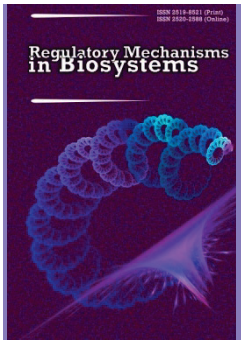

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2024, 15(3), 477–482
doi: 10.15421/022467

Production and meat quality of broiler chickens with the use of a probiotic complex of bifidobacteria and lactobacilli

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Article info

Received 09.05.2024
Received in revised form 28.06.2024
Accepted 14.07.2024

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Iakubchak, O. M., Vivych, A. Y., Hryb, J. V., Taran, T. V., & Danylenko, S. H. (2024). Production and meat quality of broiler chickens with the use of a probiotic complex of bifidobacteria and lactobacilli. Regulatory Mechanisms in Biosystems, 15(3), 477–482. doi:10.15421/022467

The prohibition of antibacterial agents in animal husbandry has spurred research into the creation and study of new probiotic supplements aimed at normalizing the gut microbiota to ensure colonization resistance of the host organism. This resistance prevents the colonization of the gastrointestinal tract by pathogenic and opportunistic microflora. Scientists from many countries have developed a range of preparations based on the normal gut microbiota, such as lactobacilli and bifidobacteria, which are used to maintain and restore the biocenosis of the digestive tract. These probiotics are also effective therapeutic and preventive agents for poultry digestive tract diseases, helping correct gut microbiocenosis. In our study, we investigated the impact of the combined probiotic preparation "TIMM-P" on the productive characteristics of poultry and the quality and preservation of broiler chicken meat. This study determined clinical indicators, absolute and average daily weight gains, the meat quality of broiler chickens, and its chemical composition under the influence of the probiotic supplement. It was found that administering "TIMM-P" in courses on days 1–5, 21–25, and 30–35 positively influences the growth of muscle tissue in poultry. Determination of the chemical composition of meat in 42-day-old experimental broiler chickens indicated a significant increase in total protein content in the muscle tissue using the probiotic. Additionally, the absolute and average daily weight gains of the broiler chickens in the experimental group significantly increased during days 15–28 and 29–42 of the study. We observed a significant impact of the probiotic preparation on extending the shelf life of poultry meat based on the study of organoleptic and chemical indicators of broiler chicken meat during refrigerated storage. On the 7th and 9th days of storage, the carcasses of the experimental group that received the probiotic showed a significant decrease in pH levels, acid number, and peroxide number of the fat in the meat. Thus, oral administration of the probiotic preparation increases live weight gains, improves meat quality indicators of broiler chickens, and extends its shelf life. The obtained results have important practical significance as they scientifically substantiate the use of probiotic preparation as a means to preserve livestock, increase live weight gains of broiler chickens, and improve poultry meat quality. Therefore, the complex probiotic preparation "TIMM-P" can be recommended for use in broiler chicken farming.

Keywords: *Bifidobacterium gallinarum*; *Lactobacillus rhamnosus*; *Lactobacillus plantarum*; *Lactobacillus paracasei* ssp. *paracasei*; meat chemical composition.

Introduction

The core principle employed during the production of food products in the context of market competition, "from farm to table," aims to provide consumers with a guaranteed safe final product of the desired quality. Currently, in developed countries, the issues of a healthy lifestyle, including healthy eating, have been elevated to the level of state policy. One of the crucial factors determining the health of the population is proper, rational, and complete nutrition. Preserving health and extending the life expectancy of a country's population is linked to providing functional nutrition for all age groups of citizens. In this context, the role of scientific support for resource-saving technologies in the production of agricultural products, ensuring their safety for humans, is increasing. These products should be free from pathogenic microorganisms, toxins, radioactive and chemical substances harmful to human health (Krysiak et al., 2021; Honchar et al., 2022). Market operators, under the supervision of veterinary medicine specialists, must ensure compliance with and implementation of legislation regarding the safety and quality control of poultry products. This includes balanced and safe production of broiler chicken meat, contributing to its safety guarantee and quality improvement for consumers (Salem et al., 2023; Vovkotrub et al., 2023).

