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Effect of probiotic biologics on morpho-biochemical parameters of broiler chicken blood

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Abstract. In the production of broiler poultry products, market operators use intensive technologies that involve the use of various, environmentally friendly nutraceuticals, in particular, probiotic preparations. In the technology of raising broiler chickens, probiotic preparations are necessary as a means for the prevention and treatment of gastrointestinal diseases, stimulating the growth and increasing the productivity of poultry. The purpose of the study is to investigate the effect of probiotic biologics on haematological parameters when it is fed to broiler chickens. The experiment used 20 broiler chickens, which were given a probiotic from 28 to 42 days in the following amounts: 0.5 g/10 dm³, 2.0 and 4.0 g/10 dm³ of water. It was found that the morpho-biochemical parameters of poultry blood corresponded to the physiological standards for broiler chickens of the established age and confirmed the absence of pathophysiological changes in their body. On days 35 and 42 of rearing, an increase in the number of leukocytes from 4.4 to 17.2% and the haemoglobin content by 3.9 and 6.2%, respectively, was observed in the blood of broiler chickens fed the probiotic at a dose of 4.0 g/10 dm³ compared to the control group. On day 35 of the study, a 1.2-fold increase in total serum protein was observed when drinking a probiotic in poultry, respectively, at doses of 2.0 and 4.0 g/10 dm³ of water, and on day 42 – 1.0 times for its drinking at a dose of 4.0 g/10 dm³ of water. It was found that the content of inorganic calcium and phosphorus in the poultry blood serum corresponded to physiological limits, which indicates a sufficient level of mineral nutrition in the body of broiler chickens. The absence of changes in the activity of alanine aminotransferase and aspartate aminotransferase, the content of total lipids, cholesterol, and creatinine in the blood serum of broiler chickens of the experimental groups indicates the hepatotoxicity and nephrotoxicity of the probiotic. Based on the results obtained, the drug under study can be recommended to increase the resistance of the poultry body and regulate metabolism

Keywords: organism resistance; balanced feeding; morphological parameters; biochemical indicators; safety

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Introduction

In the process of producing broiler meat, it is important to search for new probiotic drugs that, unlike antibiotics, would have the ability to increase poultry productivity, the activity of natural organism resistance and the reproduction of livestock (Shkodyak *et al.*, 2020). In the context of the development of intensive technologies for raising poultry, it is necessary to use high-quality food substances in feeding and compounds that have inhibitory properties in accordance with pathogenic and opportunistic strains of microorganisms and viruses. Therefore, during 2013–2023, a scientific search was conducted for alternative uses of probiotic and phytobiotic drugs that have an effect on increasing the growth of poultry and animals (Xiong *et al.*, 2023) due to pronounced effects on antioxidant indicators (Gazwi *et al.*, 2022) and immunological activity (Yuan *et al.*, 2023), and this indicates a high resistance of the body of poultry and animals to the action of pathogenic and opportunistic microorganisms.

According to M.I. Sakhatsky & Yu.V. Osadcha (2021), the development and implementation of probiotic preparations for use in the field of broiler chicken rearing is conditioned by the growing problem of antibiotic-resistant microorganisms. Moreover, when raising broiler chickens, it is necessary to take into account the stress factor, which worsens the clinical and biochemical indicators of the blood of broiler chickens for keeping in cages, while reducing the immune status of the bird's body. Probiotics based on live cultures of microorganisms increase the stimulation of biosynthetic processes in the digestive tract and increase poultry productivity (poultry growth, meat yield), affect the improvement of metabolic processes in the body of broiler chickens, and prevent stress. Currently, in accordance with the requirements of the European Community, it is not allowed to use antibiotics when feeding farm animals and poultry (Chaturvedi *et al.*, 2021).

Feeding poultry with probiotic biologics using *C. butyricum* increased the productivity of broiler chickens by improving the morphological characteristics of blood and internal organs and stimulating the development of immunity, which can be an alternative to antibiotics in poultry feed (Yang *et al.*, 2023). The study determined the expression of cytokine genes in peripheral blood mononuclear cells of broiler chickens stimulated by live probiotics. Cytokine genes in peripheral blood mononuclear cells are isolated from whole blood and stimulated by lipopolysaccharides, galactooligosaccharides, and *Lactococcus lactis* subsp. It is established that *L. lactis* has immunostimulating properties of the cytokine gene in peripheral blood mononuclear cells of chickens (Slawinska *et al.*, 2021).

As noted by C.J. Savelli *et al.* (2021), food safety management involves identifying and controlling the risks associated with animal welfare (feeding, health, maintenance, etc.) and correcting actions in the food chain to ensure safe food production. During the production of broiler chicken meat, veterinary medicine specialists and state veterinary medicine inspectors carry out risk-oriented control to obtain safe and high-quality poultry products and animal feeding, in particular, the use of probiotic drugs (Mizernytskyi, 2021). Therefore, the use of a probiotic drug is relevant in poultry farming. The purpose of the study is to experimentally and practically substantiate the effectiveness and safety of using a probiotic biological product in the rearing of broiler chickens and to investigate its effect on the morpho-biochemical parameters of poultry blood.

