Evaluation of the tolerance of probiont strains upon intracroup administration to broiler chickens

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Abstract. This paper presents the results of a study of the tolerance of indigenous probiotic strains of the genus *Lactobacillus* (*Loigolactobacillus coryniformis* VKM B-3724D and *Lactobacillus johnsonii* VKM B-3725D), isolated from the gastrointestinal tract of pheasants, to broiler chickens. It was found that the use of the studied indigenous probiotic strains, both individually and in a consortium, had no negative impact on the broiler chickens throughout the entire growing period. The biological drugs were found to not cause any signs of toxic poisoning, while an increase in the survivability and live weight of the experimental birds was observed, as well as a decrease in the feed conversion rate. It was determined that the biological drugs do not negatively affect hematopoiesis or cause inflammation. In fact, they moderately enhance redox processes in the body due to improved saturation of the blood with red blood cells and hemoglobin. A slight improvement in protein, carbohydrate, lipid, and mineral metabolism was observed in serum biochemistry. Based on the combined results of the studies, it was found that the experimental probiotic strains are safe and well tolerated by broiler chickens.

1 Introduction

Poultry farming is one of the most dynamic and strategically important sectors of agriculture, providing the population with an affordable, high-quality source of protein. However, intensive production methods, high stocking densities, and constant pressure from infectious agents have historically led to the widespread and often irrational use of antimicrobials. This has led to the emergence and global spread of antimicrobial resistance (AMR), one of the most serious threats to human and animal health in the 21st century [1].

The problem of AMR is complex and affects both veterinary and human medicine within the framework of the "One Health" concept. The long-term use of antibiotics in poultry farming, not only for therapeutic but also prophylactic and growth-promoting purposes, has led to the selection and spread of multidrug-resistant strains of pathogenic bacteria (*Salmonella, Campylobacter, E. coli, Staphylococcus aureus*) [2].

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Amid growing global concern about AMR, many countries are introducing strict legislative restrictions and complete bans on the use of feed antibiotics as growth promoters. Simultaneously, consumer demand for "clean," environmentally safe products produced without the use of antibiotics (antibiotic-free) is growing. These factors create an urgent need for the poultry industry to find and implement safe and effective alternatives to antimicrobials [3, 4]. In this context, the use of probiotic microorganisms represents one of the most promising and scientifically sound strategies. Probiotics are live, non-pathogenic microorganisms that, when administered in adequate quantities, exert a positive effect on the health of the host, primarily by normalizing its intestinal microflora [5].

Therefore, the development of effective biopreparations, particularly those based on beneficial probiotic microflora, is a promising and relevant task in addressing the global problem of antimicrobial resistance.

The aim of this study was to study the tolerance of broiler chickens to intracrotical administration of probiotic strains of the genus *Lactobacillu*s.

2 Materials and methods

Scientific experiments on poultry were conducted at the Center for Preclinical and Clinical Research of Veterinary Drugs and Feed Additives at the Russian State Agrarian University - Moscow Timiryazev Agricultural Academy.

The study utilized lyophilized forms of indigenous probiotic strains of the genus *Lactobacillus* (*Loigolactobacillus coryniformi*s VKM B-3724D and *Lactobacillus johnsonii* VKM B-3725D), isolated from the gastrointestinal tract of pheasants, with a titer of at least 1.0 x 10^{12} CFU/g of each culture. To prepare the working form of the biological product, the microorganisms were preliminarily dissolved and activated at room temperature at a dose of 1.0 g of lyophilic concentrate per 1 liter of drinking water, which amounted to at least 1.0×10^9 CFU/ml of each culture in the resulting solution.

The target biological object was fast-growing Ross 308 broiler chickens. The birds were raised for 42 days. For the first two weeks, the birds were housed in brooder-type cages, and then in three-tiered battery cages, in compliance with veterinary and technical requirements. Feeding was done manually in hanging feeders, and water was provided automatically through nipple drinkers.

For the experiment, four experimental groups were formed: the control group received standard complete feed according to the growth period and drinking water; The 1st experimental group – received standard complete feed according to the growth periods, drinking water and, in addition, individually, the birds were intracropped with 1.0 ml of the working form of the probiotic with a titer of *Lactobacillus coryniformis* VKM B-3724D of at least 1.0×10^9 CFU/ml; the 2nd experimental group – received standard complete feed according to the growth periods, drinking water and, in addition, individually, the birds were intracropped with 1.0 ml of the working form of the probiotic with a titer of *Lactobacillus johnsonii* VKM B-3725D of at least 1.0×10^9 CFU/ml; The 3rd experimental group received standard complete feed according to the growth periods, drinking water, and additionally, 1.0 ml of the working form of the probiotic with a titer of two probiotic strains of at least 1.0×10^9 CFU/ml was administered individually intracrotically to the birds. The minimum number of birds in each group was 35 heads, according to recommendations [6]. During the experiment, economically beneficial indicators were recorded in accordance with the methodological recommendations [6].

