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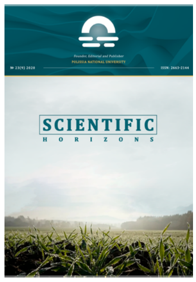

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The effect of complex application of symbiotic and biocidal preparations on the metabolic status of broiler chickens' blood

Olha Chechet, Svitlana Shulyak*, Vyacheslav Kovalenko, Maryna Romanko, Olha Haidei

State Scientific Research Institute of Laboratory Diagnostics
and Veterinary and Sanitary Expertise (SSRILDVSE)
01151, 30 Donetska Str., Kyiv, Ukraine

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Abstract. Using probiotic preparations and their complexes in poultry farming is an important step towards increasing poultry resistance and productivity, and thus producing environmentally friendly products. The purpose of the study was to investigate the effect of the complex action of symbiotic drugs and biocidal agents by investigating the clinical and biochemical parameters of blood and mineral metabolism in the body of broiler chickens under the conditions of their use according to the developed scheme. Hematologic, biochemical and immunologic blood parameters were explored by conventional methods, micro- and macroelements by optical emission spectrometry with inductively coupled plasma. Using the proposed complex of symbiotic and biocidal agents in the technology of rearing broiler chickens activates the processes of erythropoiesis, haemoglobin production, restoration of protein metabolism and normalisation of mineral status within physiological limits, which indicates the strengthening of the body's defences, increased resistance to stress factors and adverse environmental conditions, and will further have a positive effect on the growth and productivity of the target bird. The pronounced effect of the complex combination of drugs on the body of broiler chickens of experimental B is a better assimilation of many inorganic elements (by increasing the content of Calcium, Phosphorus, Cuprum, Ferrum and Selenium, Magnesium, Manganum); and, accordingly, causes the sustainable development of metabolic processes due to the prevalence of anabolic over catabolic processes (by increasing the level of total proteins against the background of a physiological increase in the number of globulin fractions; including the induction of the endogenous detoxification system – by reducing the level of production of toxic metabolites of purine metabolism: uric acid and creatinine; reduction of Chromium content; normalisation of transamination processes and deceleration of alkaline phosphatase activity) and immunobiological reactions (by enhancing erythropoiesis and haemoglobin production; induction of lysozyme, bactericidal and phagocytic activity), respectively. The proposed system of rearing young poultry using a complex of symbiotic preparations in combination with biocidal agents promotes the synergistic effect of the latter and their effective action on the body of experimental broiler chickens, which increases nonspecific resistance and restores metabolic reactions

Keywords: haematological parameters, biochemical parameters, mineral metabolism, natural resistance, probiotics, disinfectants

**Corresponding author*

INTRODUCTION

In veterinary practice, scientists propose a large number of approaches to the integrated use of drugs to increase poultry resistance and productivity, using various regimens, including antibiotics. Despite the legal ban on using antibiotics for prophylactic purposes and as growth stimulants, most researchers document their widespread use (Stoyanovskyi *et al.*, 2012; Osimani & Clementi, 2016; Romanyuk *et al.*, 2019), but the issue of their quality and safe use is still relevant.

Thus, several researchers H. El-Adawy *et al.* (2015), T.N. Nguyen *et al.* (2016) and A.A. Moawad *et al.* (2018) have proven that uncontrolled use of antibiotics, sulfonamides, coccidiostatics and numerous vaccinations results in the emergence of antibiotic resistance, immunodeficiency and poultry death. To prevent microorganisms from gaining resistance, probiotics with a different spectrum of lactobacilli and other beneficial microorganisms in their composition have recently been used to decelerate the effects of stress and increase weight gain, bringing the physiological state of the poultry body back to normal. In works (Byrd *et al.*, 2001; Babenko *et al.*, 2014; Arena *et al.*, 2017), the mechanisms of action of probiotics based on representatives of normal commensal microflora (lacto- and bifidobacteria), their antibacterial and immunomodulatory properties, and antagonistic effects against infectious agents were explored. Other researchers (Adil *et al.*, 2010; El Kady *et al.*, 2012; Babenko *et al.*, 2014) have demonstrated that the most cost-effective is using complexes of symbiotic preparations, which, due to the synergistic effect of all components, have immunomodulatory and antioxidant properties, contribute to the strengthening of nonspecific immunity, which together improves digestion, improves feed conversion, increases safety and weight gain and contributes to a better implementation of the productive potential of poultry.

Thomas, (2020), Stefańska *et al.*, (2021), Lytvynenko *et al.*, (2022) noted that effective drugs with universal properties and high-quality standards are required to protect animals from infectious diseases. Modern disinfectants should ensure the proper state of veterinary and sanitary welfare and reliable protection against infections, thus, they are one of the elements in the prevention of infectious diseases of productive animals and poultry in the technological process of their cultivation. Thus, previous studies by the authors (Chechet *et al.*, 2021; Chechet *et al.*, 2022; Kovalenko *et al.*, 2022) and several other scientists (Melnyk, 2017; Demchyshyn *et al.*, 2018) in *in vitro* and *in vivo* experiments have established the effectiveness and safety of biocides based on organic acids, peroxide compounds, chlorine dioxide, etc. As described by J. Bailly *et al.* (2017), "Biozapin", based on a mixture of cultures of probiotic bacteria *Bacillus sybtilis* and *Bacillus amyloliquefaciens* and aluminosilicate for surface treatment, has a prolonged effect due to the gradual adsorption of ammonia and urea in poultry housing. Studies by J. Bailly *et al.* (2017), O.M. Chechet *et al.* (2022) and V.L. Kovalenko *et al.* (2022) have demonstrated that the disinfectants "Diolaid" and "Biolaid" have an active antimicrobial effect against gram-positive and gram-negative bacteria, fungicidal properties, virulent effect and prevent resistance of microorganisms to the drug. V.L. Kovalenko *et al.* (2022) discusses using safe biocides in the presence of productive poultry and adding them to the water supply system, and proposes to combine the integrated use of symbiotic and disinfectants.