Literature Review

The development of modern poultry farming in Ukraine is based on the organisational and economic principles of management and optimisation of technological processes during the

production of broiler chicken meat, which provides ordinary consumers with valuable food products (Lyasota & Kolodka, 2020). In addition, the use of probiotic drugs is an urgent issue in the treatment and prevention of gastrointestinal infections of viral and bacterial aetiology, and the normalisation of intestinal microflora for the detection of dysbiosis (Delgado-Pando *et al.*, 2019). Obtaining safe meat products from broiler chickens depends on the activity of the intestines of a physiologically healthy bird due to the assimilation of nutrients from the feed that are necessary for poultry growth (Kucheruk & Zasekin, 2018).

The health of poultry depends on the safety and quality of feed, so it is necessary to monitor the feed for the content of toxins that produce harmful microorganisms, namely: *Aspergillus flavus*, *Aspergillus parasiticus speare*, *Aspergillus fumigates*, *Stachybotrys alternans* etc. (Shkodyak *et al.*, 2020). Therefore, it is necessary to stabilise the balance of the bird's intestines by using probiotics that stimulate feeding processes due to the normalisation of microflora, which represents adjuvant-active compounds that enter the blood and stimulate the immune system (Nwaigwe *et al.*, 2020).

Pre-colonisation of the intestines of broiler chickens with probiotic cultures that are considered competitive leads to a decrease in the formation of pathogenic microflora in the intestine, that is, probiotics act as a competitive replacement. This process minimises the occurrence of infectious gastrointestinal diseases in 65-85% of young poultry, and therefore, probiotic preparations have a positive effect on the quality and safety of poultry products (Sarangi *et al.*, 2016). Probiotic preparations for feeding poultry not only increase its productivity, but also meet the needs of the necessary components of the body of ordinary consumers in high-quality and safe nutrition (Podolian, 2016).

Probiotics based on bacilli, in particular, their introduction into special feed mixtures and preparations that are complex, have become widespread in veterinary practice. They are prescribed for the prevention of salmonellosis in poultry during farmstead cultivation. The high efficiency of the use of representatives of the genus *Bacillus* was also established during the prevention and treatment of salmonellosis, purulent, colibacillosis, and staphylococcal infections (Zhou *et al.*, 2015). The use of the probiotic Probiol in the amount of 0.25 g/t of mixed feed when feeding broiler chickens of the cross Ross-308 for 42 days has a positive effect on the morphological and biochemical parameters of the blood. Researchers have found an increase in the level of haemoglobin by almost 15.0%, the content of red blood cells and white blood cells in the blood of poultry, an increase in the content of total protein in the blood serum by almost 5.0% and glucose by 30%. However, these indicators were within the physiological norm for broiler chickens (Podolian, 2017).

It is believed that the addition of probiotics to the poultry diet further increases the titer of antibodies against infectious bursal disease (IBD) and increases the immune response – the response of broiler chickens to this disease. Feed supplements affected the blood counts of broilers on day 35 of rearing. At the same time, the content of total protein, albumins, globulins, cholesterol, and the activity of alanine aminotransferase in blood serum remained unchanged ($P<0.01$). Blood glucose levels were significantly reduced ($P<0.05$), triglycerides increased, and alanine aminotransferase activity also decreased ($P<0.01$) (Rehman *et al.*, 2020). M.M. Khubeiz & A.M. Shirif (2020) found changes in blood counts when feeding chickens natural preparations. An increase in the number of basophils and eosinophils indicated changes in the immune system of chickens under the influence of coriander seed powder. An

increase in the number of lymphocytes also indicated a possible improvement in the immune system. The number of basophils and eosinophils increased by 2.5%, and lymphocytes – by 1.5%. The use of probiotic preparations in poultry farming improves their productivity, namely, the increase in carcass weight, the quality and safety of meat and offal, which provides the human body with nutrients and is quickly absorbed.

Materials and Methods

To establish the efficacy and safety of the probiotic biological product Subtiform in the rearing of broiler chickens of the cross SOBB-500, the tests were conducted at the LLC Skibinetska Poultry Farm, Skibintsi village, Tetiiv district, Kyiv Region, during 2022-2023.

At the facilities of the LLC Skibinetska Poultry Farm, the technology used is the rearing of COBB-500 broilers on a floor with deep litter. The broiler chickens are housed in large-sized poultry houses in large single-age batches (up to 50 animal units). The lighting of poultry farm premises is automated; all production processes of poultry rearing in poultry houses are mechanised: feed distribution, water drinking, poultry heating, and droppings cleaning. The basic principle when raising poultry is to control the optimal microclimate in the poultry house: temperature of 28-30°C and humidity level of 70-80%. The poultry farm has developed a special mixed feed for poultry, which includes corn, wheat, several types of meal, a complex of vitamins and minerals, bone meal, fat, yeast, salt, and chalk. To improve the overall nonspecific resistance of the poultry body, broiler chickens were given vitamin preparations for 6-12 days of life in accordance with the requirements of use. In the period from 12 to 14 days of life, broiler chickens were given preventive measures for coccidiosis.

