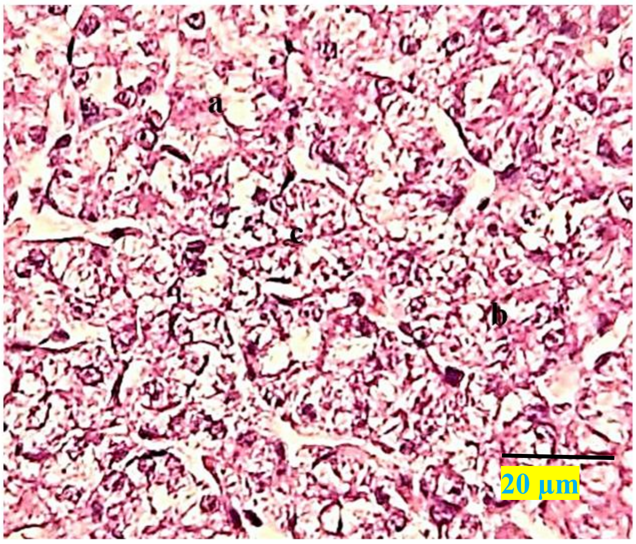

Figure 9 Fragment of the microscopic structure of a liver lobule of 42-day-old broiler chickens of the control group: a – liver parenchyma; b – dyscomplexation of hepatocytes of hepatic tubules; c – hepatocytes with foamy cytoplasm. Hematoxylin and eosin.

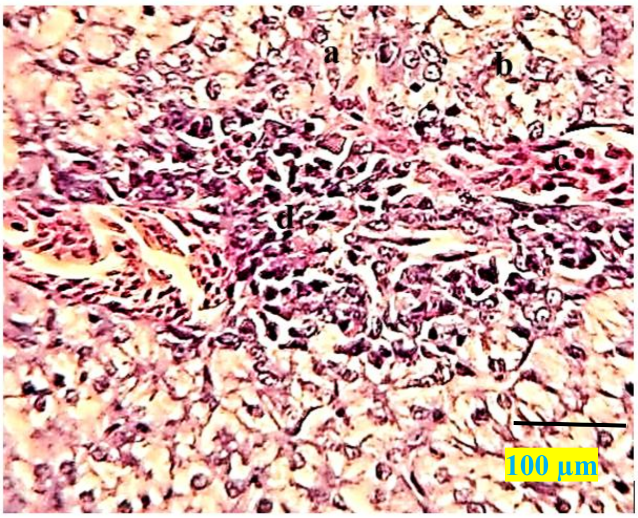

Figure 10 Fragment of the microscopic structure of a liver lobule of 42-day-old broiler chickens of the control group: a – liver parenchyma; b – fine granularity in the cytoplasm of hepatocytes; c – central vein; d – perivascular clusters of polymorphic cells. Hematoxylin and eosin.

In most cases, hepatocytes were found to have large and transparent intracytoplasmic vacuoles, indicating the development of hepatosis, characterized by the presence of fatty liver dystrophy (Figure 11). In such a pathological condition, in which excessive lipid accumulation occurred in liver cells, the characteristic cytoarchitecture of the liver was destroyed (Figure 11). Cells with signs of apoptosis (chromatin marginalisation - its accumulation on the nuclear membrane, loss of intercellular contacts) and necrosis (karyopyknosis, reticular or "blurred" cytoplasmic structure) were often found. Sinusoidal capillaries of the liver lobules formed local blood-filled dilations (Figure 12). Fatty liver dystrophy is the most common pathology in broiler chickens, which complicates the course of infectious pathology **[38]**. At the same time, fatty dystrophy, for example, of goose liver, is a desirable phenomenon in cooking, as it has better taste characteristics and is highly valued in the consumer market **[39]**, and its usefulness is determined not only by its content, but also by the ratio of polyunsaturated fatty acids **[40]**.

