# Biochemical parameters of blood of broiler chickens when using probiotic normosil

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**Abstract.** Experimental data are presented that the total protein in the I Experimental group, which consumed Normosil in the amount of $10^7$ CFU, was 10.5% higher than the values of the control group, while the difference in the indicators in the IV Experimental group, in which the bird consumed a probiotic in a dose of $10^6$ CFU with a break of 4 days after 10 days of giving over the control was 42.3% higher. At the same time, at the age of 40 days, the chickens of I Experimental Group sank in terms of both albumins and globulins. The glucose level in the experimental groups was higher by 6.2; 8.2; 32.0; 52.6% ($p \leq 0.05$), respectively. In terms of the amount of calcium at 35 days of age, the superiority of all chickens that consumed probiotics was by 18.61; 3.47; 8.83; 23.66% ($p \leq 0.05$), respectively, compared with the control group.

## 1 Introduction

It is difficult to overestimate the role of poultry farming in providing the population with high-quality protein and increasing the availability of food. Poultry produces two main food products – meat and egg. The quality of these products must be at the proper level so as not to harm human health, because antibiotics are used to treat animals, the misuse of which is harmful. The likelihood of using preparations can be reduced by introducing probiotics into feeding diets, because today there is enough evidence that the introduction of certain strains of microorganisms has a positive effect on various aspects of poultry life. Thus, Y. Wang, et al. (2017), determined that probiotics can improve the taste of chicken meat and increase the diversity of the intestinal microbiota [15]. The Toyocerin probiotic mitigates the negative effects of salmonella infection [5]. In addition, probiotics reduce the risk of developing negative consequences during vaccination and stimulate metabolic processes. It turned out that some probiotics introduced into chicken diets are able to stimulate the synthesis of low-molecular-weight antioxidants responsible for maintaining redox homeostasis without adversely affecting liver metabolism in chickens [12].

The effectiveness of using various probiotics as a preventive agent and a growth stimulator of poultry has been established [14]. For example, the probiotic preparation Vetom 1.1 in the diet normalizes metabolic processes and increases average daily gains [4].

The inclusion of a probiotic preparation in the poultry diet in doses of 0.5 kg, 0.7 kg, and 1.0 kg per 1 ton of feed increased the digestibility of crude protein by 0.84-0.88%, crude fat

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by 1.64-1.72%, crude fiber by 0.12-0.17%, nitrogen-free extractive fraction by 0.46-0.77% [3].

Although it has been proven that the use of microorganisms in poultry feeding can affect the reduction of serum cholesterol in broiler chickens [6]. In the works [4], in control chickens, by the 4th month of life, there was a tendency to metabolic disorders and an increased likelihood of liver damage, while the experimental bird increased average daily gains with normal metabolic rates.

Probiotics can affect the immune system's performance and thereby reduce morbidity [9]. In the experiments of Safonov V. A. [10], the use of the probiotic Bacell-M increased the level of plasma proteins, which indicates the stimulation of the immune response.

The addition of phytase and probiotic showed a significant increase in total blood protein, albumin, glutathione peroxidase, superoxide dismutase, catalase, and total antioxidant capacity, while reducing levels of triglycerides (TG), cholesterol (CHL), aspartate transferase, alanine aminotransferase, alkaline phosphatase, and malondialdehyde compared with control chickens [1].

The introduction of probiotics into the diet of laying hens had a significant effect on uric acid levels, as well as reduced AST, ALT, and creatinine levels, increasing the levels of total protein, albumin, and globulin [14].

Thus, the effect of probiotic preparations on the biochemical parameters of poultry blood definitely exists. At the same time, the mechanisms of probiotic action have not yet been fully studied, and the effect on the biochemical parameters of blood in birds has not been sufficiently studied. This is further facilitated by the fact that each probiotic is unique in composition, which causes a diverse effect on biochemical parameters [2, 8, 11].

Objective: to study the effect of the probiotic Normosil on the biochemical parameters of the blood of Arbor Acres cross broiler chickens.