The task was set to experimentally substantiate the feasibility of using and determine the optimal dose of probiotic biologics among the studied doses (0.5, 2.0, and 4.0 g/10 dm³ of water) for broiler chickens during their feeding. A control group of broiler chickens (birds were not given a probiotic preparation) and 3 experimental groups (20 birds each) were established, which were provided with feed and given probiotics in the specified amounts: experimental group 1 – 0.5 g/10 dm³ of water; experimental group 2 – 2.0 g/10 dm³ of water; experimental group 3 – 4.0 g/10 dm³ of water in the period from 28 to 42 days. Control and experimental groups of poultry were fed nutritious feed according to the established standards, considering the age of broiler chickens. The clinical condition of the birds was monitored daily. Blood samples were taken from six broiler chickens from each group on days 28, 35, and 42 rearing and for further research in the accredited research laboratory of the Department of Veterinary and Sanitary Expertise and Laboratory Diagnostics (certificate of compliance of the measurement system with the requirements of DSTU 10012:2005 (2007) and the inter-faculty laboratory of histochemical and biochemical methods research of the Bila Tserkva National Agrarian University. The effect of probiotic biologics on the morphological parameters of the blood of broiler chickens was investigated: the number of red blood cells and white blood cells counted in the Goryaev chamber, and the haemoglobin concentration was also determined (Levchenko, 2017).

The biochemical parameters of blood serum were determined: the concentration of total serum protein by the Kingel-Weikselbaum biuretic reaction method, the content of albumins by the Nephelometric (turbidimetric) method using a photoelectrocolorimeter (KFK-3); the content of total lipids by the Zlatkis-Zak method, total cholesterol by the

Trinder method by fermentation; creatinine by the Jaffe-Popper method due to deproteinisation with picric acid; the concentration of uric acid – by the colour reaction using phosphor – Wolfram reagent on KFK-3; activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) – by Reitman-Frenkel dinitrophenylhydrazine method; content of triacylglycerols (TAG) and total calcium content – by photometric method, inorganic phosphorus – by reduction of phosphor-molybdenum acid (Levchenko, 2002). Stabilised blood and its serum were used to analyse morpho-biochemical parameters in broiler chickens.

Characteristics of the probiotic biological product Subtiform. Probiotic biological product Subtiform is a drug of symbiotic nature. The composition of the drug includes bacteria of the genus *Bacillus subtilis* and *Bacillus licheniformis*, the content of which is 2.5×10^9 CFU/g, filler – dry milk whey. Immunobiological properties: probiotic preparation is used as an auxiliary agent for the treatment and preventive measures for gastrointestinal infections of bacterial and viral aetiology, stability of intestinal microflora in dysbiosis, growth stimulation, to improve the safety and productivity of livestock and poultry. Probiotic bacteria *Bacillus subtilis* and *Bacillus licheniformis* which are part of the feed additive, show sensitivity to the following antibiotics: gentamicin, neomycin, colistin sulphate, amoxicillin, and streptomycin. When combined with antibiotics with a gram-positive spectrum of action, a partial decrease in the effectiveness of the drug may occur; it can be combined with coccidiostatics.

Experimental studies were conducted in accordance with modern methodological approaches and in compliance with the requirements and national standards, namely DSTU ISO/IEC 17025:2019 (2021) and in accordance with Directive 2010/63/EU (2010), which were approved by the conclusion of the commission

on ethics and bioethics of the Faculty of Veterinary Medicine of the Bila Tserkva National Agrarian University dated 12.04.2023. The animals were kept and all manipulations were carried out in accordance with the provisions of the European Convention for the protection of vertebrate animals used for experimental and scientific purposes (1986, March).

The importance of research for their implementation was confirmed by the use of certified equipment, modern methods, and in the process of statistical processing of the results obtained. Variational and statistical processing of the results was carried out using Microsoft Excel and Maple-12 (Maplesoft, 2008) software suites. The Student's t-test was considered in the case of a normal data distribution, and the following differences were assumed statistically significant: $P < 0.05$; $P < 0.01$, $P < 0.001$.

Results and Discussion

According to the clinical examination of broiler chickens, the natural position of their bodies was established, the chickens also reacted to external stimuli, actively moved, and consumed water and feed. The condition of the visible mucous membranes of chickens had a pale pink colour; the condition of the feather cover was marked by cleanliness; the condition of the beak was dry; discharge from the eyes and cloaca was not observed; dryness was found on the surface of the limbs, without visible damage. No wheezing was detected during the breathing acts of broiler chickens. The body temperature corresponded to physiological limits, namely the range of 40.5–42°C. During the testing period, no cases of disease or deaths of broiler chickens were recorded. The results of the studied haematological parameters of broiler chickens for the use of probiotic biologics on days 35 and 42 of rearing in the control and experimental groups are shown in Figures 1, 2.