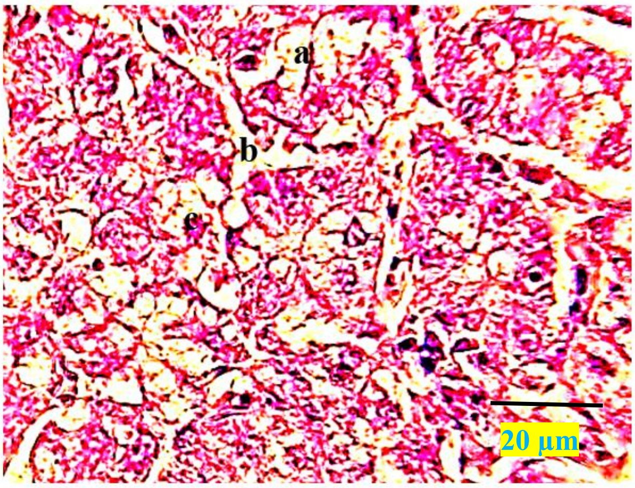

Figure 11 Fragment of the microscopic structure of a liver lobule of 42-day-old broiler chickens of the control group: a – liver parenchyma; b – sinusoidal capillaries; c – intracytoplasmic vacuoles. Hematoxylin and eosin.

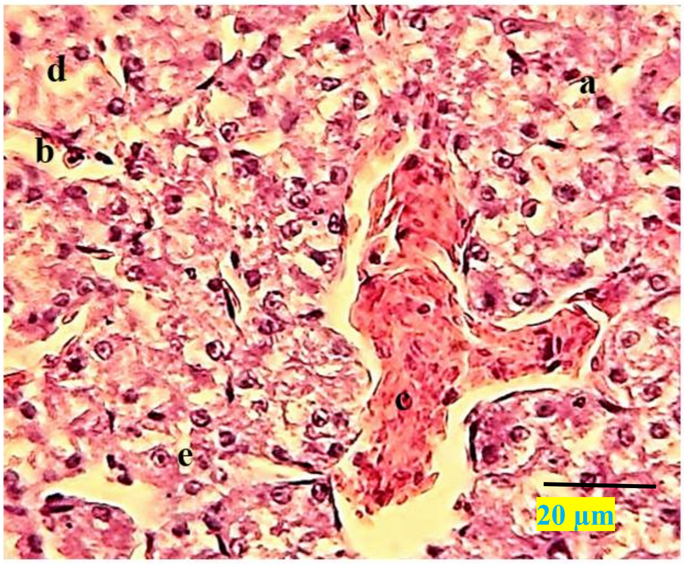

Figure 12 Fragment of the microscopic structure of a liver lobule of 42-day-old broiler chickens of the control group: a – liver parenchyma; b – sinusoidal capillaries; c – local blood-filled dilations; d – hepatocyte necrosis; e – hepatocyte nuclei. Hematoxylin and eosin.

Thickening of the walls and overflow of blood vessels of the portal tracts and central veins of the lobules were observed (Figure 13). In the interlobular connective tissue, especially in the portal tracts, signs of interstitial inflammation with infiltration of cells of the lymphoid-macrophage series were detected. The bile ducts of the liver in the portal tracts are significantly dilated (Figure 14).

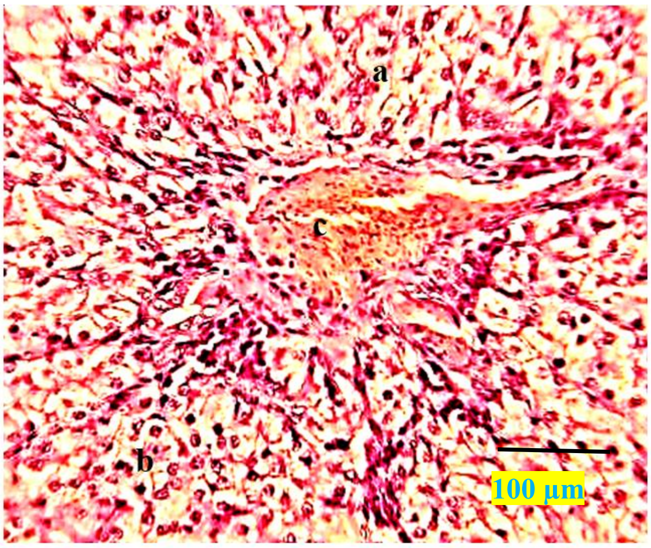

Figure 13 Fragment of the microscopic structure of the liver lobule of 42-day-old broiler chickens of the control group: a – liver parenchyma; b – hepatocytes; c – central vein; Hematoxylin and eosin.