Antibiotics are often used for therapeutic purposes, as well as for disease prevention and growth stimulation in animals, which presents a significant environmental issue. The European Union banned the use of antimicrobial agents as growth promoters for animals in 2006 under Regulation 1831/2003/EC. This event initiated similar practices in other countries, including Ukraine. In recent years, governments and professional organizations have developed various legislative acts, requirements, and recommendations based on the "One Health" concept (Kosenko et al., 2022). As an alternative to antibiotics in poultry farming, nutraceuticals are used, with a growing interest in probiotics that positively affect the organism and productivity of broiler chickens (Awad et al., 2009; Tomczyk et al., 2024). Researchers report that the effects of probiotics are not limited to the digestive tract; they also improve the overall condition of the organism (Kadam et al., 2023; Larsberg et al., 2023). Probiotics can increase the production of digestive enzymes and even exhibit detoxifying functions in the diet, leading to improved growth and enhanced poultry productivity (Soumeh et al., 2021). The term "probiotic" originates from Latin and means "for life." In 1992, probiotics were described as "a feed additive of live microorganisms that beneficially affect the intestinal flora of the host animal" (Miranda et al., 2023). According to the Food and Agriculture Organization (FAO) and the World Health Organization

(WHO), probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (Elshaghabee et al., 2017). Probiotics must meet certain requirements: microorganisms should be part of the intestinal microflora, resistant to high acidity, easily attach to the intestinal epithelium, and positively impact the digestive tract microbiome. The beneficial effects of probiotics on poultry are established through optimizing the digestion process and nutrient absorption, resulting in improved meat quality by establishing an optimal balance of amino acids and fatty acids (Selle et al., 2023).

Currently, various taxonomic groups of microorganisms are used in the production of probiotics, including *Bacillus subtilis*, *Bifidobacterium bifidum*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and bacteria of the genus *Streptococcus* (Kothari et al., 2019; Khalid et al., 2022). With the development of modern biotechnologies, research on creating new complex probiotic supplements is being intensified. It has been established that the greatest effect is achieved when using complex multi-species probiotics – multiprobiotics (Abd El-Hack et al., 2020; Vovkotrub et al., 2024). Combined probiotics contain bacteria from different taxonomic groups that enhance each other's effects, demonstrating better metabolic and preventive actions compared to mono-species probiotics. Current European regulatory requirements in the field of probiotics necessitate studies on the biological activity of individual probiotic species as well as their complexes when developing new probiotic supplements.

It is known that some probiotics can affect the histology of the intestinal mucosa. Research on the effect of *Bacillus subtilis* probiotics on broiler chickens showed an increase in villus height in the duodenum of the birds, improved protein assimilation, and better productivity indicators (Afsharmanesh & Sadaghi, 2014). Scientists report a positive impact on the intestinal microflora of broiler chickens from the probiotic *Bacillus coagulans*. The developed dietary supplement positively influenced the height of intestinal villi in birds, improving feed conversion ratios throughout the research period (Hung et al., 2012).The composition and activity of chicken microbiota are closely related to various factors such as diet, environmental factors, localization in the digestive tract, breed, age, and sex of the bird, resulting in significant variability in research data. The primary functions of microbiota in animal and poultry bodies include participation in metabolism and maintaining pH levels, production of biologically active compounds, immunogenic function, provision of colonization resistance, and detoxification. Poultry differs from other agricultural animals in the structure of their digestive systems and has a high metabolic rate, significantly influenced by the enzymes of the gastrointestinal microbiota. At the time of hatching, the chicks' intestinal tract is sterile and gets colonized by environmental microorganisms in the first hours of life. Young poultry are more susceptible to colonization by pathogens due to the undeveloped gut microbiocenosis. Therefore, ensuring the rapid and complete formation of the digestive system microbiota in young birds is crucial for obtaining healthy poultry flocks.