Thus, in the context of the intensification of the poultry industry, the health of productive poultry can be maintained by using complexes of safe probiotics, biocides and nutraceuticals, which will optimise metabolic processes in the body and increase natural resistance, effectively preventing the development of infectious diseases.

Thus, the purpose of the study was to explore the effect of a complex of symbiotic preparations in combination with biocidal agents on the dynamics of clinical and biochemical parameters and features of mineral metabolism in the body of broiler chickens in the technological scheme of rearing broiler poultry.

MATERIALS AND METHODS

A system of rearing young broiler chickens with the oral use of the symbiotic preparation "Biomagn" with the probiotic "Biozapin" (by spraying in the air) in combination with the biocidal agents "Diolide" (with the addition of water supply) and "Biolide" (disinfection of the room in the presence of poultry) is proposed. "Biomagn" contains a mixture of probiotic bacteria *Bacillus subtilis*, *Bacillus licheniformis*, *Enterococcus faecium* and lyophilized fermentation products of microorganisms *Lactococcus Lactis*, *Bacillus subtilis* and *Bacillus licheniformis*. In addition, "Biomagn" contains aluminosilicates, which effectively absorb mycotoxins from fodder and bacterial toxins, and suppress mutagenic microflora; betaine, which has a hepatoprotective effect, helps to reduce fats in hepatocytes, neutralises toxic elements and their compounds; silymarin, which has antioxidant properties, stimulates the synthesis of proteins and phospholipids in the affected liver cells; mannan oligosaccharides – exhibit probiotic properties and contribute to the normalisation of intestinal microflora by binding mannose residues to the receptors of pathogenic bacteria, respectively.

The experiment was performed in the vivarium of the State Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise ("SRILDVSE", Kyiv). According to the principle of analogues, 2 experimental (A; B) and one control (n = 21) were established from broiler chickens of the COBB-500 cross in the amount of 63 heads, 5 days old. The birds were fed complete fodder "Starter" (first 14 days) and "Grover" until the end of the experiment. Birds of experimental A and B were fed fodder with the addition of the symbiotic preparation 'Biomagn" from the first to the 7th and from the 22nd to the 27th day of rearing at a rate of 0.5 mg per kg of fodder, respectively.

Therewith, chickens of experimental B were given a solution of the biocidal preparation "Diolaid" (based on chlorine dioxide) 1.0 mg/l (by chlorine dioxide) with water throughout the experiment, which corresponds to a concentration of 0.0004% (Table 1). Disinfection in the experimental B poultry houses was performed with "Biolaid" biocidal preparation 0.2% (based on hydrogen peroxide, supralactic acid, and lactic acid) with an exposure time of 60 minutes. The technological rearing scheme included the probiotic preparation "Biozapin", which was used once every 2 weeks after disinfection and sprayed evenly in the poultry house at a rate of 10-30 g/m^2.

***Table 1**. Scheme of application of symbiotic with probiotic and in combination with biocidal preparations in experiments on broiler chickens*

Drugs/concentration	experimental A (probiotics) (n = 21)	experimental B (probiotics + + biocides) (n = 21)	Control (n = 21)
1	2	3	4
"Biomagn"	1-7th, 22-27th day	(1-7)th, (22-27)th day	Supplement was not provided
"Biozapin"	7th; 22th day	7th; 22th day	Supplement was not provided
"Diolaid"/0,0004%	Supplement was not provided	during the experiment	Supplement was not provided
"Biolaid"/0.2%	Supplement was not provided-	1time/week	Supplement was not provided
Antibiotics	Not provided-	–	(5-10)th day
Vitamins in mg per head	Not provided	–	(5-10)th day

***Source:** developed by authors*

The control birds received a standard rearing scheme. Blood samples were collected from poultry on the 10th, 30th and 40th day of rearing in vivo, by puncture from the subcubital vein using a heparinized cannula-free needle, following the rules of asepsis and antisepsis. The collected whole blood samples were divided into two parts: one for the determination of micro- and macroelements, and the second was stabilised with an anticoagulant (1% heparin solution) at the rate of 2-3 drops per 10-12 ml of blood for further determination of biochemical parameters.

Hematologic, biochemical, and immunologic blood parameters were explored by conventional methods. The content of total haemoglobin in the blood was determined by the haemoglobin cyanide method by colourimetric analysis, the number of red blood cells and leukocytes by the test tube method, in a chamber with a Goryaev grid (Hunchak, 2013; Fersunin, 2014). To determine the bactericidal activity of blood serum (BAC), a daily broth culture of *E. coli*, serovar O26, grown on Hottinger broth according to the method of O.V. Smirnova and T.A. Kuzmina (Danchuk *et al.*, 2013) was used. The determination of serum lysozyme activity ("SLA") was performed by the photonephelometric method of V.G. Dorofeichuk (Kotsiumbas *et al.*, 2012; Hunchak, 2013). The test culture used for the study was *Micrococcus lysodeikticus*, strain 2665. The phagocytic activity (PA) of blood leukocytes was explored in heparin-stabilised blood using a daily broth culture of *E. coli* (strain VKM) according to the Gostev method (Danchuk *et al.*, 2013).

Total proteins, albumin, uric acid, creatinine and the level of enzymatic activity [AST (K. F. 2.6.1.1); ALT (K. F. 2.6.1.2); AP (K. F. 3.1.3.1) activity] in plasma were determined using CORMAY (Poland, 2011) reagent kits as described in the manufacturer's manual (Vlizlo *et al.*, 2014). Spectrophotometric measurements were performed using a SHIMADZU UV-1800 instrument (Japan, 2017).

The content of micro- and macronutrients was explored by inductively coupled plasma optical emission spectrometry using an optical emission spectrometer (ICP-OES) PlasmaQuant PQ 9000 (Germany, 2018). Ultrapure nitric acid (Merck, Germany), certified multi-element standard solutions for atomic emission spectrometry (Merck, Germany) with certified ion content were used to prepare samples and prepare background and calibration solutions (El Kady *et al.*, 2012; Chechet *et al.*, 2022). The solvent was ultra-pure deionised water according to ISO 11885:1996 (1996), prepared by the Atrium 631 UV purification system (Sartorius, Germany). For the mineralisation of blood samples, it was used the Milestone Ethnos Easy system with HPR-1000/10S high-pressure rotor autoclaves (Milestone, Italy, 2021).