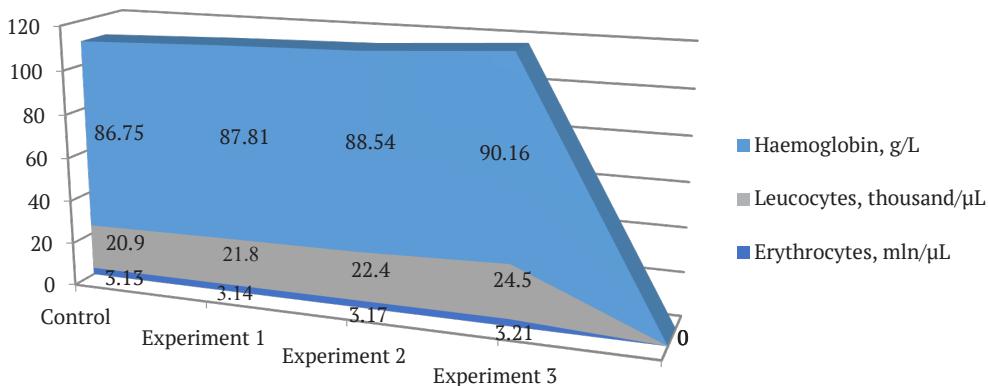


Figure 1. Dynamics of morpho-biochemical parameters of whole blood of broiler chickens on day 35 of rearing

Source: developed by the author

The content of red blood cells in the blood of broiler chickens of the 2nd and 3rd experimental groups increased by 1.2% ($P<0.05$) and 2.6%, respectively, according to the indicators of the control group, but these indicators were within physiological limits. In the blood of broiler chickens of the 1st, 2nd and 3rd experimental groups, there was a tendency to increase the number of white blood cells, which indicated an increase

in the state of general resistance of the body of broiler chickens and the mobilisation of the corresponding cellular factors, namely on the 35th day of poultry rearing, respectively-by 4.4%, 7.2 and 17.2% ($P<0.05$) in accordance with the indicators of the control group. The haemoglobin content in the blood of poultry of the experimental groups increased, respectively, by 1.2%, 2.1, and 3.9% compared to the control group.

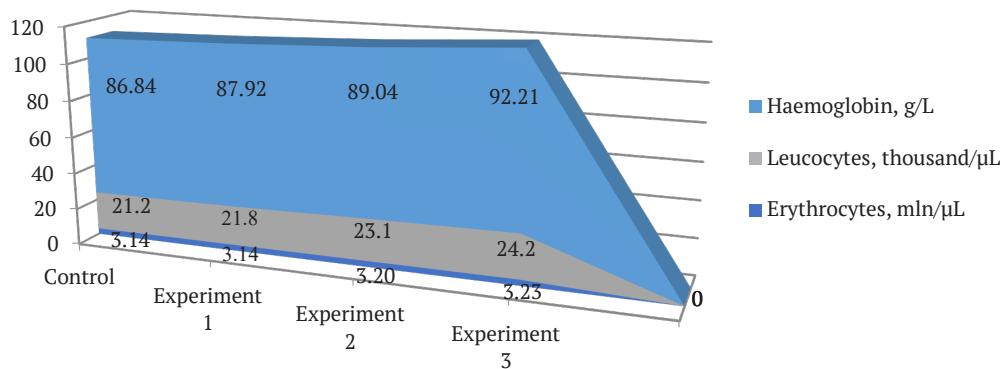


Figure 2. Dynamics of morpho-biochemical parameters of blood of broiler chickens on day 42 of rearing

Source: developed by the author

The number of red blood cells in the blood of chickens of the 2nd and 3rd experimental groups increased by 1.9% ($P<0.05$) and 2.9%, respectively,

on the 42nd day of rearing compared to the control indicators. In the blood of broiler chickens of the 1st, 2nd and 3rd experimental groups, there

was a tendency to increase the number of white blood cells, which indicated an increase in the resistant state of their body and a corresponding increase in cellular factors, namely, on the 42nd day of rearing, respectively – by 2.4%, 9.0 and 14.2% ($P<0.05$) compared to the control group. The studied indicators were within physiological limits. The haemoglobin content in the blood of poultry in the three groups increased by 1.2%, 2.5 and 6.2%, respectively, compared to the control group of broiler chickens.

Thus, the use of probiotic preparations in the production of broiler chickens to produce organic food is a topical issue in the global livestock industry. W. Jiang *et al.* (2017) claim that the use of probiotics exhibits antibacterial properties and immunomodulatory effects on the body of broiler chickens. They had a positive effect on the blood parameters of poultry on the 42nd day of rearing with a significant increase in the number of red blood cells by 2.4% ($P<0.01$), the number of white blood cells by 6.2% ($P<0.01$), and the haemoglobin content by 3.7% compared to the control group of poultry. W. Jiang *et al.* (2017) proved that the introduction of new feed additives into the diet of broiler chickens can cause stress for poultry,

and also found that during the rearing of poultry, blood parameters slightly differed with the control group – the number of red blood cells significantly increased by 1.3 times ($P<0.01$), the number of white blood cells by 1.5 times ($P<0.01$), and the haemoglobin content – by 1.2 times ($P<0.01$). When a probiotic drug was introduced into the diet of broiler chickens in combination with thyroxine, the total number of white blood cells increased (11.5×10^3 per 1 μL) relative to the control group of poultry (10.7×10^3 per 1 μL). Researchers have proven an increase in the natural resistance of the body of broiler chickens to the use of probiotics.