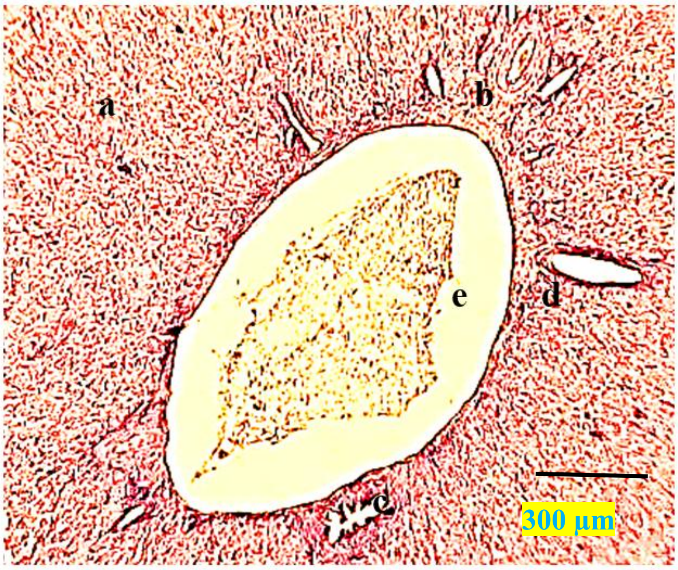

Figure 14 Fragment of the microscopic structure of the liver of 42-day-old broiler chickens of the control group: a – liver lobule; b – portal tract; c – artery; d – vein; e – dilated bile duct. Hematoxylin and eosin.

Along with signs of interstitial inflammation, in sinusoidal capillaries on the periphery of the organ and in the subcapsular zone, aggregation of erythrocytes and platelets was observed, and sludge formation was detected in various parts of the venous bed (Figure 15), which indicates a violation of microcirculation in the form of stasis. Such changes in the liver of healthy broiler chickens may, to some extent, reflect the characteristic state of hypoxia, which is the cause of myopathies in high-yielding meat crosses of poultry, including the Cobb-500 cross **[41]**.

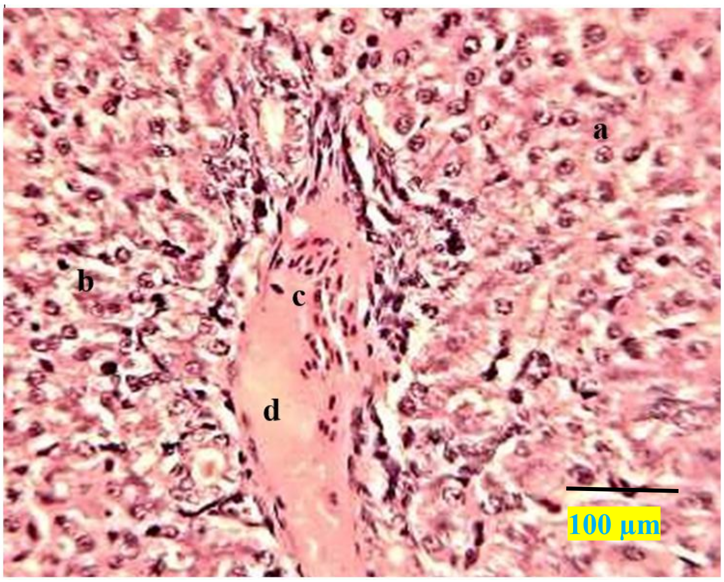

Figure 15 Fragment of the microscopic structure of a liver lobule of 42-day-old broiler chickens of the control group: a – liver parenchyma; b – dyscomplexation of hepatocytes of hepatic tubules; c – vessel; d – hemolysis of erythrocytes (sludge phenomenon). Hematoxylin and eosin.