The results are presented as mean ± standard error (x ± SE). To compare the difference in mean parameters between the control and experimental groups, the authors of the research used the Tukey test, where the differences were considered statistically significant at $P<0.05$ for all the data.

All experimental studies were conducted according to modern methodological approaches and in compliance with the relevant requirements and standards, in particular, they correspond to the requirements of DSTU ISO/IEC 17025:2005 (2006). The animals were kept and all manipulations were performed according to the provisions of the Procedure for conducting experiments and experiments on animals by scientific institutions (Law of Ukraine No. 249, 2012), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European convention…, 1986).

RESULTS

Haematological parameters in experimental and control chickens were within their reference level and changed during the experiment within physiological limits. A gradual increase in the number of red blood cells in the blood of experimental poultry fed a probiotic agent and in combination with biocidal drugs was established. On the 10th day, the number of red blood cells in the blood of experimental A birds increased by 8.2%, and in experimental B – by 8.1%, respectively, compared to the control values of the indicator. By the end of the experiment, the tendency to increase the number of red blood cells within physiological limits compared to the control was maintained, which on day 40 was 8.8% for experimental A and 9.6% ($P < 0.05$) for experimental B birds, respectively.

It was established, within the physiological standard, a slight tendency to increase leukocytes in broilers of experimental A and B after disinfection on the 5th and 10th day by 0.89% and 1.70%, respectively, compared to the control. An increase in the content of total haemoglobin (Fig. 1) was established within the physiological standard, which is consistent with the dynamics of the number of red blood cells. Thus, on the 10th and 30th day of the experiment, the concentration of total haemoglobin in the blood of experimental A birds increased by 4.2 and 6.1% and experimental B – by 4.4 and 14.3% ($P < 0.05$), respectively, compared to the control. This trend persisted until the end of the experiment: on day 40, the most significant increase in the level of the index in experimental B birds was recorded, which was 16.9% ($P < 0.05$) compared to the control. Therewith, the increase in total haemoglobin in the blood of experimental A birds was determined to be only 6.1%, respectively.

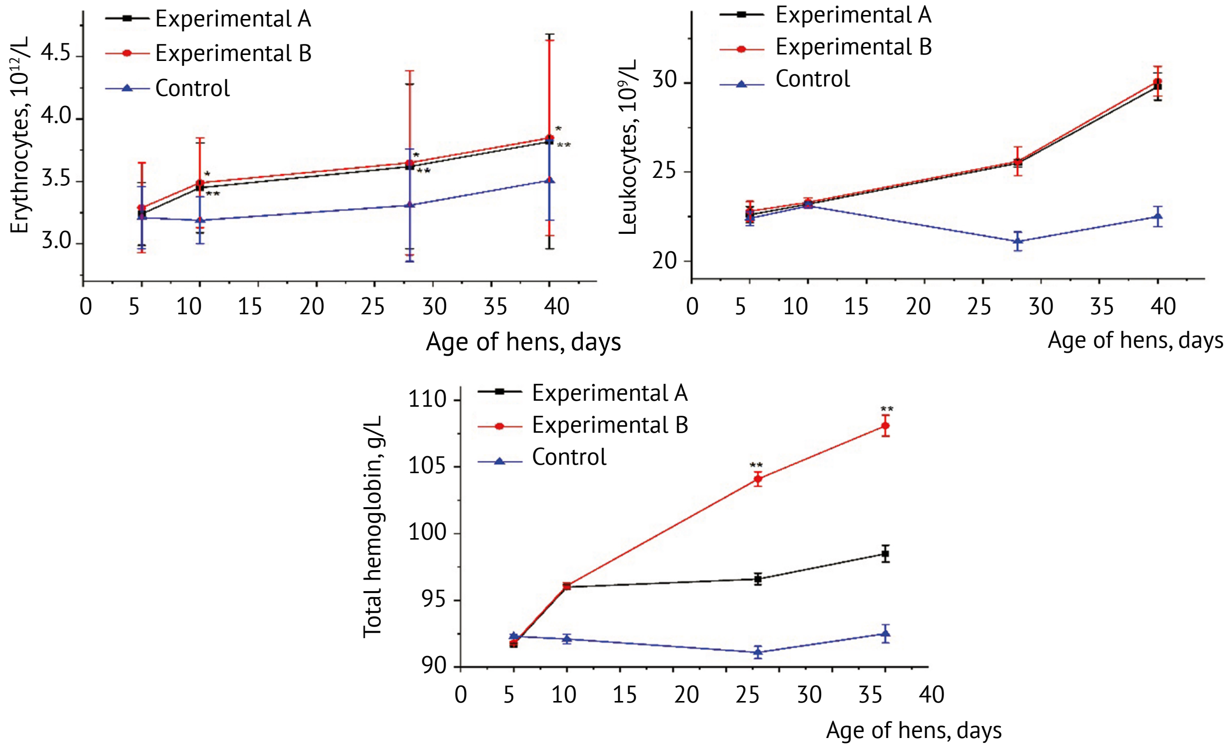

***Figure 1**. Dynamics of haematological parameters in the blood of broiler chickens under the influence of symbiotic with probiotic and in combination with biocidal preparations:*
***Notes:** Experimental A ("Biomagn"+"Biozapin"); Experimental B ("Biomagn"+"Biozapin" in the complex "Diolide"+"Biolide"); control (standard rearing diet); (x±SE; n=21); * – the mark indicates significant changes in the values of the indicator in the bird of the experimental group relative to that in the control group (P<0.05) according to the results of the ANOVA*
***Source:** developed by authors*

As a result of the study of nonspecific resistance indicators, a significant increase in the level of serum lysozyme activity and phagocytic activity (Fig. 2) in experimental B birds was established, which averaged

25.4% and 13.1% (P < 0.01) relative to the control values of indicators. Therewith, the value of the bactericidal activity of blood serum (Fig. 2) increased in experimental A and B birds from day 30 to day 40 inclusive, which at the end of the experiment averaged 26.1 and 24.1% (P < 0.01) relative to the control.