To achieve this goal, the following tasks were considered, which established the dependence of the effect of different doses of the probiotic under study:

- on blood serum protein levels (total protein, albumin, globulins)
- on the cholesterol content;
- on the alkaline phosphatase level;
- on the mineral composition of blood serum macronutrients (calcium, phosphorus, sodium, and potassium).

## 2 Materials and Methods of research

The composition of the Normosil feed additive is a complex of bacterial strains: Lactobacillus brevis (No. 17) - probiotic against colibacteriosis Lactobacillus plantarum (No. 16) Lactobacillus acidophilus - probiotic, (No. 15) - bacteriocin producer, Enterococcus faecium (No. 3) - probiotic, producer of B group vitamins, killed yeast - saccharomycetes strain 3K is a non-specific immunomodulator and enterosorbent. The developer is RIC BashInkom LLC. The new feed additive Normosil has been insufficiently studied [2].

The research was conducted on broiler chickens of Arbor Acres cross.

To conduct the research, 5 groups of 35 day-old broiler chickens were formed in each according to the principle of analogues.

All the birds used in the experiment were clinically healthy. The bird intended for the experiment was individually weighed. The difference in average weight between the groups at daily age did not exceed 3%. The growing period was 42 days. During the experiment, the conditions of detention corresponded to the regime accepted at the farm. The bird had free access to food and water.

The control group was fed with standard compound feed recommended by All-Russian Research and Technological Institute of Poultry Farming.

The first experimental group of broiler chickens with drinking water was given the probiotic feed additive Normosil at a concentration of $1x10_7$ CFU/l daily, the second experimental group of broiler chickens with drinking water was given at a concentration of $1x10^7$ CFU/l for 10 days, followed by a 4-day break from daily to 42 days of age. The III-experimental group of broiler chickens with drinking water was given the probiotic feed additive Normosil at a concentration of $1x10^6$ CFU/l daily, and the IV-experimental group of broiler chickens Normosil was administered at a concentration of $1x10^6$ CFU/l for 10 days, followed by a 4-day break from daily to 42-days of age.

Blood was collected 3 hours after morning feeding. The serum blood fluid was analyzed on a Stat Fax 3300 biochemanalyzer using Olvex test kits in the laboratory of feed analysis at the Department of Physiology, Biochemistry, and Animal Feeding of Bashkir State Agrarian University. Before the analysis, the blood serum was precipitated from the shaped blood elements and centrifuged at a speed of 1200 g for 15 minutes. The total protein content was determined by the endpoint colorimetric method with a biuretic reagent, the albumin concentration - by the colorimetric method with bromocresol green, cholesterol - by the enzymatic colorimetric method, alkaline phosphatase by the kinetic method with a diethanol-amine buffer, the calcium concentration by the colorimetric method with an o-cresolphthalein complex, potassium - by the turbidimetric method without deproteination, sodium - by the colorimetric method by endpoint with precipitation, and phosphorus – by UV method without deproteination.

## 3 Research results

The most important factor affecting poultry productivity is carbohydrate metabolism. Glucose indicators can be used to assess its intensity and the possibilities of glycogen deposition in poultry muscles and liver, as well as the course of detoxification processes [13].

The most significant changes in glucose levels in the body of experimental chickens were detected at 21 days of age - at this time there was a clear tendency for the indicator to increase in the groups. Thus, if in the control group the indicator was 9.7 mmol/l, then in the experimental groups the results were higher by 6.2; 8.2; 32.0; 52.6% ($p \leq 0.05$), respectively. This indicates that the chickens have increased the possibilities of gluconeogenesis, which affected the intensity of the gains.

Nevertheless, at the age of 40 days, the indicators of experimental groups I and II were lower than the control by 7.5 and 5.6%, while Experimental Group III exceeded the control by 12.2%, and Experimental Group IV – by 17.3% ($p \leq 0.05$). Perhaps this is due to the fact that in the first two experimental groups, excessive colonization of the intestine by microorganisms that are part of Normosil occurred, which led to a decrease in the composition and quantity of its own microflora and had a depressing effect on growth and development.