In 42-day-old broiler chickens of the experimental group, the histoarchitectonics of the liver were similar to those of the animals of the control group. Microscopically, the liver had a not completely preserved lobular structure, and hepatic triads were visualised at the border of two and three hepatic lobules: arteries, veins, and bile ducts (Figure 16).

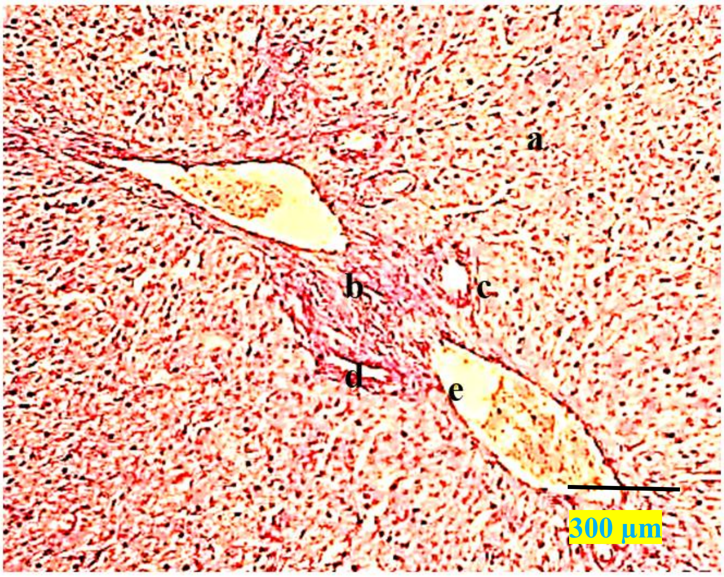

Figure 16 Fragment of the microscopic structure of the liver of 42-day-old broiler chickens of the experimental group: a – liver lobule; b – portal tract; c – artery; d – vein; e – dilated bile duct. Hematoxylin and eosin.

According to cytological analysis, the edges of their hepatocytes were indistinct, and their somewhat granular cytoplasm had a foamy appearance. The cytoplasm of most cells showed clearly visible granularity, which may be associated with the activity of protein metabolism in the body. In such cases, the zonality of the hepatocyte cytoplasm was often disrupted, and the cells merged with one another. In some areas of the histopreparation, as well as in control animals, transparent vacuoles were observed in the cytoplasm of the cells, indicating the development of fatty liver dystrophy. The cell nuclei were typically rounded in shape, located centrally or eccentrically in the cytoplasm of the cells. Nucleoli were clearly visualised in the nuclei of the hepatocytes, and the karyoplasm was filled with coarsely dispersed nuclear chromatin. Such changes in the liver of clinically healthy broiler chickens may be associated with the effects of genetic selection aimed at promoting rapid growth in birds and increasing meat yield after slaughter **[42]**. In some studies, the relationship between the lipid profile of the liver and the pectoral muscles of broiler chickens with the "wooden breast" syndrome was studied, and, although no significant relationship was established, changes in lipid metabolism and fatty acid composition in the liver were confirmed **[43]**.

In such areas of the histopreparation, not completely preserved hepatic tubules were detected (Figure 17).