Materials and methods

All experimental studies were conducted in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes of 1986, as well as the Law of Ukraine "On the Protection of Animals from Cruelty" dated February 21, 2006, No. 3447-IV, as revised on August 4, 2017.

For the study, 100 day-old Cobb 500 broiler chickens were selected. These chickens were divided into two groups based on the principle of analogs: one control group and one experimental group, each consisting of 50 birds (Table 1).

Table 1
Study design scheme

Groups	Number of birds per group	Feeding regimen
Control	50	Balanced feed, drinking water without a probiotic supplement
Experimental	50	A balanced feed with the addition of the probiotic "TIMM-P" according to the application course

Broiler chickens were raised on litter until 42 days of age. Throughout the experiment, the chickens were fed a standard ration providing their nutritional and biologically active substance requirements. Access to drinking water was unrestricted and provided via cup drinkers.

The functional additive "TIMM-P" consists of a complex of microorganisms including various strains of lactic acid bacteria and intestinal bifidobacteria, isolated from healthy poultry. The preparation contains 5 highly active strains: *Bifidobacterium gallinarum*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus paracasei* ssp. *paracasei*. These strains are natural microorganisms that have not undergone any genetic modification. They actively synthesize various enzymes that significantly improve digestion. "TIMM-P" is a dry, powdery substance ranging in color from cream to light brown, free from foreign inclusions. The probiotic easily mixes with feed and dissolves well in water, with moisture content not exceeding 5%. The preparation contains not less than $1x10^9$ CFU/g of lactic acid bacteria and a similar concentration of bifidobacteria.

In the experimental group of broiler chickens, "TIMM-P" was administered by drinking water courses: on days 1–5, 21–25, and 30–35 of the study, administered for 2 hours in the morning before the first feeding. Throughout the study period, sanitary and hygienic parameters of poultry management were monitored daily. Temperature and humidity in the facility housing the chickens were measured using the VIT-2 hygrometer (produced by "Sklolprylad", Ukraine), with temperature and humidity levels recorded three times a day (at 6:00, 14:00, and 22:00).

To determine the air movement speed in the premises, a cathetometer was used. For measuring the air velocity in ventilation ducts with a natural draft, a vane anemometer ASO-3 (manufactured by "TK PROMEL", Ukraine) was employed.

The concentration of carbon dioxide was determined using the Subbotin-Nagorsky method, which involves the absorption of carbon dioxide by a barium hydroxide solution, forming barium carbonate. The amount of barium combined with carbon dioxide was determined by titration with oxalic acid. Barium hydroxide (from China) and oxalic acid (from Italy) were utilized. For rapid assessment of harmful gases, a universal gas analyzer UG-2 ("Analit Pribor", Ukraine) was employed. This device is designed to detect carbon dioxide, ammonia, hydrogen sulfide, and other gases. It operates based on passing a specific volume of air through indicator powder tubes, where the color changes proportionally to the amount of gas present in the air. The magnitude of color change on the indicator powder scale was measured in mg/m^3.

Physiological indicators of broiler chickens were monitored, including respiratory rate (counting breaths per minute) and body temperature using a veterinary electronic thermometer ("Kerbl", Germany).

The study on the effect of the probiotic on broiler chicken survival involved daily inspections and record-keeping of the birds. Live body weight was measured on days 1, 14, 28, and 42 of the study using Aurora AU 309 scales (from China) with a measurement accuracy of ± 1 g. A calculation method was applied to determine the absolute and average daily weight gain (P_1) using the formula: $P_1 = (V_1 – V_0) / T$, where P_1 is the average daily gain; V_1 is the live body weight at the end of the study period, in kg; V_0 is the live body weight at the beginning of the study period, in kg; T is the duration of the study in days.