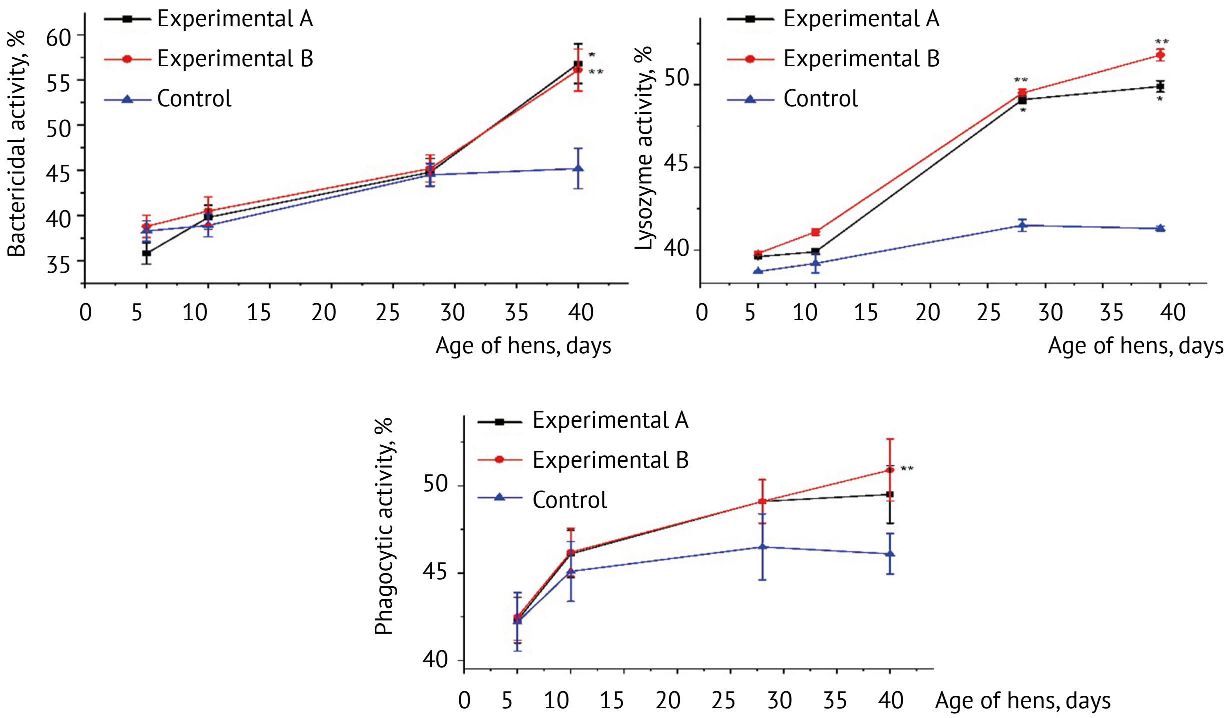

***Figure 1**. Dynamics of bactericidal (BAC, %;), lysozyme (SLA, %;) serum activities and phagocytic activity (PA, %;) in broiler chickens under the influence of symbiotic with probiotic and in combination with biocidal preparations*
***Notes:** experimental A ("Biomagn"+"Biozapin"); experimental B ("Biomagn"+"Biozapin"+ in the complex "Diolide"+"Biolide"); control (standard rearing diet); (x ± SE; n = 21); * – the mark indicates significant changes in the values of the indicator in the bird of the experimental group relative to that in the control group (P < 0.01) according to the results of the Tukey test*
***Source:** developed by authors*

Table 2 presents the results of studies of the level of biochemical parameters in the blood serum of broiler chickens in the dynamics under the influence of symbiotic preparations and biocidal agents. It was established that the content of total proteins in the blood serum of experimental A and B birds increased on day 10 of the experiment and was higher than the control by 10.9% and 19.1% (P < 0.01), respectively (Table 2). In addition, the same trend was observed at the end of the experiment, where the content of total proteins in the blood serum of experimental A and B birds on day 40 was higher than their control values by an average of 7.6% and 2.9%, respectively. Notably, on the 10th and 40th day of the experiment, the values of the index in birds of experimental group B exceeded those in experimental A by an average of 8.1% and 3.1%, respectively. Thus, it is evident that the increase in the level of SLA and PA (P < 0.01) in experimental B birds is caused by lysozyme secreted by phagocytes, which is a nonspecific factor of the immune system due to its pronounced hydrolytic, bactericidal effect, phagocytosis and antibody production. Therewith, the level of BAC increased in experimental A and B birds (P < 0.01), starting from day 30 to day 40 inclusive.

***Table 2**. Dynamics of biochemical parameters in the blood serum of broiler chickens under the conditions of exposure to symbiotic with probiotic and in combination with biocidal preparations (x ± SE; n = 21)*

Indicator	Poultry group (n = 21)	Reference level	10th day Of the Experiment	40th day of the experiment
1	3	2	4	5
Total proteins, g/L	Experimental A	25-40	28.53 ± 1.36[ab]	28.37 ± 1.30[a]
	Experimental B		30.61 ± 1.41[b]	29.21 ± 1.39[a]
	Control		25.72 ± 1.05[a]	26.23 ± 1.07[a]