According to the obtained haematological parameters, an increase in the level of antibacterial properties of the body protection of broiler chickens of the 3rd experimental group was established on the 35th and 42nd days of rearing when feeding a probiotic biological product at a dose of 4.0 g/10 dm³. Table 1 shows the biochemical parameters of the blood of broiler chickens of the 1st, 2nd, and 3rd experimental groups when feeding probiotic biologics in the amount of 0.5 g/10 dm³ of water, 2.0 g/10 dm³ of water, and 4.0 g/10 dm³ of water for the 35th day of rearing.

Table 1. Dynamics of biochemical parameters of blood serum of broiler chickens when using a probiotic biological product on day 35 of poultry rearing, $M \pm m$, $n=6$

Indicator	Control group	Day 35 of rearing		
		Experimental group 1	Experimental group 2	Experimental group 3
Total protein, g/L	37.26 ± 1.27	37.35 ± 1.12	$43.57 \pm 1.44^{**}$	$44.09 \pm 1.39^{**}$
Albumins, %	21.56 ± 0.84	22.62 ± 1.02	22.51 ± 1.01	23.01 ± 0.89
Total lipids, g/L	13.19 ± 0.47	13.20 ± 0.47	13.19 ± 0.43	13.23 ± 0.47
Total cholesterol, mmol/L	3.20 ± 0.09	3.19 ± 0.04	3.18 ± 0.05	3.20 ± 0.05
Creatinine, mmol/L	25.71 ± 0.59	26.13 ± 0.34	26.19 ± 0.28	26.25 ± 0.34
Uric acid, mmol/L	0.27 ± 0.04	0.26 ± 0.01	0.21 ± 0.01	0.19 ± 0.01
Alanine aminotransferase, U/L	4.92 ± 0.64	5.42 ± 0.42	5.52 ± 0.29	5.57 ± 0.65
Aspartate aminotransferase, U/L	77.88 ± 1.74	$84.16 \pm 1.66^{*}$	$85.33 \pm 1.72^{**}$	$85.68 \pm 1.68^{**}$
Triacylglycerols, mmol/L	1.84 ± 0.10	1.70 ± 0.09	1.61 ± 0.10	1.44 ± 0.06

Table 1. Continued

Indicator	Control group	Day 35 of rearing		
		Experimental group 1	Experimental group 2	Experimental group 3
Calcium, mmol/L	2.47±0.05	2.61±0.02	2.60±0.02	2.65±0.02
Inorganic phosphorus, mmol/L	2.40±0.03	2.29±0.02**	2.45±0.02	2.47±0.04

Notes: *** $P<0.05$; ** $P<0.01$; *** $P<0.001$ compared to the control group of broiler chickens

Source: developed by the author

The total protein content in the blood serum of poultry of the 2nd and 3rd experimental groups increased slightly on the 35th day of rearing, respectively, by 1.17 times ($P<0.01$) and 1.18 times ($P<0.01$) compared to the control group. There was no statistically significant difference in the content of albumins and total lipids in the blood serum of poultry with the control group. The content of cholesterol in the blood serum of chickens of experimental groups on the 35th day of rearing was within the reference values. This is due to the fact that cholesterol synthesis is reduced by the action of a probiotic. S. Mookiah *et al.* (2014) proved that feeding broiler chickens a probiotic drug improved the poultry's metabolism, reduced serum cholesterol by 2.9% ($P<0.001$), and increased the number of white blood cells by 16.0%.

Serum creatinine levels in broiler chickens were not statistically significant compared to the control group (they were within the physiological norm). A.A. Koronowicz *et al.* (2016) state that when using probiotic drugs, the content of total serum protein slightly increased by 1.2% ($P<0.01$), albumins by 1.1%, and total lipids was within the normal range and at the level of the control group. The results of this study were consistent with the results of Yu.M. Podolian (2017), who found that the serum cholesterol content of broiler chickens fed a probiotic decreased by 7.1%, the total protein content increased by 3.5%, the haemoglobin content increased by 1.1 times, the number of red blood cells increased by 1.2 times, and the

number of leukocytes increased by 1.0 times compared to the control.

The concentration of uric acid in the blood serum of chickens of experimental group 1 was within the control values and corresponded to the values of the control group of poultry, and in experimental groups 2 and 3 it slightly decreased. A 1.3-fold decrease in uric acid content ($P<0.01$) in the blood serum of chickens for setting probiotics is consistent with the results of C. Li *et al.* (2022). The researchers also found that in the control and experimental groups of broiler chickens that were given probiotic drugs, the concentration of urea in the blood serum in its values corresponded to the limits of physiological fluctuations. This confirms the positive effect of probiotics of bacteria of the genus *Bacillus subtilis* and *Bacillus licheniformis* on the blood parameters of broiler chickens and an increase in their productivity, which affected the improvement of the quality of meat of broiler chickens. According to the conducted study, the activity of the AST enzyme in the blood serum of broiler chickens of the experimental groups significantly increased. In experimental group 1 – by 8.1% ($P<0.05$), in experimental group 2 – by 9.6% ($P<0.01$), in experimental group 3 – by 10.0% ($P<0.01$) compared to the control. Indicators of inorganic phosphorus content in the blood serum of poultry of experimental group 1 significantly decreased by 4.6% ($P<0.01$) in accordance with the control group. These indicators suggested that there were no violations of the Ca : P ratio, which was in the range from 1.07 to 1.14.