To investigate the impact of probiotic supplementation on gut microbiota and the condition of internal organs, slaughter was conducted on 5 chickens each from the control and experimental groups on the 14th and 28th days. A meat quality assessment of broiler chickens was performed after the slaughter on the 42nd day. The selection of birds for this study followed a group-analog principle, with 10 birds selected per group. Before slaughter, stunning was performed using electrical stunning equipment Le Reve ("FAF", France).

Organoleptic assessment of broiler chicken meat was conducted according to current standard methodologies: assessing odor, external appearance of carcasses, degree of feather removal, condition of skin covering and skeletal system, meat consistency, color of muscle tissue, skin, subcutaneous and intra-abdominal fat tissue. Each sample was analyzed individually under daylight and room temperature conditions. During laboratory examinations, chemical and microscopic indicators of chicken meat were determined on days 3, 5, 7, and 9 of refrigerated storage at 4 ± 1 °C. The pH value of chicken meat was measured using a pH meter of pH 211 (HANNA instruments, Romania). Other freshness indicators,

such as acid number of fat, peroxide number of fat, and reaction with copper sulfate to determine primary breakdown products of proteins in meat broth, were determined using generally accepted methodologies. To calculate the quantity of microorganisms and assess the degree of muscle tissue breakdown, smears were prepared and stained with Gram stain, followed by microscopic examination using a binocular microscope XS-3320 with LED illumination MICROmed (China), after drying.

Additionally, to assess meat freshness on the 9th day of storage, the content of volatile fatty acids was determined as an additional method for comprehensive meat quality assessment using an enhanced method. Volatile fatty acids are formed during amino acid deamination and breakdown of intramuscular fat. For this parameter, an improved device was used, incorporating a water trap with a tube for water drainage at the initial stage of the reaction, preventing excessive water from entering the flask with the meat mixture. This design enhancement avoids the collection of water droplets affecting the accuracy of results, unlike the conventional method where the water trap is placed above the flask with the meat mixture and lacks a drain for water drops. The diagram of the enhanced device is shown in Figure 1.

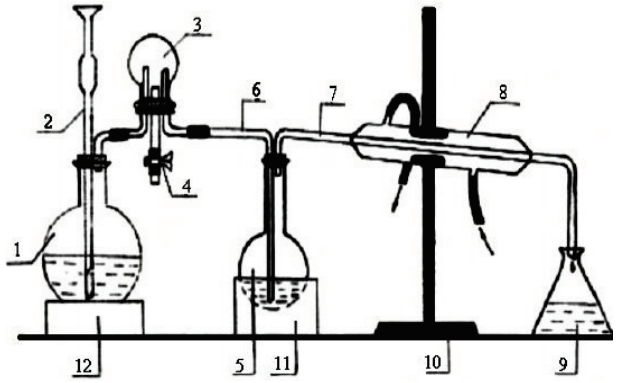

Fig. 1. Diagram of the enhanced device for distilling volatile fatty acids: *1* – flat-bottom flask (vaporizer); *2* – safety tube with neck; *3* – water trap (vapor catcher) with tube for steam release; *4* – clamp; *5* – round-bottom flask for distillation; *6, 7* – steam vents; *8* – Liebig water condenser; *9* – receiver; *10* – stand; *11* – flask heater; *12* – electric hot plate

Prepared samples of 25 ± 0.01 g of minced meat, weighed on laboratory scales ADC6200C (Ukraine), were placed into a round-bottom flask and supplemented with 150 cm^3 of sulfuric acid solution with a concentration of 20 g/dm^3. The flask contents were mixed and sealed with a stopper. A conical flask, with a capacity of 250 cm^3 and marked to indicate a volume of 200 cm^3, was placed under a Liebig condenser. Distilled water in the flat-bottom flask (vaporizer) was brought to a boil, and volatile fatty acids were distilled off by steam until 200 cm^3 of distillate was collected in the receiving flask. During distillation, the flask containing the sample was heated. Titration of the entire distillate volume was performed with 0.1 mol/dm^3 potassium hydroxide (or sodium hydroxide) solution using a phenolphthalein indicator until a persistent pink color appeared for 30 seconds.