Protein metabolism determines the gains and indicators of bird immunity [12]. In our studies, we evaluated total protein, albumins, and globulins, and found the ratio of albumin/globulin fractions. If there were no significant changes in the studied parameters during the first 7 days, then starting from the 3rd week of the experiment, the total protein in Experimental Group I was 10.5% higher than the values of the control group, and the indicators of Experimental Group II and III were almost at the same level, while surpassing the indicators of their peers. The difference in performance in the IV experimental group over the control group was as much as 42.3% ($p \leq 0.005$) (Figure 1).

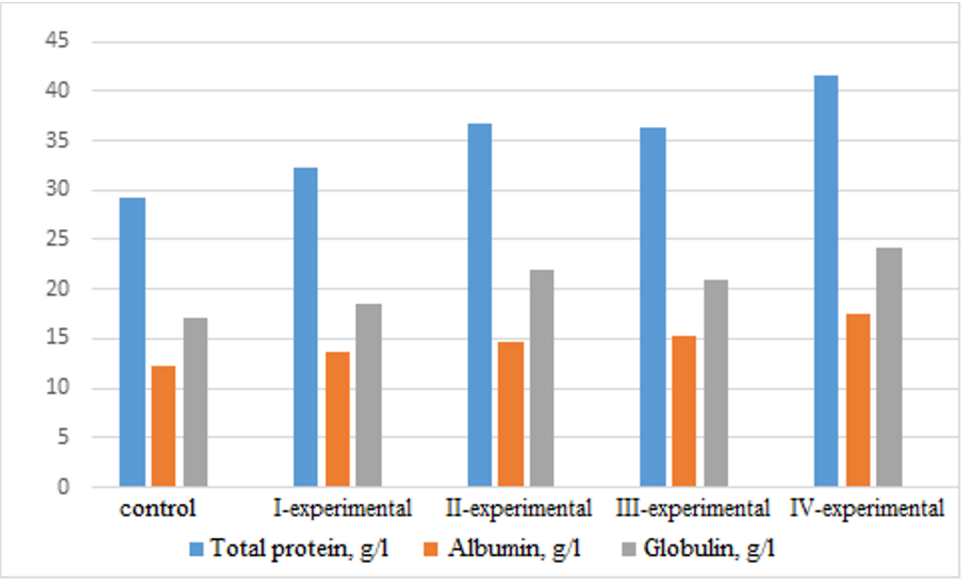


**Fig. 1.** Indicators of protein metabolism at the age of 21 days.

Nevertheless, the situation changed somewhat over time – on the 40th day of the experiments, the bird from the Experimental Group I had a result 20.5% lower than the control, and the difference between the other groups was only 2.5; 0.5; and 5.7% (Figure 2).