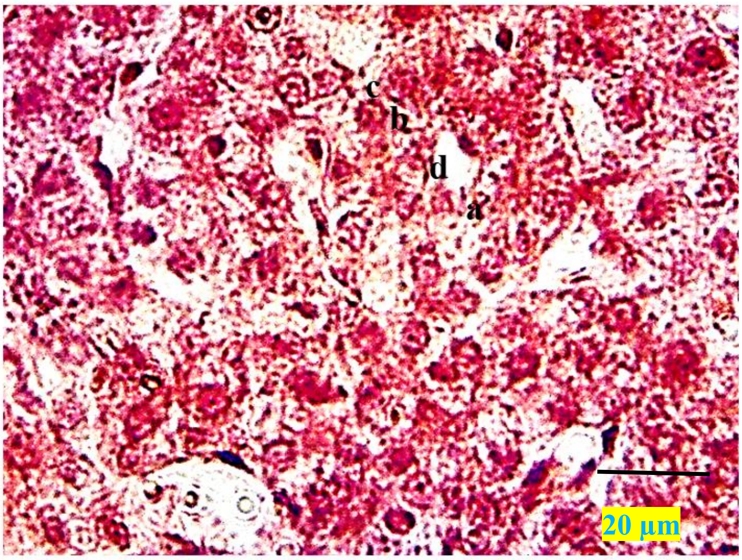

Figure 17 Fragment of the microscopic structure of the liver lobe of 42-day-old broiler chickens of the experimental group: a – hepatic tubules; b – hepatocytes; c – hepatocyte nuclei; d – bile capillary. Hematoxylin and eosin.

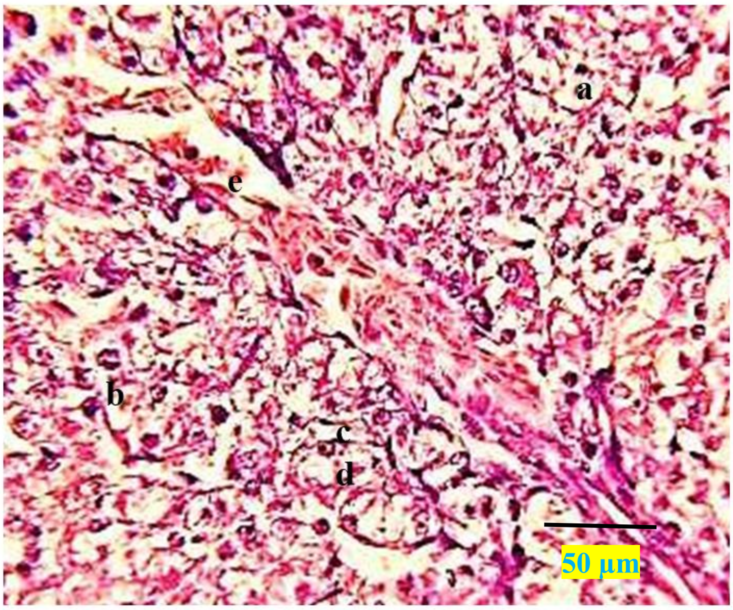

Figure 18 Fragment of the microscopic structure of a liver lobule of 42-day-old broiler chickens of the experimental group: a – liver parenchyma; b – hepatocytes; c – hepatocyte nuclei; d – foamy cytoplasm; e – sinusoidal capillary (longitudinal section). Hematoxylin and eosin.

In some animals, complete destruction of the tubular structure of the liver lobules was observed, where extensive accumulations of polymorphic cells were found in the parenchyma of the lobules (Figure 19). In addition, perivascular infiltration by polymorphic cells was observed around the central veins of the liver lobules (Figure 20).