Simultaneously, a control experiment was conducted under the same conditions to determine the consumption of alkali for titration of distillate without meat.

The amount of volatile fatty acids (X) in milligrams of potassium hydroxide per 25 g of meat was calculated using the formula: $X = (v - v_0) \times K \times 5.61$, where v is the volume in cm^3 of 0.1 mol/dm^3 potassium hydroxide solution used to titrate 200 cm^3 of distillate from the meat sample, v_0 is the volume in cm^3 of 0.1 mol/dm^3 potassium hydroxide solution used for titration of 200 cm^3 of distillate from the control analysis, K is the correction factor for 0.1 mol/dm^3 potassium hydroxide solution, and 5.61 is the amount of potassium hydroxide contained in 1 cm^3 of 0.1 mol/dm^3 solution, in milligrams.

The final research results were based on the arithmetic mean of two parallel determinations.

Calculations were conducted with an error not exceeding 0.01 mg of potassium hydroxide. Meat was considered to be of questionable freshness if it contained volatile fatty acids from 4 to 9 mg of potassium hydroxide, and unfit for consumption if above 9 mg.

The chemical composition of broiler chicken meat (breast muscles and thigh muscles) in the control and experimental groups was determined by analyzing its moisture content, fat content, protein content, and ash content. Moisture content was determined by successive weighing and drying of muscle tissue samples in a drying cabinet SP-30K (Riva-Stahl LLC, Ukraine). Dry matter in meat samples was calculated based on moisture content data. Nitrogen content in poultry meat was determined by the Kjeldahl method, with sample mineralization performed using a DK 6 moist mineralizer (Velp Scientifica, Italy), and ammonia distillation carried out on a semi-automatic Kjeldahl steam distillation apparatus UDK 129 (Velp Scientifica, Italy). Protein content in meat was calculated after nitrogen determination. Ash content in meat was determined by combustion of samples in a Nabertherm L15 muffle furnace (Germany). Fat content was determined by Soxhlet extraction of meat samples using diethyl ether with an automatic extraction apparatus SER 148 (Velp Scientifica, Italy).

Statistical analysis of the obtained results was performed using ANOVA software, and data in tables are presented as x ± SD (mean ± standard deviation). The difference between groups was considered significant for $P < 0.05$ (taking into account the Bonferroni correction).

Results

The microclimate parameters in the room where the broiler chickens were kept corresponded to optimal indicators: relative humidity varied within 62–70%, and the temperature for the chicks was maintained between 28–30 °C for the first 14 days, gradually decreasing to 20 °C thereafter. The CO_2 concentration in the aviary housing the birds ranged from 0.07–0.08%, with NH_3 content up to 5 mg/m^3 and H_2S at 2–3 mg/m^3. The provision of a probiotic supplement to the experimental broiler chicks did not significantly affect water consumption or feed intake compared to the control throughout the entire 42-day experiment.

The body temperature and respiratory rate of the daily chickens corresponded to physiological parameters. The body temperature of the control group chicks was 40.65 ± 0.08 °C, and for the experimental group, it was 40.75 ± 0.06 °C. The resting respiratory rate in the control group chicks was 34.77 ± 1.34, while in the experimental group, it was 35.73 ± 0.70. At the end of the study, on day 42, the body temperature readings in the control group chicks were 41.02 ± 0.04 °C and in the experimental group, 41.03 ± 0.05 °C. The pulse rate in the control group of broiler chicks was 189.92 ± 1.94 and in the experimental group, it was 189.71 ± 2.20. The respiratory rate in the chickens was 35.46 ± 1.35 for the control group and 34.21 ± 1.48 for the experimental group.