The clinical and physiological condition of the broiler chickens was monitored daily by inspecting the flock, paying particular attention to behavior, mobility, feather cover, feed intake, and water consumption.

At the end of the study (on day 42), a complete blood count and biochemical analysis of the test birds were performed. Blood was collected from the axillary vein in special tubes (vacuettes) for whole blood and serum.

The data were analyzed and interpreted using biometric statistics. Results were processed using Microsoft Excel 2019 (Windows 10 operating system). The following parameters were calculated for the statistical analysis: arithmetic mean (M), standard error of the mean (m), and standard deviation (σ). Comparisons of quantitative parameters between two independent samples were performed using Student's t-test. The level of statistical significance of differences was determined by the p-value; differences were considered statistically significant at $p \leq 0.05$.

3 Results and discussion

3.1 Study of the safety, live weight, and palatability of compound feed in broiler chicken farming

To conduct the scientific and farm experiment, groups of clinically healthy broiler chickens were formed on the first day of birth, with live weights nearly identical to those required. Clinical analysis showed satisfactory results, with no signs of toxicity from the strains. Live weight, growth, feed consumption, and survival data are presented in Table 1.

Table 1. The studied economic indicators of broiler chickens of the Ross 308 cross (n=35).

Indicator	Group			
	control	1st experimental	2nd experimental	3rd experimental
Bird survival rate, %	91.4	94.2	97.1	100.0
Live weight dynamics, g				
Daily allowance	43.1±0.6	43.6±0.7	44.2±0.5	45.1±0.6
7th day	191.7±2.1	187.1±1.9	192.1±2.0	189.7±2.4
14th day	481.3±4.6	485.1±5.1	489.2±4.2	493.6±4.8
21st day	884.2±7.9	895.3±7.4	894.2±8.3	898.2±8.1
28th day	1457.3±10.3	1479.6±9.7	1485.1±9.5	1498.1±11.5
35th day	2295.1±15.2	2354.3±16.3	2349.2±15.5	2378.7±14.4
42nd day	2654.2±14.2	2743.9±15.4	2735.3±14.3	2780.3±15.2*
Live weight gain during the growing period (0-42 days)				
One head on average, g	2611.1	2700.3	2691.1	2735.2
Feed consumption during the growing period (0-42 days)				
1 head, g	4536.7	4570.2	4589.1	4601.4
Conversion, kg	1.73	1.69	1.70	1.68
* – the difference with the control group is significant ($p \leq 0.05$)				

A study of the economic performance of Ross 308 broiler chickens demonstrated that intracrotical administration of probiotic strains increased the survival rate of young birds in experimental groups 1–3 compared to the control group. In the control group, 3 birds died during the entire growing period, while in experimental group 1, bird viability during the growing period was 2.8% higher (2 birds died). In experimental group 2, 1 bird died, a 5.7% increase, and in experimental group 3, there was no mortality. Consequently, the survival rate was 100.0%, 8.6% higher than in the control group. Bird mortality in the control and experimental groups was natural, and autopsy revealed no changes that could have been caused by the studied dietary supplements.

A study of poultry live weight dynamics revealed that the studied indicator increased

uniformly across the experimental groups at each weighing period. No statistically significant differences were found between groups up to day 35, but positive trends in live weight gain were observed in the experimental birds. Thus, on day 14 of individual weighing, the average weight in the first experimental group was 0.9% higher than in the control group, 1.6% higher in the second experimental group, and 2.6% higher in the third experimental group. On the 21st day, the live weight of young broiler chickens in the experimental groups averaged 895.3±7.4 g (1st experimental group), 894.2±8.3 g (2nd experimental group) and 898.2±8.1 g (3rd experimental group), which is 1.3%, 1.1% and 1.5% more than in the control group. On the 28th day, when weighing the birds, the weight of the experimental birds was higher than in the control birds by 1.5% (1st experimental group), 1.9% (2nd experimental group) and 2.8% (3rd experimental group). On the 35th day of poultry weighing, the weight of broiler chickens in the 1st, 2nd and 3rd experimental groups was 2354.3±16.3 g, 2349.2±15.5 g and 2378.7±14.4 g, which is 2.6%, 2.3% and 3.6% higher than the control group, respectively. At the last weighing on the 42nd day, it was revealed that the weight of broiler chickens in the 1st and 2nd experimental groups, as in the early periods, had a positive trend in changing the studied indicator without significant shifts and amounted to 2743.9 ± 15.4 g and 2735.3 ± 14.3 g, which is higher than in the control group by 3.4% and 3.1%, however, in the 3rd experimental group, these changes were higher by 4.7% and had statistically significant changes compared to the control (at $p \leq 0.05$), which confirms the direct positive effect of the use of the studied joint composition of probiont strains on the analyzed indicator.