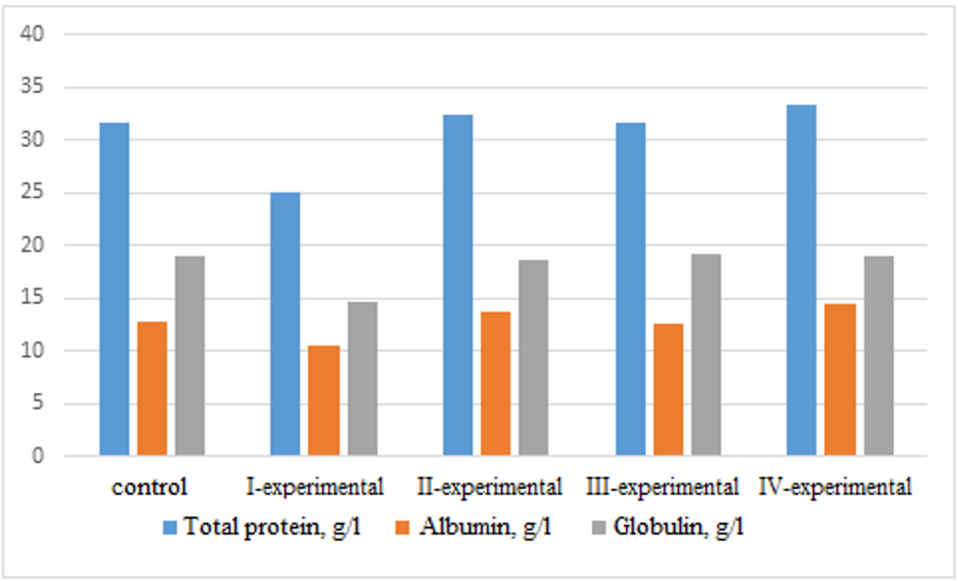


**Fig. 2.** Indicators of protein metabolism at the age of 40 days.

At the same time, the weight of chickens that consumed Normosil was significantly higher than the control at 40 days of age by 65-88 grams ($p \leq 0.05$). It is likely that the microorganisms included in the probiotic affect the reactions of carbohydrate metabolism by reducing the consumption of glucogenic amino acids, which are used for protein synthesis and provide increased growth rates of chickens. There is also a possibility that the mechanism is associated with an increase in the formation of biotin, which is produced by the microbiota.

Increased synthesis of B vitamins is also likely, in particular $B_1$, $B_2$, $B_3$, and $B_5$, which are limiting in glucose metabolism reactions. There is a possibility of increased niacin production, which leads to increased pyruvate production and increased efficiency of Krebs cycle reactions. Vitamin K, which is formed in the intestine with the participation of the microbiome, can also improve metabolism, since it participates in the work of the mitochondrial respiratory chain. It is also known from the literature that probiotic supplements are capable of synthesizing up to 57 different substances, including amino acids and natural antibiotics [11].

By changing the content of protein fractions in the blood serum, it is possible to judge the intensity of protein metabolism in the body of chickens. Albumins and globulins are the main proteins, the amount of which affects the growth of poultry. The growth of albumins and globulins by week is shown in Fig. 3.

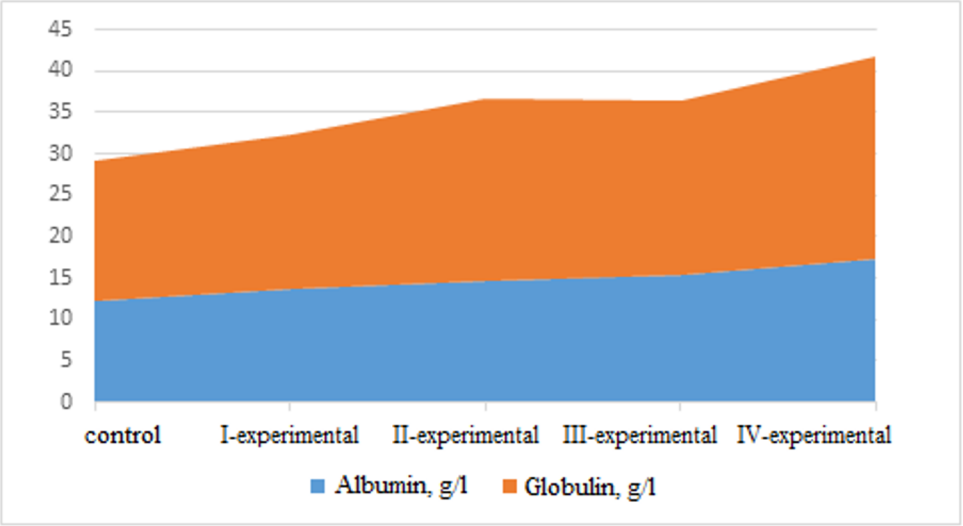


**Fig. 3.** Indicators of albumins and globulins in blood serum, g/l at 21 days of age.

According to the graph, it is obvious that at the age of three weeks, an increased number of protein fractions was inherent in poultry in the experimental groups. At the same time, at the age of 40 days (Figure 4), the chickens of Experimental Group I sank in terms of both albumins and globulins.