Table 2, Continued

Indicator	Poultry group (n = 21)	Reference level	10th day Of the Experiment	40th day of the experiment
1	3	2	4	5
Albumins, g/L	Experimental A	15-22	10.21 ± 0.45	10.12 ± 0.41
	Experimental B		10.98 ± 0.38[a]	10.07 ± 0.40
	Control		13.45 ± 0.64	10.25 ± 0.42
Uric acid, mkmol/L	Experimental A	230-360	279.02 ± 11.52[a]	206.08 ± 14.51[a]
	Experimental B		271.41 ± 13.12[a]	205.10 ± 15.12[a]
	Control		314.71 ± 14.15	278.12 ± 11.88
Creatinine, mkmol/L	Experimental A	8.8-35.4	23.02 ± 3.25	20.04 ± 1.17[a]
	Experimental B		22.25 ± 2.04[a]	21.02 ± 1.19[a]
	Control		24.12 ± 1.07	23.48 ± 1.25
AST activity, U/L	Experimental A	2-8	4.02 ± 0.21[a]	3.02 ± 0.01[a]
	Experimental B		3.25 ± 0.32[a]	3.13 ± 0.03[a]
	Control		5.17 ± 0.02	5.25 ± 0.02
ALT activity, U/L	Experimental A	200-300	254.25 ± 9.12	294.32 ± 15.43
	Experimental B		253.12 ± 9.05	289.11 ± 16.05
	Control		255.52 ± 10.29	295.63 ± 18.41
AP activity, U/L	Experimental A	1900-19000	14139.45 ± 113.29	2570.48 ± 62.27[a]
	Experimental B		14320.12 ± 97.56	2620.25 ± 43.71[a]
	Control		13471.22 ± 123.14	4170.36 ± 71.18

Note: *experimental A (Biomagn + Biosapin); experimental B (Biomagn + Biosapin in the complex "Diolide" + "Biolide"); control (standard rearing diet); (x ± SE; n = 21); * – letters indicate significant differences between the groups in each period within column at (P < 0.05) according to the results of the Tukey test*
Source: *developed by authors*

A temporary tendency to increase the content of albumin in the blood serum of poultry of the control group was recorded. It was established that on day 10 of the study, the amount of albumin in the blood of experimental A and B birds was lower than in the control by an average of 19.6% (P<0.01) and 5.1%, respectively. However, the determined values of the index on the 40th day of the experiment did not vary statistically in experimental and control chickens and were almost at the same level. It was established that using symbiotic and biocidal preparations in the experimental chicken-rearing system significantly affected the dynamics of the end products of protein degradation in the poultry body – uric acid and creatinine, respectively (Table 2). It is known that urea synthesis in hepatocytes is the main way to neutralise nitrogen-containing compounds produced in the process of amino acid deamidation, and its concentration in the blood characterises the functional state of the liver and kidneys. The content of uric acid in the blood serum of chickens of experimental A and B on the 10th and 40th day of the experiment gradually decreased relative to the control values, which at the end of the experiment averaged 25.9% and 26.2% (P<0.01), respectively. In addition, the creatinine content in the blood serum of poultry decreased relative to the control, and its values were significantly lower on day 40 of the experiment than the control level in chickens of both experimental groups by an average of 14.6% and 10.6% (P < 0.01), respectively.

An important indicator of the functional state of the liver is the activity of the cellular enzymes alanine aminotransferase (ALT activity) and aspartate aminotransferase (AST activity), which catalyse metabolic processes and participate in the biochemical transformations of carbohydrates, lipids, nucleic acids and amino acids (Hilal *et al.*, 2016). In birds of experimental A and B, during the experiment, the values of enzymatic AST activity gradually decreased within the physiological standard, and on day 40 of the experiment were lower than the control level of the enzyme by an average of 42.5 and 40.4% (P < 0.01), respectively (Table 2).

However, the values of ALT activity in the blood serum of broiler chickens of the experimental groups in the dynamics of the experiment did not statistically vary from the control level and were within the physiological limits of the indicator.

The activity of alkaline phosphatase (AP activity) in the blood serum of experimental poultry was characterised by an upward trend at the beginning and its probable inhibition at the end of the experiment (Table 2). It was established that a moderate increase in AP activity within physiological limits relative to the control at the initial period of the study (day 10), in the future (day 40 of the experiment) the enzyme values significantly decreased relative to the previous ones, which for chickens of experimental A and B and control was 81.8% and 81.7% and 69.0% (P < 0.01), respectively.

Therewith, a significant decrease in the level of activity of this enzyme relative to the control values was recorded in chickens of experimental A and B on day 40 of the experiment, which averaged 38.4 and 37.2% ($P < 0.01$), respectively.

As a result of the analysis of the data obtained on the trace element composition of the blood of control and experimental poultry during the experiment, some changes in the indicators were established (Table 3). It was established that the concentration of Calcium, Phosphorus, Kalium, Magnesium, Manganum, Natrium, Zinc, Ferrum and Selenium in the blood of control chickens physiologically increased during the experiment, and its values were within the reference level of indicators. The values of such elements as Cobaltum, Chromium and Cuprum in the blood of control chickens at all study periods did not vary statistically and were within the reference level of these indicators.

***Table 3**. Dynamics of biochemical parameters in the blood serum of broiler chickens under the conditions of exposure to symbiotic with probiotic and in combination with biocidal preparations (x ± SE; n = 21)*