When assessing the weight gain of experimental birds over the entire rearing period, it was found that in the control group this indicator was 2611.1 g, while in the 1st experimental group the increase was 2700.3 g, in the 2nd experimental group 2691.1 g, and in the 3rd 2735.2 g, which, respectively, exceeded the analyzed indicator in the biological control by 3.4%, 3.0%, and 4.6%.

When calculating daily feed costs for the entire period of keeping birds in the control and experimental groups, it was found that feed consumption in the 1st, 2nd, and 3rd experimental groups was, respectively, higher than in the control by 0.7%, 1.2%, and 1.4%. Despite higher feed consumption by the experimental birds, due to their higher growth energy, the feed consumption per kg of gain (conversion) was lower than in the control group (1.73 kg). It was 1.69 kg in the 1st experimental group, 1.70 kg in the 2nd experimental group, and 1.68 kg in the 3rd experimental group, representing a decrease of 2.3%, 1.7%, and 2.9%, respectively.

Thus, the use of biopreparations based on live cultures of indigenous lactobacilli had no negative impact on the broiler chickens throughout the entire growing period. No signs of toxic poisoning were recorded. However, it contributed to increased survivability and live weight of the experimental birds, as well as a decrease in feed conversion. The best values of economically useful indicators were revealed in the 3rd experimental group, where broiler chickens were additionally given a joint composition of *Loigolactobacillus coryniformis* VKM B-3724D and *Lactobacillus johnsonii* VKM B-3725D.

3.2 General clinical blood test

A complete blood count (CBC) provides a comprehensive assessment of the body's condition, detecting inflammation, anemia, hematopoietic and coagulation disorders, and assessing immune system function. The results of the CBC analysis of Ross 308 broiler chickens from the control and experimental groups after administration of probiotic strains are presented in Table 2.

Table 2. Results of clinical blood analysis of Ross 308 broiler chickens (n=10).

Indicator	Group			
	control	1st experimental	2nd experimental	3rd experimental
Red blood cells, 10^{12}/L	2.49±0.02	2.50±0.01	2.53±0.02	2.60±0.04
Hemoglobin, g/L	81.26±1.26	82.76±1.34	83.48±1.36	84.03±1.40
Hematocrit, %	40.23±0.95	41.94±0.94	42.94±1.01	43.12±0.89
Platelets, 10^9/L	64.58±1.05	65.28±0.97	65.68±0.77	65.47±0.88
Leukocytes, 10^9/L	24.85±0.54	25.12±0.43	25.36±0.46	25.47±0.51

The results of the general clinical blood analysis of broiler chickens for the studied parameters demonstrated a positive dynamics of change in the experimental groups compared to the control group; however, these changes did not have statistically significant shifts. Thus, red blood cells (erythrocytes) and hemoglobin in the blood reflecting the body's ability to receive and transport oxygen and carbon dioxide in the experimental groups were slightly higher than in the control group: in the 1st experimental group by 0.4% (by erythrocytes) and 1.8% (by hemoglobin), in the 2nd experimental group by 1.6% (by erythrocytes) and 2.7% (by hemoglobin), in the 3rd experimental group – 4.4% (by erythrocytes) and 3.4% (by hemoglobin). The hematocrit index, reflecting the proportion of red blood cells in the total blood volume, also showed a positive trend in its change in the experimental birds compared to the control ones and was higher by 4.2% (1st experimental group), 6.7% (2nd experimental group) and 7.1% (3rd experimental group). The change in the platelet count in birds, responsible for the state of the hemostasis system, as well as leukocytes, reflecting the state of the immune system and the general response of the body to stress, inflammation and other pathological processes, was minimal across groups and amounted to 64.58±1.05×10^9/l for platelets in the control group, 65.28±0.97×10^9/l in the 1st experimental group, 65.68±0.77×10^9/l in the 2nd experimental group and 65.47±0.88×10^9/l in the 3rd group; For leukocytes, the respective values were 24.85±0.54×10^9/L, 25.12±0.43×10^9/L, 25.36±0.46×10^9/L, and 25.47±0.51×10^9/L.

Thus, the results of the general clinical blood analysis of Ross 308 broiler chickens demonstrated that the use of these probiotic strains in the experiments did not negatively affect hematopoiesis or cause inflammation, but rather moderately enhanced oxidation-reduction processes in the body due to improved saturation of the blood with erythrocytes and hemoglobin.

3.3 Blood biochemistry test

The primary and most informative "marker" for intravital diagnostics of the condition of organs, tissues, metabolism, and metabolic rate in a living subject are the results of a biochemical blood test. Therefore, at the end of the study, a blood serum sample was collected from the bird. The results of individual serum parameters are presented in Table 3.