W. Jiang *et al.* (2017) found that when broiler chickens were given probiotics in the blood serum, the content of phosphorus (5.77 mmol/L compared to the control group of poultry – 5.17 mmol/L; $P=0.0121$) and glucose (256.9 mg/dm³ compared to the control group – 219.3 mg/dm³; $P<0.0001$).

The period of action of the probiotic did not affect the concentration of blood calci-

um, total protein, albumin, globulins, total cholesterol, triacylglycerols, AST and ALT activity ($P<0.05$). Table 2 shows the biochemical parameters of the blood serum of broiler chickens of the 1st, 2nd, and 3rd experimental groups when feeding probiotic biologics in the amount of 0.5 g/10 dm³ of water, 2.0 g/10 dm³ of water, and 4.0 g/10 dm³ of water for the 42nd day of rearing.

Table 2. Dynamics of biochemical parameters of blood serum of broiler chickens with the use of probiotic biological product on day 42 of poultry rearing, $M\pm m$, $n=6$

Indicators	Control group	Day 42 of rearing		
		Experimental group 1	Experimental group 2	Experimental group 3
Total protein, g/L	37.26±1.27	37.16±1.57	37.66±2.92	38.36±3.07**
Albumins, %	21.56±0.84	22.64±1.02	22.44±0.95	23.94±1.13
Total lipids, g/L	13.19±0.47	13.18±0.46	13.08±0.51	13.24±0.44
Total cholesterol, mmol/L	3.20±0.09	3.17±0.04	3.18±0.05	2.89±0.08*
Creatinine, mmol/L	25.71±0.59	26.25±0.44	26.51±0.31	26.73±0.36
Uric acid, mmol/L	0.27±0.04	0.17±0.01*	0.15±0.03*	0.14±0.04
Alanine aminotransferase, U/L	4.92±0.64	6.19±0.59	6.36±0.62	6.57±0.65
Aspartate aminotransferase, U/L	77.88±1.74	93.17±1.64***	99.43±1.32***	102.34±0.82***
Triacylglycerols, mmol/L	1.84±0.10	1.64±0.07	1.53±0.09	1.31±0.15
Calcium, mmol/L	2.47±0.05	2.55±0.02	2.57±0.02	2.61±0.02
Inorganic phosphorus, mmol/L	2.40±0.03	2.30±0.01**	2.32±0.03	2.49±0.02*

Notes: *** $P<0.05$, ** $P<0.01$; *** $P<0.001$ compared to the control group of broiler chickens

Source: developed by the author

The total protein content in the blood serum of poultry of experimental group 3 on the 42nd day of poultry rearing significantly increased by 1.0 times ($P<0.01$), the albumin content changed slightly, and in experimental group 3 it slightly increased – by 1.1 times. There is no statistical difference in the amount of albumin content with the indicator of the control group. This indicated that the broiler chickens remained clinically healthy and their bodies maintained proper colloidal and osmotic blood pressure, regulation of water metabolism, the process of binding and transporting lipids, carbohydrates, minerals, and vitamins. Lipids for the body of living things are

an energy material. In particular, when 1 g of fat is completely broken down, about 9.6 kcal of energy is released, which is almost 2.2 times more than similar carbohydrates and proteins. The bird's body is provided with energy at the expense of fats due to a lack of carbohydrates. The cell membranes of animal organs and tissues contain a significant amount of lipids (Youssef *et al.*, 2022), and therefore, they also perform a structural function.

The results obtained in this study were consistent with the results of L.W. Chen *et al.* (2021), who found that when feeding broiler chickens probiotics *Bacillus amyloliquefaciens*

and *Saccharomyces cerevisiae* had a positive effect on intestinal morphological features and regulation of fat metabolism in poultry, an increase in the carcass weight of broiler chickens by 0.771 g, as well as changes in blood serum parameters: an increase in total protein content by 1.4% ($P<0.05$), albumins – by 1.4% ($P<0.01$), reducing cholesterol by 1.1% ($P<0.05$) and uric acid by 1.2% ($P<0.05$), as well as increased activity of alanine aminotransferase by 1.3 times ($P<0.01$) and aspartate aminotransferase by 1.6 times ($P<0.05$). As can be seen from Table 2, there was no statistical difference in the cholesterol content in the blood serum of poultry of experimental groups 1 and 2 with the control group. But in poultry of experimental group 3, this indicator decreased by 1.1 times ($P<0.05$) compared to the control. This is due to the fact that cholesterol synthesis is reduced by the action of a probiotic (Al-Khalafah *et al.*, 2022). The creatinine content in the blood serum of broiler chickens on days 35 and 42 of rearing was within the physiological norm. It was found that creatinine is actively involved in energy processes in cells between mitochondria, and its content in the blood depends on the level of formation and excretion, and this had a positive effect on the muscle development of broiler chickens.