Element	Duration of the experiment, day	experimental A (n = 21)	experimental B (n = 21)	control (n = 21)
1	2	3	4	5
Magnesium, mg/kg	10th	0.57 ± 0.01	0.59 ± 0.01[a]	0.46 ± 0.06
	30th	1.79 ± 0.01	1.83 ± 0.01[a]	1.67 ± 0.03
	40th	1.79 ± 0.02	1.84 ± 0.02[a]	1.66 ± 0.11
Natrium, mg/kg	10th	41.06 ± 2,48	42.85 ± 2.55	44.13 ± 2.62
	30th	71.15 ± 5.68[a]	88.74 ± 6.71	88.40 ± 6.58
	40th	78.60 ± 3.12[a]	109.00 ± 7.58	107.70 ± 6.98
Kalium, mg/kg	10th	24.18 ± 1.68	25.00 ± 1.59	25.93 ± 1.58
	30th	37.23 ± 1.75	38.36 ± 1.75	38.77 ± 1.80
	40th	34.73 ± 1.70[a]	37.20 ± 1.71	39.15 ± 1.68
Calcium, mg/kg	10th	0.43 ± 0.05[a]	0.45 ± 0.06[a]	0.38 ± 0.043
	30th	1.15 ± 0.06[a]	1.12 ± 0.05[a]	0.95 ± 0.051
	40th	1.58 ± 0.08	1.57 ± 0.08[a]	1.42 ± 0.060
Chromium, mg/kg	10th	0.68 ± 0.06	0.60 ± 0.05a	0.72 ± 0.07
	30th	0.60 ± 0.06[a]	0.62 ± 0.06[a]	0.69 ± 0.063
	40th	0.64 ± 0,06[a]	0.64 ± 0.06[a]	0.71 ± 0.069
Manganum, mg/kg	10th	0.11 ± 0.04	0.20 ± 0.01[a]	0.12 ± 0.004
	30th	0.20 ± 0,05[a]	0.66 ± 0.01[a]	0.60 ± 0.006
	40th	0.17 ± 0.01[a]	0.68 ± 0.02[a]	0,57 ± 0.009
Ferrum, mg/kg	10th	4.25 ± 0.035	4,94 ± 0.03	4.63 ± 0.04
	30th	19.73 ± 0.81[a]	19.58 ± 1.62[a]	15.22 ± 0.61
	40th	22.01 ± 1.15[a]	22.22 ± 1.01[a]	15.45 ± 0.81
Cobaltum, mg/kg	10th	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.01
	30th	0.036 ± 0.020	0.04 ± 0.01	0.04 ± 0.03
	40th	0.021 ± 0.001[a]	0.025 ± 0.002	0.025 ± 0.002
Cuprum, mg/kg	10th	82.69 ± 1.68[a]	70.21 ± 1.78	71.83 ± 1.88
	30th	89.18 ± 1.85[a]	73.38 ± 2.32	72.28 ± 2.68
	40th	77.92 ± 1.28	76.08 ± 1.15	75.67 ± 1.08
Zinc, mg/kg	10th	71.61 ± 5.69	74.30 ± 5.77	71.99 ± 5.56
	30th	148.80 ± 11.18	152.30 ± 9.98	150.80 ± 9.78
	40th	173.50 ± 12.55	174.80 ± 12.68	173.90 ± 11.80
Selenium, mg/kg	10th	11.15 ± 0.035	13.64 ± 0.04[a]	9.63 ± 0.07
	30th	15.73 ± 1.81[a]	16.98 ± 0.12[a]	13.22 ± 1.11
	40th	21.01 ± 1.12[a]	23.22 ± 2.01[a]	16.45 ± 0.81

Table 3, Continued

Element	Duration of the experiment, day	experimental A (n = 21)	experimental B (n = 21)	control (n = 21)
1	2	3	4	5
Phosphorus, mg/kg	10th	15.59 ± 1.10ª	17.86 ± 1.20ª	13.55 ± 0.98
	30th	46.57 ± 3.23ª	46.87 ± 3.72ª	30.19 ± 2.16
	40th	46.39 ± 1.85ª	47.01 ± 1.97ª	34.42 ± 1.07
	30th	71.15 ± 5.68ª	88.74 ± 6.71	88.40 ± 6.58
	40th	78.60 ± 3.12ª	109.00 ± 7.58	107.70 ± 6.98

Note: *experimental A (Biomagn + Biosapin); experimental B (Biomagn + Biosapin in the complex "Diolide" + "Biolide"); control (standard rearing diet); (x ± SE; n = 21); * - letters indicate significant differences between the groups in each period within column at (P < 0.05) according to the results of the Tukey test*
Source: *developed by authors*

The results obtained indicate that at all periods of research, the level of such elements as Calcium and Phosphorus in the blood of experimental chickens gradually increased relative to their control values: on the 10th, 30th and 40th day of the experiment in experimental A birds it was 13.2%, 21.1% and 11.3% (P<0.01) and 15.1%, 54.3% and 34.8% (P < 0.01), respectively, and experimental B – 18.4%, 17.9% and 10.6% and 31.8%, 55.3% and 36.6%, respectively (Table 3). The data obtained, in general, indicate an intensification of the accumulation of Calcium and Phosphorus in the body of the experimental poultry.

In addition, a steady increase in the content of Ferrum and Selenium in the blood of experimental chickens was recorded, but for experimental A birds, it was recorded from day 20 to day 40 inclusive, which averaged 36.1% and 23.8% (P < 0.01), respectively, compared to the control (Table 3). In contrast to the chickens of experimental A, in the blood of experimental B birds, a positive trend in the increase in the content of Ferrum and Selenium began to be established from day 10 until the end of the experiment, which averaged 36.3% and 37.0% (P < 0.01), respectively, relative to the control level of indicators.

However, the values of such elements as Zinc in the blood of experimental broiler chickens in the dynamics of the experiment did not statistically vary from the control level, respectively, but increased during the experiment, which is physiological and within the reference range of the indicator. However, the bioavailability of Zinc is related to the action of phytic acid and the concentration of Cuprum. Consequently, the content of Cuprum in the blood of the experimental birds increased in value with their age. Thus, the highest concentration of the element was recorded on the 10th and 30th day of the experiment in chickens of experimental A, on average by 15.1% and 23.4% (P<0.01), respectively, compared to the control, which is obviously due to its active participation in the processes of hematopoiesis. After that, there was a significant decrease in the concentration of Cuprum in the blood of birds of this experimental group.

When determining the content of Magnesium and Manganum, a positive gradual dynamics was established in increasing the values of these elements only in the blood of experimental B birds, starting from day 10 and up to day 40 of the experiment inclusive (Table 3). Thus, the increase in the concentration of Magnesium and Manganum in the blood of chickens of experimental B relative to the control during the experiment was on average 12.7% and 18.6% (P < 0.01), respectively.

Notably, under the influence of Biomagn and Biosapin alone, the blood of experimental A chickens, on the contrary, decreased the content of such elements as Kalium, Manganum, Natrium and Cobaltum, which averaged 11.3%, 67.8%, 23.7% and 16.0% (P < 0.01), respectively, relative to the control values of these elements (Table 3). Notably, this was recorded in most cases at the last stages of the study (day 30 and/or 40 of the experiment). The content of Chromium in the blood of experimental A and B birds decreased on the 30th and 40th and on the 10th, 30th and 40th day of the experiment compared to the control values by an average of 11.4% and 12.7% (P < 0.01), respectively (Table 3).