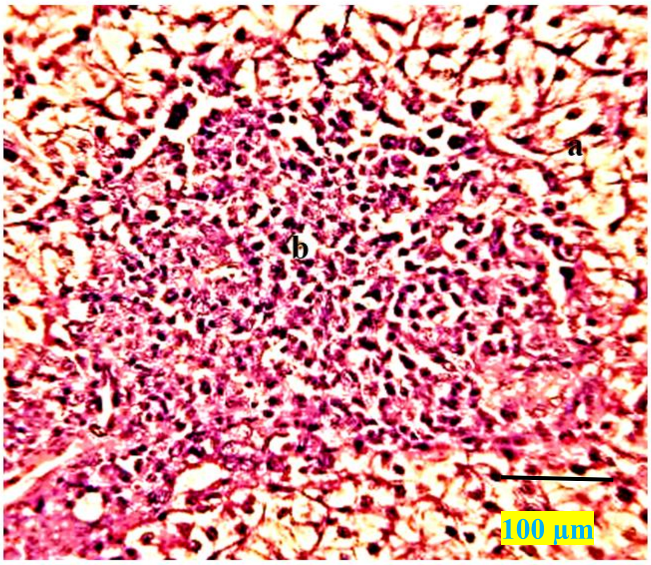

Figure 19 Fragment of the microscopic structure of the liver lobule of 42-day-old broiler chickens of the experimental group: a – liver parenchyma; b – extensive accumulations of polymorphic cells. Hematoxylin and eosin.

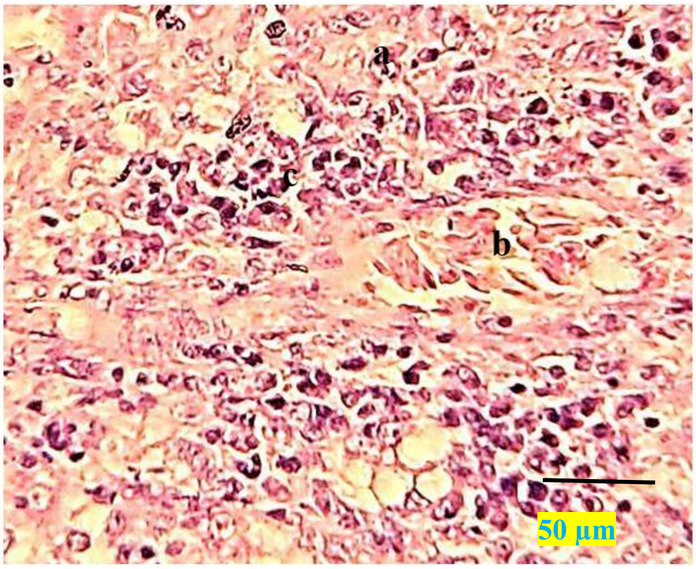

Figure 20 Fragment of the microscopic structure of the liver lobule of 42-day-old broiler chickens of the experimental group: a – liver parenchyma; b – central vein; c – accumulation of polymorphic cells. Hematoxylin and eosin.

The liver stroma in the perivascular areas is edematous, there is partial desquamation of the bile duct epithelium (Figure 21), which may indicate the development of cholongitis, hepatosis and perivascular micronecrosis of hepatocytes. The bile ducts in the areas of the hepatic triads are dilated. Around the dilated bile ducts, many vessels of the hemomicrocirculatory bed and perivascular accumulations of polymorphic cells are detected (Figure 21, Figure 22).

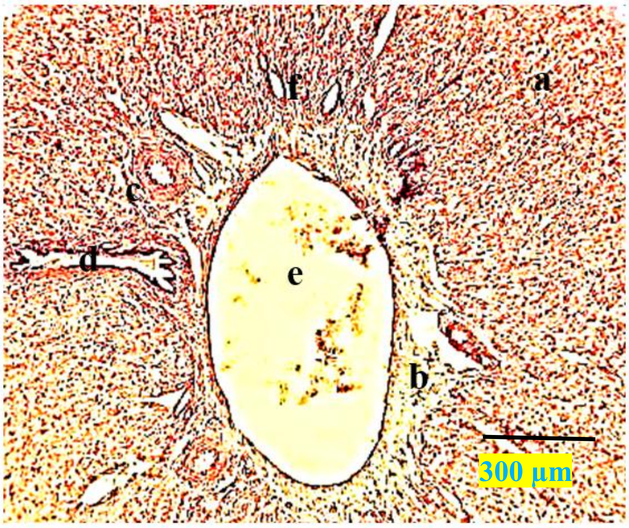

Figure 21 Fragment of the microscopic structure of the liver of 42-day-old broiler chickens of the experimental group: a – liver parenchyma; b – hepatic triads; c – artery; d – vein; e – dilated bile duct; f – vessels of the hemomicrocirculatory bed. Hematoxylin and eosin.