The concentration of uric acid in the blood serum of poultry of experimental groups 1 and 2 decreased by 1.6 times ($P<0.05$) and 1.8 times ($P<0.05$) on the 42nd day of rearing, respectively, compared to the control group. The uric acid content in the blood serum of broiler chickens of the control and experimental groups was within the physiological norm. The results obtained in this paper are consistent with the results of A. Nada *et al.* (2023) that the concentration of uric acid in the blood serum when feeding broiler chickens a probiotic drug decreased by 1.3-1.9% ($P<0.05$). This is indicated by the stability of the balance of microflora in the intestines of poultry, stimulating feeding

processes due to the normalisation of microflora, which acts as a source of adjuvant-active substances that enter the blood and stimulate the immune system of broiler chickens. In the future, this leads to an increase in poultry productivity, an increase in the weight of carcasses and internal organs, a decrease in the content of microorganisms in meat and products of slaughter of broiler chickens, and also has a positive effect on the microstructure of muscle tissue, an improvement in the content of amino acid and fatty acid composition of poultry meat.

According to the ALT activity (Table 2) in the blood serum of broiler chickens of the experimental groups, no statistical significance was established with the control group. The results obtained were consistent with data of G. Bazaka *et al.* (2019), who found that probiotic supplements increased serum alanine aminotransferase activity by 2.2% ($P<0.01$) and aspartate aminotransferase 2.0% ($P<0.05$). The content of white blood cells and red blood cells in the blood of broiler chickens increased by 5.6 and 4.8%, respectively. The AST activity in the blood of poultry of experimental groups significantly increased (Table 2). In experimental group 1 – by 19.6% ($P<0.001$), in experimental group 2 – by 27.8% ($P<0.001$), in experimental group 3 – by 31.4% ($P<0.001$) compared to the indicators in the control group. The indicator of AST activity signifies the activation of the formation of substitutable amino acids by reamination during prolonged feeding of the probiotic, and this process is characterised by an increase in muscle tissue weight, which indicates an increase in weight gain in broiler chickens on day 42 of rearing. The content of calcium in the blood serum of poultry on the 42nd day of rearing was at the level of the control group, which indicated that there was no violation of the Ca : P ratio (it was in the range from 1.05 to 1.11).

C. Li *et al.* (2022) found that for feeding broiler chickens a probiotic *Bacillus amyloliquefaciens*

CGMCC18230 during poultry rearing up to 42 days, a higher concentration ($P<0.05$) of phosphorus and alkaline phosphatase activity in the blood serum were observed, but the calcium content did not change. When the experimental groups of broiler chickens were fed the biological product at a dose of 4.0 g/10 dm³ of distilled water, it was found that their growth improved due to the effect of the probiotic on the activation of processes in the intestine for microbial modulation and increased accumulation of phosphorus and calcium.

C.U. Nwaigwe *et al.* (2020) found that according to the biochemical parameters of the blood serum of poultry, which are significant in assessing the physiological status of the body of broiler chickens, as well as the presence of stress factors and various diseases of poultry, it is possible to assess the state of metabolic processes in the body of broiler chickens. The results obtained in this study regarding the positive effect of probiotic dietary supplements on the morpho-biochemical parameters of the blood of broiler chickens are consistent with the results of other researchers. F.U. Memon *et al.* (2022) found that adding probiotics *Bacillus subtilis* to the diet of broiler chickens had a positive effect on maintaining intestinal homeostasis, modelling its microflora and preventing the development of certain intestinal infections of poultry, as well as increased the immune status and productivity of poultry and improved the quality of slaughter products.

According to the results of experiments that provided for feeding probiotic biologics to broiler chickens in different doses, the biochemical parameters of blood serum on the 35th and 42nd days of their cultivation corresponded to the limits of physiological fluctuations and were characteristic of the corresponding age groups. The results obtained are also consistent with the data of N.R. Sarangi *et al.* (2016) and Yu. M. Podolian (2017). Feeding of the probiotic

biological product for 14 days, i.e., from 28 to 42 days of rearing, provided an increase in body weight by 2.0-8.2%, an increase in average daily body weight gain from 96 to 104 g, and feed consumption per 1 kg of weight gain decreased by 1.97-7.55%. According to this study, it was found that feeding probiotic biologics (*Bacillus subtilis* and *Bacillus licheniformis*) to broiler chickens stimulates their productivity.