The results of the total protein, reflecting the process of protein metabolism in the bird's body showed that the introduction of the studied probiont strains separately into the bird's body contributes to a slight positive increase in the analyzed parameter without statistically significant values and was higher than in the control group by 2.5% (in the 1st experimental group) and 5.2% (in the 2nd experimental group). However, in the 3rd experimental group, which received a mixture of strains, the value of total protein in the blood serum was statistically significantly higher than in the control group by 4.46 g / l or 11.3% (at $p \leq 0.05$). The value of cholesterol, as a "marker" of lipid metabolism, similar to total protein, had better dynamics of change in the experimental groups compared to the control group. Thus, in the 2nd and 3rd experimental groups, a reliable decrease in this indicator was

found compared to the control group by 1.9% and 3.2% ($p \leq 0.05$). In the first experimental group, cholesterol levels also showed a positive picture, but without significant differences, although they were 0.5% lower. Uric acid in poultry, being the end product of nitrogenous compound breakdown, reflects protein metabolism activity and also indicates kidney and liver function. An increase in this indicator may indicate the deposition of nitrogenous compounds in the bird's body, which may lead to the development of gout. However, when analyzing uric acid in the blood serum of experimental birds, no abnormalities were recorded in any of the studied groups, and the value was within the range of 80.38-82.67 μmol/L. In experimental groups 1-3, the value was slightly lower than in the control by 1.3%, 2.7%, and 2.5%. Carbohydrate metabolism was assessed based on the amount of glucose in the blood of broiler chickens. It was established that no significant changes in this indicator were recorded across the experimental groups and that it was within the physiological norm and amounted to 8.46±0.32 mmol/l in the control, 8.50±0.36 mmol/l in the 1st experimental group, 8.45±0.39 mmol/l in the 2nd group and 8.42±0.41 mmol/l in the 3rd experimental group. The condition and function of the liver of the experimental broiler chickens were assessed by the most informative indicators, namely, the level of alkaline phosphatase, AST and ALT in the blood serum. It was established that no significant increase or decrease in the analyzed indicators was found in the experimental groups compared to the control group; they were within the reference values. Mineral metabolism in poultry was analyzed through changes in the level of calcium and phosphorus in the blood serum. The results showed that the values of these parameters increased slightly in the experimental groups, but this shift in favor of the experimental birds was not reliably confirmed. Thus, the calcium content in experimental birds from the 1st group was 0.5% higher than in the control group, 1.4% higher in the 2nd experimental group, and 1.6% higher in the 3rd group; phosphorus levels were 1.1% higher (for the 1st experimental group), 0.7% higher (for the 2nd experimental group), and 2.8% higher in the 3rd experimental group.

Table 3. Results of biochemical blood analysis of Ross 308 broiler chickens (n=10).

Indicator	Group			
	control	1st experimental	2nd experimental	3rd experimental
Total protein, g/L	39.38±0.63	40.37±0.52	41.42±0.59	43.84±0.67*
Cholesterol, mmol/L	3.75±0.04	3.73±0.06	3.68±0.03*	3.63±0.05*
Uric acid, μmol/L	82.67±1.03	81.56±0.97	80.38±1.08	80.58±1.05
Glucose, mmol/L	8.46±0.32	8.50±0.36	8.45±0.39	8.42±0.41
Alkaline phosphatase, U/L	2365.34±60.14	2386.11±67.28	2347.78±69.11	2345.70±63.44
AST, U/L	207.14±3.25	209.42±2.87	210.38±2.98	205.54±3.05
ALT, U/L	23.76±0.32	23.27±0.27	22.27±0.28	22.76±0.30
Calcium, mM/L	9.37±0.32	9.42±0.24	9.50±0.26	9.52±0.25
Phosphorus, mmol/L	4.27±0.11	4.32±0.09	4.30±0.09	4.39±0.14
* – the difference with the control group is significant ($p \leq 0.05$)				

4 Conclusion

Thus, the results of a research study examining the tolerance of probiotic strains isolated from the gastrointestinal tract of pheasants to intracrotical administration in broiler chickens demonstrated that the Lactobacillus species used were safe in in vivo studies on the target biological object and were well tolerated by the birds. The experiments revealed that the use of the studied indigenous probiotic strains—either individually or as part of a consortium—did not have a negative impact on the broiler chickens throughout the entire rearing period.

No signs of toxic poisoning were recorded. Simultaneously, increased flock survival, increased live weight, and improved feed conversion rates were observed. Blood analysis showed that the biopreparations did not disrupt hematopoiesis or provoke inflammatory reactions. Moreover, they promote a moderate enhancement of redox processes by increasing red blood cell and hemoglobin levels, and are accompanied by a slight improvement in protein, carbohydrate, lipid, and mineral metabolism. Therefore, these lactobacilli can serve as a basis for the development of microbial biopreparations with probiotic action for use in broiler chickens.

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