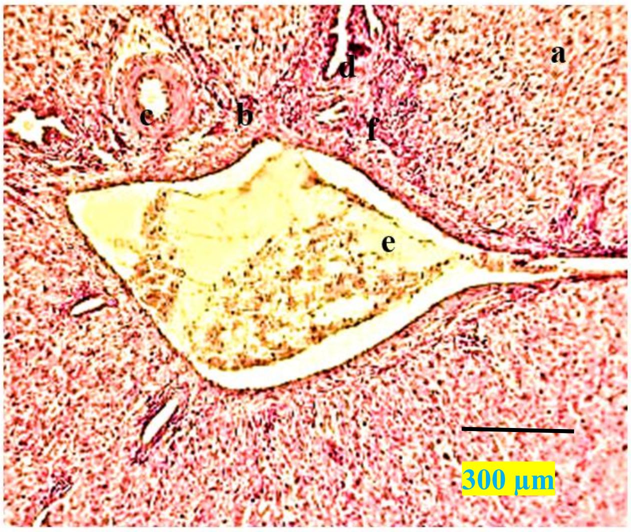

Figure 22 Fragment of the microscopic structure of the liver of 42-day-old broiler chickens of the experimental group: a – liver parenchyma; b – hepatic triads; c – artery; d – vein; e – dilated bile duct; f – perivascular clusters of polymorphic cells. Hematoxylin and eosin.

According to the results of morphometry, the average diameter of hepatic tubules in 42-day-old broiler chickens of the control group did not differ from the experimental animals. There was also a slight tendency to increase the average diameter of hepatic tubules of liver lobules, relative to such indicators in 28-day-old chickens (Table 1).

According to the cytometry analysis of the results, the volumes of hepatocytes and their nuclei in 42-day-old broiler chickens were compared with the corresponding cytological parameters of hepatocytes in 28-day-old chickens. The volumes of hepatocytes and their nuclei increased by 1.2 times (Table 1). At the same time, there was a tendency to reduce the NCR of liver hepatocytes in chickens of the experimental groups compared with the control (Table 1)

Thus, the perivascular infiltration by polymorphic cells around the central veins of the liver lobules and periportal tracts and the tendency to reduce the NCR of liver hepatocytes in broiler chickens of the experimental group, compared with the control, is likely associated with the strengthening of the immune status of the organism **[44]**, as well as is a consequence of improved intestinal barrier function **[45]**, **[46]**, normalisation of lipid metabolism in tissues **[47]** and hepatoprotective effect of probiotic complex of bifidobacteria and lactobacilli, which contributed to increasing liver resistance and restoring its functions **[48]**, **[49]**.

Our assumption is confirmed by data obtained by other researchers, which revealed a decrease in the severity of histopathological changes in the liver of chickens exposed to aflatoxin B with the use of a probiotic preparation consisting of yeast, lactic acid bacteria, and Saccharomyces cerevisiae **[50]**. This finding is consistent with the data of other scientists, who believe that lactic acid bacteria and yeast from probiotics are capable of effectively binding aflatoxins and other toxins. This effect of probiotics contributed to the reduction of the intake of toxic substances to the liver, kidneys, and other organs and prevented or reduced the severity of degenerative changes. Another study **[51]** confirmed that the use of a complex probiotic to chickens, which included *S. cerevisiae* RC016, as well as *L. rhamnosus* RC007, showed a protective effect in aflatoxicosis. At the same time, against the background of histopathological changes in the liver of chickens treated with aflatoxin B, bile duct proliferation and hepatocellular degeneration were detected. In contrast, in the case of probiotic use, the absence of microvacuolar fatty degeneration of the liver was noted. The improvement of the histostructure of the liver of broiler chickens with probiotics occurred not only due to a decrease in the amount of toxic components entering the liver, but also in the morphology and function of the intestine **[52]**.