The results obtained are important for the implementation of the Food Safety System – HACCP (Hazard Analysis and Critical Control Points) at enterprises that process and sell meat and meat products. The basis of this system is the establishment of control over the safety of food products in determining dangerous risks and ensuring the prevention of toxic infections. Providing consumers with safe food products is regulated by national and international laws and regulations. Communicating information to consumers about the implementation of HACCP and traceability systems, as well as its proper functioning, problems, and advantages in implementing these systems throughout the food chain, should provide consumers with an informed choice of a safe and high-quality food product in order to meet the consumer's nutritional needs (Sharma *et al.*, 2019). Risk-based control over the technology of feeding broiler chickens is carried out by veterinary medicine specialists, including dosed delivery of probiotic drugs, as well as improving the conditions of keeping poultry, eliminating the occurrence of stress (Vojir *et al.*, 2012). The introduction of HACCP and traceability systems at broiler chicken facilities will ensure proper control of poultry rearing with balanced feeding using probiotic biologics and obtaining safe products for slaughtering broiler chickens.

Conclusions

The use of the probiotic preparation in the rearing of broiler chickens for 14 days at the

recommended doses of 0.5 g/dm³, 2.0 and 4.0 g/dm³ did not reveal any negative changes in the studied morphological and biochemical parameters of the poultry blood. In the blood of broiler chickens of experimental groups 1, 2, and 3, there was a tendency to increase the number of white blood cells on the 35th and 42nd days of rearing, which indicated a sufficient immune status of the body of broiler chickens and an increase in natural resistance against the background of an increase in the number of corresponding cellular factors, namely, on the 35th day of rearing poultry, respectively – by 4.4%, 7.2 and 17.2%; on the 42nd day, respectively, white blood cells – by 2.4%, 9.0 and 14.2% (P<0.05) compared to control indicators. The haemoglobin content in the blood of poultry of the experimental groups (on days 35 and 42) increased, respectively, by 1.2%, 2.1 and 3.9% and by 1.2%, 2.5 and 6.2% compared to the control indicators. The content of total protein in the blood serum of poultry of experimental groups 2 and 3 on the 35th day of cultivation slightly increased, respectively, by 1.2 times (P<0.01) compared to the control indicators; in experimental group 3 on the 42nd day of cultivation – by 1.0 times

(P<0.01); the content of albumins in the study groups on the 35th and 42nd days was within the control values. The content of inorganic calcium and phosphorus corresponded to the limits of physiological parameters, which indicated a sufficient level of mineral supply in the control and experimental groups of broiler chickens. The activity of alanine aminotransferase and aspartate aminotransferase, as well as the content of creatinine and total cholesterol in the blood serum of broiler chickens of the experimental groups did not differ from the control group, which indicated the absence of hepatotoxic and nephrotoxic effects of the probiotic drug.

Prospects for further study – to conduct post-slaughter veterinary and sanitary inspection of broiler chicken slaughter products, to establish the biological value of meat and its chemical composition for the use of probiotic biologics.

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None.

Conflict of Interest

None.

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Вплив пробіотичного біопрепарату на морфо-біохімічні показники крові курчат-бройлерів

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Анотація. За виробництва продукції бройлерного птахівництва оператори ринку застосовують інтенсивні технології, що передбачають використання різноманітних, екологічно нешкідливих нутрієвтиків, зокрема, пробіотичних препаратів. У технології вирошування курчат-бройлерів пробіотичні препарати необхідні як засоби для профілактики і лікування шлунково-кишкових захворювань, стимуляції росту та збільшення продуктивності птиці. Мета роботи – дослідити вплив пробіотичного біопрепарату на гематологічні показники за його випоювання курчатам-бройлерам. У досліді використали 20 голів курчат-бройлерів, яким випоювали пробіотик із 28 до 42 доби у кількостях: 0,5 г/10 дм³, 2,0 і 4,0 г/10 дм³ води. Встановлено, що морфо-біохімічні показники крові птиці відповідали фізіологічним нормативам для курчат-бройлерів встановленого віку та підтверджували відсутність патофізіологічних змін в їх організмі. На 35 та 42 добу вирошування у крові курчат-бройлерів за випоювання пробіотика у дозі 4,0 г/10 дм³ відзначали збільшення кількості лейкоцитів від 4,4 до 17,2 % і вмісту гемоглобіну – на 3,9 та 6,2 %, відповідно, порівняно з контрольною групою. На 35 добу дослідження за випоювання птиці пробіотика спостерігали збільшення вмісту загального білка у сироватці крові в 1,2 раза, відповідно в дозах 2,0 і 4,0 г/10 дм³ води, а на 42 добу – в 1,0 раза за його випоювання у дозі 4,0 г/10 дм³ води. Встановлено, що у сироватці крові птиці вміст кальцію й фосфору неорганічного відповідали фізіологічним межам, що вказує на достатній рівень мінерального живлення організму курчат-бройлерів. Відсутність змін активності аланінамінотрансферази та аспартатамінотрансферази, вмісту загальних ліпідів, холестерину та креатиніну в сироватці крові курчат-бройлерів дослідних груп свідчить про гепато- і нефронетоксичність пробіотику. За результатами роботи дослідженій препарат можна рекомендувати для підвищення резистентності організму птиці та регуляції обміну речовин

Ключові слова: резистентність організму; збалансована годівля; морфологічні показники; біохімічні показники; безпечність