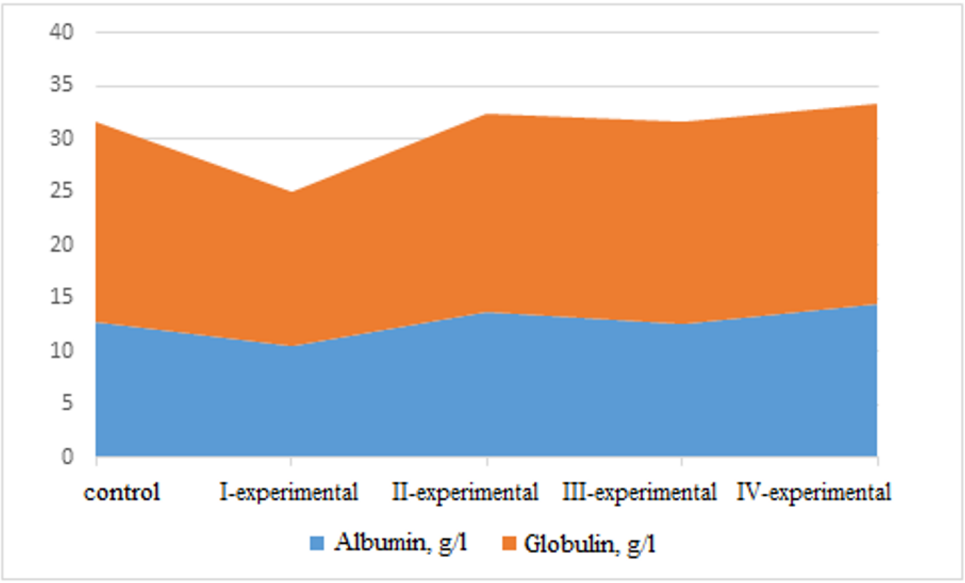


**Fig. 4.** Indicators of albumins and globulins in blood serum, g/l at the age of 40 days.

Moreover, the difference between them and the control bird was 7.7% in albumins and reached 22.3% in globulins. This fact may indicate the probiotic toxicity when used without interruptions at a dosage of $10^7$ CFU/l.

We tracked the fat metabolism indicators by cholesterol levels. We noticed that starting from the 21st day of the experiment, the cholesterol level in all Experimental groups was below the control. At the same time, the digital indicators were almost equal in the intact and III groups, and the difference between the data in the comparison group and the Experimental I, II, IV was 4.2; 2.9; 9.7%.

The largest gap between the groups was established on the 40th day of the experiment. The difference between the experimental birds and the representative sample was -7.7; -4.3; -0.7; -2.9.

Probably, an improvement in carbohydrate metabolism leads to a decrease in lipid metabolism, which includes cholesterol. Along with this, a mechanism for faster and increased synthesis of steroids from it is possible, and therefore less of it is found in the blood serum.

Being the mineral basis of the body, electrolytes are involved in supporting blood homeostasis, many biological processes, poultry development and bone mineralization, metabolism of other elements, energy metabolism and acid-base balance, etc. The exchanges of Ca, P, Na, and K are closely related, and therefore the lack of one of them affects productivity. Their deficiency prevents the achievement of maximum productivity during the intensive growth period. The mineral composition of the blood serum is shown in Table 1.

**Table 1.** The content of calcium, phosphorus, sodium, and potassium in the blood.

| Age | Macronutrients, mmol/l | | | | |
|---|---|---|---|---|---|
| | Calcium | | | | |
| Age 7 days | 3.23±0.48 | 3.48±0.68 | 2.95±0.26 | 2.76±0.68 | 3.54±0.56 |
| Age 21 days | 3.67±0.31 | 4.02±0.55 | 3.35±0.38 | 3.14±0.32 | 3.17±0.27 |