The hepatoprotective function of probiotics in the body of broiler chickens exposed to toxic components, particularly aflatoxins, is also confirmed by studies **[53]** that used the probiotic Protexin, which contained 9 bacterial strains, including *Streptococcus salivarius spp. thermophilus, Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus rhamnosus, Lactobacillus plantarum, Bifidobacterium bifidum, Candida pintolopesii* та *Aspergillus oryzae*. In the liver of broiler chickens treated with this probiotic, a decrease in the number of mononuclear and eosinophil cells was found, accompanied by an expansion of sinusoids and lipid vacuoles. Based on these research results, it can be assumed that probiotics not only have a positive effect on the health of productive poultry but also on the health of consumers who consume meat products containing probiotic cultures **[54]**.

In addition to probiotics, as an alternative to antibiotics to improve growth and productivity of broiler chickens, as well as to obtain high-quality meat with biological value, a number of plant-based supplements are used, which have antioxidant, hepatoprotective and immunostimulating effects, the effectiveness of which in the bird's body is assessed not only by biochemical indicators, but also by histostructural analysis of individual vital organs, in particular the liver **[55]**, **[56]**, and **[57]**. However, in these studies, only the livers of broiler chickens at 35 days of age were analysed, which does not allow for a full comparison of the dynamics of changes in the histostructural structure of the liver when using probiotics in this study. Thus, the occurrence of various degrees of destructive changes in the liver of broiler chickens when using an intensive growing system and complete feed is inevitable. The use of the probiotic preparation TIMM-P partially mitigates the negative influence of technological factors on the liver's structure.

CONCLUSION

1. The histoarchitectonics of the liver of broiler chickens was characterised by a lobular structure and formed by parenchyma and poorly developed interlobular connective tissue.

2. The parenchyma of the liver lobules of broiler chickens is formed by branched hepatic tubules radially oriented to the central vein, built of mononuclear hepatocytes. Binuclear cells were often found in the liver's structure, indicating the processes of its physiological regeneration.

3. The intensive system of growing broiler chickens using complete feed causes violations of the histoarchitectonics of the liver, the development and severity of which are more related to the age aspect and to a lesser extent to the use of a probiotic preparation: on the 14th day of growth in broiler chickens that received the basic diet, the cytoplasm of hepatocytes had a reduced optical density, and individual small droplets of lipid inclusions were detected in it. In broiler chickens that were given the complex probiotic "TIMM-P", during the same period of growth, the cytoplasm of hepatocytes was characterised by uniform and intense colouration with preserved radiality of the tubular structure of the liver lobules; on the 28th day of growth, the development of fatty liver dystrophy and focal perivascular infiltration with polymorphic cells were observed in broiler chickens of the control group. Administration of the complex probiotic to broiler chickens via drinking was associated with the development of small-droplet fatty liver dystrophy, as well as the presence of isolated focal round lymphoid formations between the hepatic tubules of the parenchyma: – on the 42nd day of growth, hepatocytes with signs of apoptosis, necrosis and fatty dystrophy were detected in broiler chickens of the control group, in the areas of the portal tracts – a manifestation of interstitial inflammation with infiltration by cells of the lymphoid-macrophage series, the bile ducts of the liver of the portal tracts were significantly dilated, in various areas of the venous bed there was sludge formation, which indicated a violation of microcirculation in the form of stasis. When using a complex probiotic in broiler chickens, fuzzy edges of hepatocytes were noted, their cytoplasm acquired a foamy appearance, and its

consistency was often disturbed. In some animals, complete destruction of the tubular structure of the liver lobules occurred, as well as perivascular infiltration by polymorphic cells.

4. According to the results of morphometry, the average diameter of hepatic tubules increases with the age of broiler chickens. The use of a complex probiotic during the cultivation of broiler chickens did not significantly affect this indicator.

5. The volume of hepatocytes and their nuclei in broiler chickens increased with age, and the nuclear-cytoplasmic ratio of hepatocytes was characterised by a tendency to increase. The use of a complex probiotic contributed to the tendency to decrease the nuclear-cytoplasmic ratio of hepatocytes, which indicated an increase in liver resistance and restoration of its function.

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