DISCUSSION

Based on the results obtained, notably, the increase in natural resistance in the body of experimental B chickens is more pronounced and is characterised by a gradual increase in total haemoglobin, SLA, BAC, PA and total proteins (P < 0.01) under the influence of a complex of symbiotic preparations in combination with biocidal agents, and confirms the ability of the latter to inhibit and neutralise microbial agents. In addition, Kotsiumbas *et al.* (2012) and Bogusławska-Tryk *et al.* (2016) noted that the addition of probiotics to fodder in combination with biocide spraying stimulates the lymphatic system, the establishment of immunoglobulins, increases the amount of complement, macrophage and lysozyme activity, and the resistance of vascular and tissue barriers to toxins. Thus, due to the induction of oxidative processes, metabolism in the body of young poultry during intensive growth is intensified. The nature of the changes is consistent with the state of biochemical parameters in the blood of chickens of the experimental groups.

It should be emphasised that feeding the symbiotic "Biomagn" with the probiotic "Biozapin" and in combination with biocides in experimental B chickens, through the restoration of proteinogram indicators (with an increase in the content of total proteins and albumin; ($P < 0.01$)), has a positive effect on erythropoiesis and haemoglobin development, indicating an improvement in the nutritional, respiratory and enzymatic functions of the blood and contributes to an increase in the body's defences. Stimulation of protein-synthetic processes in the poultry body is a sign of the prevalence of anabolic (synthetic) processes over catabolic ones, and a sign of increased metabolic intensity is a decrease in the establishment of purine metabolism products ($P < 0.01$). Thus, the concentration of creatinine in the blood depends on the intensity of protein proteolysis in muscle tissue, which, along with a decrease in uric acid levels, indicates an activation of the functionality of endogenous detoxification systems in the body of chickens. It is consistent with the decrease in AST ($P < 0.01$) in experimental A and B birds. In control poultry, the rearing scheme which included the addition of an antibiotic to the diet, hyperenzymemia of AST was recorded, which is a sign of impaired liver reamination and detoxification functions, and it is argued that the dynamics of aminotransferases is an indicative and informative test of hepatocyte cell membrane permeability during liver damage by exogenous or endogenous toxins (Romanovich *et al.*, 2017; Chechet, 2021).

AP activity in the blood of experimental poultry is characterised by an increase at the beginning of the experiment and a decrease at the end ($P < 0.01$). Evidently, the probiotic "Biomagn" has a significant effect on the neurohumoral regulation of the activity of this enzyme, and according to Denbow (2000; 2013) and Bailly *et al.* (2017), by improving the digestion and absorption of vitamins, trace elements and other biologically active substances in the gastrointestinal tract, it potentially enhances its activity. Therewith, the decrease in AP activity relative to the control in chickens of experimental A and B can be explained by the intensification of Calcium and Phosphorus metabolism between bone tissue and macroorganism and the increased functioning of osteoclasts, which occurs during physiological growth and development of poultry, as described by Danchuk *et al.* (2013) Notably, the dynamics of changes in Calcium and Phosphorus in the body of experimental poultry are more pronounced in experimental B chickens, indicating a more intensive absorption of these elements from fodder under the combined effect of probiotic preparations with biocidal ones. Boikiv *et al.* (2001) and Bhattacharya *et al.* (2016) associate the active accumulation of Calcium and Phosphorus in the blood with the intensification of absorption from the gastrointestinal tract as a result of the improvement of the poultry digestive system and biocenosis in it.

On the other hand, the positive effect of the drug complex on the immunobiological reactivity of experimental poultry can be explained by the biological role of inorganic elements. The authors of the research established an increase in the level of Magnesium and Manganum in the blood of experimental B birds during the experiment. Thus, according to Devrim *et al.* (2010), El Kady *et al.* (2012) and Laur *et al.* (2020), the positive effect of the complex of drugs may be to improve redox processes, tissue respiration, bone development, hematopoiesis, biosynthesis of nucleic acids, proteins, antibodies, and affect the growth and development of young broilers. Thus, the content of Zinc was higher in the blood of experimental B birds compared to experimental A and control, which Hofmann & Hagey (2008) attributed to the dynamics of protein metabolism. As proved by Jegede *et al.* (2011) and Hajjarmanesh *et al.* (2022), the bioavailability of Zinc depends on the levels of phytic acid and Cuprum. Thus, the content of Cuprum in the blood of experimental poultry increases ($P < 0.01$), which is associated with the active participation of this element in the processes of hematopoiesis and is consistent with the dynamics of haematological parameters in chickens. According to Kalinichenko (2013), Cuprum is a hematopoietic bioelement, participating actively in the synthesis of haemoglobin and the establishment of other iron porphyrins, plays an important role in the conversion of Ferrum into an organically bound form, stimulates the maturation of reticulocytes and their transformation into red blood cells (Wang & Pantopoulos, 2011; El Sabry *et al.*, 2021). Thus, a positive trend in the increase in the content of Ferrum and Selenium ($P < 0.01$) in the blood of experimental B chickens during the experiment was established. It has been proved (Tsynovyi, 2013; Zapata, 2016) that Ferrum is a part of cytochrome C heme and is regulated in the body primarily by modulating its intestinal absorption and depends on the state of the intestinal microbiocenosis. Other researchers (Zwolak & Zaporowska, 2012) have demonstrated that increased absorption of Ferrum and Selenium in the body indicates a lack of their content in the poultry diet during intensive rearing. In the works of WHO. The European Health Report (2002), Li & Yang (2018), Dalia *et al.* (2020) and Sobolev *et al.* (2022) describe Selenium's ability to stimulate the production of antibodies, its role in liver function, protein synthesis and protection of the body from toxic minerals, participation in prostaglandin metabolism, and antioxidant and immunomodulatory properties. Researchers Klaenhammer *et al.* (2012) and Jaishankar *et al.* (2014) proved the possibility of influencing the biotransformation of such a heavy metal as Chromium and its significant reduction during critical physiological periods through the optimisation of poultry rearing technology by using probiotics. It is consistent with the determined decrease during the experiment in the content of Chromium ($P < 0.01$) in the blood of poultry of both experimental groups compared to the control.