| | | | | | |
|---|---|---|---|---|---|
| Age 35 days | 3.17±0.22 | 3.76±0.48 | 3.28±0.25 | 3.45±0.18 | 3.92±0.23* |
| Phosphorus | | | | | |
| Age 7 days | 2.25±0.23 | 2.34±0.34 | 2.86±0.27 | 2.28±0.36 | 2.48±0.24 |
| Age 21 days | 2.84±0.26 | 2.28±0.41 | 3.24±0.38 | 2.36±0.28 | 3.62±0.21* |
| Age 35 days | 3.17±0.45 | 2.94±0.18 | 3.12±0.24 | 2.17±0.22 | 2.93±0.31 |
| Sodium | | | | | |
| Age 7 days | 117.3±15.8 | 122.5±22.3 | 128.4±17.4 | 118.2±16.3 | 128.7±20.4 |
| Age 21 days | 113.6±8.6 | 109.2±17.5 | 115.9±21.2 | 126.7±14.1 | 146.7±10.4* |
| Age 35 days | 109.3±12.6 | 131.7±18.3 | 127.3±22.8 | 131.2±16.4 | 116.7±17.3 |
| Potassium | | | | | |
| Age 7 days | 4.24±0.48 | 4.31±0.31 | 4.22±0.59 | 4.31±0.41 | 4.28±0.22 |
| Age 21 days | 4.82±0.36 | 4.75±0.48 | 4.34±0.71 | 4.72±0.62 | 5.15±0.43 |
| Age 35 days | 4.56±0.21 | 5.11±0.74 | 4.82±0.52 | 4.44±0.77 | 5.33±0.23* |

As a result of the research of indicators of all studied macronutrients in the blood serum of broiler chickens, no statistically significant results were found after 7 days of drinking the probiotic Normosil.

In terms of the amount of calcium at 35 days of age, the superiority of all chickens that consumed probiotics was by 18.61; 3.47; 8.83; 23.66% ($p \leq 0.05$), respectively, compared with the control group.

In terms of sodium content, superiority was also determined in the Experimental Groups – by 20.50; 16.47; 20.04; 6.77.

The phosphorus concentration in the blood serum of the experimental broilers was, on the contrary, lower than that of the intact group. Thus, the difference in results between the control and experimental groups was lower by 7.3; 1.6; 31.55; 7.6%. These results correlated with the indicators of alkaline phosphatase.

For potassium – 12.06; 5.70; 16.89%, and only in the Experimental Group there was a decrease of 2.6% compared with the control.

Thus, the alkaline elements in the blood serum exceed the control, and the acidic element, phosphorus, is reduced. This may indicate that the buffer systems of an experienced bird work better. This mechanism may also be due to better absorption of minerals from feed.

## 4 Conclusions

Studies conducted on the effect of different doses and methods of giving the probiotic Normolax in the diet of Acros broiler chickens on blood serum levels have established the effect of the feed additive on the level of glucose, total protein, and protein fractions, cholesterol and macronutrients. The results make it possible to suggest the mechanism of action of bacterial cultures that make up the preparation. The microorganisms included in the studied supplement activate metabolic processes of an anabolic nature in the bird body.