But, under the influence of drugs in the blood of chickens of experimental A, on the contrary, at the end of the experiment, there is a decrease in Kalium, Manganum, Natrium and Cobaltum ($P < 0.01$) compared to the control.

According to Klaenhammer *et al.* (2012) and Kivit (2014), the biological role of Cobaltum is associated with its participation in the enzymatic function of vitamin B12, thus, evidently, the decrease in its content in the blood of experimental poultry is a consequence of the physiological maturity of its body and, accordingly, its lower need.

Based on the results obtained and according to (Kovalenko, 2008; Kalinichenko, 2013; Romanovich *et al.*, 2017), it can be emphasised that using a complex of probiotic and biocidal agents in the presence of birds will allow for an active action on pathogenic pathogens by reducing the microbial load in the environment, and will contribute to the stimulation of hematopoiesis, immune and metabolic reactions in the target bird.

CONCLUSIONS

Using the symbiotic "Biomagn" with fodder in combination with the probiotic "Biozapin" and in combination with the biocidal agents "Diolaid" and "Biolaid" promotes the synergistic effect of these drugs and their effective action, which activates the processes of haemoglobin development and protein metabolism. In particular, a gradual increase in the number of red blood cells and total haemoglobin in the blood of poultry of both experimental groups within physiological limits was found by 8.8% and 5.2% for experimental A and 9.6% and 9.4% ($P < 0.05$) for experimental B, respectively, compared to the control. It was established that the content of total proteins in the blood serum of experimental A and B birds increased by an average of 7.6 % and 2.9 % ($P < 0.01$), respectively, compared to the control, which, along with an increase in the level of BAC ($P < 0.01$), and SLA and PA ($P < 0.01$) – only in experimental B birds, characterises a significantly pronounced induction of immunobiological reactivity in the body of chickens of this group.

A decrease in the establishment of protein degradation products in the poultry body – uric acid and creatinine ($P < 0.01$) in the blood of experimental chickens of both experimental groups indicates an increase in the intensity of metabolism in the poultry body under the influence of a complex of preparations of symbiotic/probiotic and biocidal action. The content of uric acid and creatinine in the blood serum of chickens of experimental A and B decreased by an average of 25.9% and 26.2% ($P < 0.01$) and 14.6% and 10.6% ($P < 0.01$), respectively, compared to the control values.

Analysis of the results of blood trace element composition indicates normalisation of mineral status, enhancement of endogenous detoxification activity and body defences, which increases resistance to stress factors and adverse environmental conditions, and in combination with an increase in total protein content in experimental chickens is a sign of the prevalence of anabolic (synthetic) processes over catabolic ones, which will further positively affect the growth and productivity of broilers. Considering the results obtained, it is advisable to further explore the quality and safety of poultry products obtained as a result of using this rearing scheme.

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Вплив комплексного застосування симбіотичних та біоцидних препаратів на метаболічний статус крові курчат-бройлерів

Ольга Миколаїна Чечет, Світлана Валеріївна Шуляк, Вячеслав Леонідович Коваленко, Марина Євгенівна Романько, Ольга Сергіївна Гайдей

Державний науково-дослідний інститут лабораторної діагностики
та ветеринарно-санітарної експертизи (ДНДІЛДВСЕ)
01151, вул. Донецька 30, м. Київ, Україна

Анотація. Використання у птахівництві пробіотичних препаратів, а також їх комплексів, є важливим кроком до підвищення резистентності, продуктивності птиці, а отже, і отримання екологічно чистої продукції. Метою роботи було вивчення впливу комплексної дії симбіотичних препаратів та біоцидних засобів шляхом дослідження клініко-біохімічних показників крові та мінерального обміну в організмі курчат-бройлерів за умов їх застосування відповідно розробленої схеми. Гематологічні, біохімічні та імунологічні показники крові досліджували загально-прийнятими методами, мікро- макроелементи – методом оптико-емісійної спектрометрії з індуктивно зв'язаною плазмою. Застосування запропонованого комплексу симбіотичних та біоцидних засобів у технології вирощування курчат-бройлерів сприяє активізації процесів еритропоезу, гемоглобіноутворення, відновленню показників білкового обміну та нормалізації мінерального статусу в фізіологічних межах, що свідчить про підсилення захисних сил організму, підвищення стійкості до стресових факторів і негативних умов зовнішнього середовища, та у подальшому буде позитивно впливати на ріст та продуктивність цільової птиці. Виражений вплив комплексного поєднання препаратів на організм курчат-бройлерів experimental B полягає у кращому засвоєнні багатьох неорганічних елементів (за підвищенням вмісту Calcium, Phosphorus, Cuprum, Ferrum і Selenium, Magnesium, Manganum); та відповідно зумовлює сталий розвиток метаболічних процесів через превалювання анаболічних над катаболічними (за підвищенням рівня загальних протеїнів на тлі фізіологічного збільшення кількості глобулінових фракцій; включаючи індукцію ендогенної детоксикаційної системи – за зниженням рівня утворення токсичних метаболітів пуринового обміну: сечової кислоти та креатиніну; зменшенням вмісту Chromium; нормалізацією процесів переамінування та уповільнення alkaline phosphatase activity) та імунобіологічних реакцій (за посиленням еритропоезу та гемоглобіноутворення; індукцією лізоцимної, бактерицидної та фагоцитарної активності) відповідно. Запропонована система вирощування молодняка птиці із застосуванням комплексу симбіотичних препаратів у поєднанні із біоцидними засобами сприяє синергічному впливу останніх та їх ефективну дію на організм експериментальних курчат-бройлерів, що веде до підвищення неспецифічної резистентності та відновлення метаболічних реакцій

Ключові слова: гематологічні показники, біохімічні показники, мінеральний обмін, природня резистентність, пробіотики, дезінфектанти