## References

1. M. Derakhshan, S.O. Gasemian, M. Golami-Akhangaran, *The effect of probiotics and phytase on growth rates, biochemical parameters and antioxidant capacity in broiler chickens*, Vet Med Sci., **9(2),** 860-866 (2023). (in Russ) doi: 10.1002/vms3.1075. Published on January 20, 2023 Review in the journal: Vet Med Sci. 2024 Sep; 10(5): e1566 doi: 10.1002/vms3.1566. PMID: 36669151; PMCID: PMC10029878.
2. S.S. Nikolenko, A.I. Ivanov, *The use of the probiotic "Normasil" and its effect on biochemical parameters in cattle pasteurellosis*, Agricultural sciences and the agro-industrial complex at the turn of the century, **10** (2015) (in Russ). URL: https://cyberleninka.ru/article/n/primenenie-probiotika-normasil-i-ego-vliyanie-na-biohimicheskie-pokazateli-pri-pasterelleze-krupnogo-rogatogo-skota (date of access: 02/26/2025).
3. L.Yu. Topuria, G.M. Topuria, *The digestibility of feed nutrients and the state of metabolism in ducklings when using Immunoflor*, Agricultural science, **2,** 67-72 (2025) (in Russ). https://doi.org/10.32634/0869-8155-2025-391-02-67-
4. A.V. Trebukhov, A.E. Demeneva, *The effect of the Vetom probiotic 1.1. The physical and biochemical parameters of chickens*, Innovation and food safety, **2,** 49-56 (2023) (In Russ) https://doi.org/10.31677/2311-0651-2023-40-2-49-56
5. A.A. Abudabos, M.A. Murshed, M.M. Qaid, A.G. Abdelrahman, *Effect of probiotics on serum biochemical and blood constituents in chicken challenged with Salmonella enterica Subsp Typhimurium*, Trop J Pharm Res, **15(3),** 461-467 (2016) doi: 10.4314/tjpr.v15i3.5.
6. A.E. Andreeva, T.A. Sedykh, R.S. Gizatullin, F.C. Khaziakhmetov, *The use of enterosorbent "Priminkor" in the diets of laying ducks*, Fundamental research, **2-14,** 3110-3115 (2015) EDN UBQLBP.
7. A. Alkhalf, M. Alhaj, I. Al-Homidan, *Influence of probiotic supplementation on blood parameters and growth performance in broiler chickens*, Saudi J Biol Sci., **17(3)**, 219-25 (2010) doi: 10.1016/j.sjbs.2010.04.005. Epub 2010 Apr 18. PMID: 23961081; PMCID: PMC3730717.
8. A. Basharov, E. Andriyanova, A. Andreeva, A. Khabirov, I. Yumaguzin, *Comparative assessment of forage legume and grass-legume mixture quality ensiled with biological and chemical preservatives*, Proceedings of the National Academy of Sciences India Section B - Biological Sciences, **91(4),** 839-845 (2021).
9. T. Ebeid, I. Al-Homidan, M. Fathi, R. Al-Jamaan, M. Mostafa, O. Abou-Emera, A. Alkhalaf, *Impact of probiotics and/or organic acids supplementation on growth performance, microbiota, antioxidative status, and immune response of broilers*, Italian Journal of Animal Science, **20(1),** 2263-2273 (2021) https://doi.org/10.1080/1828051X.2021.2012092.
10. V.A. Safonov, Y.V. Mikhailov, S.N. Semenov, et al., *Influence of probiotic bacella-m® on immuno-biochemical indicators of blood of ross-308 cross broiler chickens during vaccination*, Journal of Agriculture and Environment, **12(40)** (2023) URL: https://jae.cifra.science/archive/12-40-2023-december/10.23649/JAE.2023.40.20 (accessed: 26.02.2025). DOI: 10.23649/JAE.2023.40.20
11. Y.L. Tsai, T.L. Lin, C.J. Chang, et al., *Probiotics, prebiotics and amelioration of diseases*, J Biomed Sci, **26,** 3 (2019) https://doi.org/10.1186/s12929-018-0493-6.

12. O. Katarzyna, M. Krauze, E. Cholewińska, K. Abramowicz, *The Effect of a Probiotic Containing Enterococcus faecium DSM 7134 on Redox and Biochemical Parameters in Chicken Blood*, Annals of Animal Science, **17,** (2017) 10.1515/aoas-2016-0097.
13. N. Halder, J. Sunder, A.K. De, et al., *Probiotics in poultry: a comprehensive review*, JoBAZ, **85,** 23 (2024). https://doi.org/10.1186/s41936-024-00379-5.
14. L. Adriani, A. Mushawwir, N. Mayasari, A. Sekar Rosiyanti, *Effect of Probiotic Consortium Administration in Improving Organ Function and Blood Biochemistry in Laying Chickens*, Pakistan Journal of Biological Sciences, **27,** 493-498 (2024) DOI: 10.3923/pjbs.2024.493.498
15. Y. Wang, J. Sun, H. Zhong, et al., *Effect of probiotics on the meat flavour and gut microbiota of chicken*, Sci Rep, **7,** 6400 (2017) https://doi.org/10.1038/s41598-017-06677-z.