The live weight of daily chicks at the beginning of the study averaged 45 g in both the control and experimental groups. During the first day of the study, one chick each from the control and experimental groups died. Re-weighing was conducted every 14 days. At two weeks of age, the live weight of broilers in the control group was 2.54% higher compared to the experimental group, a difference that was not statistically significant. This difference varied with age. At 28 days, the experimental group of broiler chickens exceeded the control group by 2.69% in live weight, a statistically significant difference (Table 2). By day 42, the live weight of the experimental group birds was also higher than the control, by 3.46%.

Table 2
The dynamics of live weight gains in broiler chickens using the probiotic preparation (g, x ± SD)

Age, days	n	Live weight of broiler chicken	
		control group	experimental group
1	50	45.1 ± 2.3	44.9 ± 2.2
14	49	323.7 ± 32.9	315.7 ± 36.3
28	44	1213.7 ± 66.3	1246.3 ± 45.2*
42	39	2911.7 ± 132.7	3012.5 ± 155.3*

Note: * – $P < 0.05$ – relative to the control group of broiler chickens; two groups were compared using one-way ANOVA.

According to the live weight of chickens in the experimental group, statistically higher values of absolute and average daily weight gains were

observed on days 15–28 of the study (Table 3). Over the entire study period from day 1 to day 42, the average daily weight gains in the control group were 68.35 g/day, while in the experimental group, this figure was higher at 70.68 g/day.

Table 3
Dynamics of absolute and average daily body weight gains of broiler chickens using probiotic supplement (g, x ± SD)

Age, days	n	Control group	Experimental group
		Absolute weight gains	
1–14	49	278.5 ± 32.8	270.7 ± 36.0
15–28	44	892.6 ± 73.6	934.1 ± 66.4*
29–42	39	1699.6 ± 173.2	1763.8 ± 145.3
		Average daily weight gains	
1–14	49	19.9 ± 2.3	19.3 ± 2.6
15–28	44	63.8 ± 5.3	66.7 ± 4.7*
29–42	39	121.4 ± 12.4	126.0 ± 10.4

Note: * – P < 0.05 – relative to the control group of broiler chickens; two groups were compared using one-way ANOVA.

After slaughtering the chickens from the experimental and control groups, the weight of internal organs, feathers, heads, extremities, and separately the weight of eviscerated carcasses were determined (Table 4). The carcass weight in the experimental group was significantly higher by 64.5 g compared to the broiler chickens in the control group.

Table 4
Slaughter yield of poultry (x ± SD, n = 39)

Indicators	Control group	Experimental group
Eviscerated carcass weight, g	2245 ± 112	2310 ± 109*
Weight of internal organs, feathers, heads, and lower extremities, g	666 ± 24	703 ± 47**
Slaughter yield, %	77.08 ± 0.59	76.68 ± 0.53

Note: * – P < 0.05, ** – P < 0.01 – relative to the control group of broiler chickens; two groups were compared using ANOVA.

Organoleptic studies of poultry slaughter products have shown that all examined carcasses were well bled, with a clean surface, without blood clots, intestinal remnants, or reproductive organs inside. The skin was clean, whitish-yellow in color, without scratches, tears, stains, or bruises. The odor of the meat was specific and characteristic of fresh poultry meat. The skeletal system of the broiler chickens in both the experimental and control groups showed no fractures or deformations. The muscle consistency of the broiler chickens in both groups was dense and elastic; when pressed with a spatula or finger, the depression quickly flattened out. The muscle tissue was pale pink. Subcutaneous and internal fat were yellowish. Thus, based on organoleptic indicators, the carcasses of the broiler chickens in the experimental and control groups met the requirements for fresh meat. Therefore, the addition of the probiotic "TIMM-P" did not affect the organoleptic characteristics of the experimental poultry carcasses.

Regarding the chemical composition of the meat from broiler chickens, it was found that the moisture content in the meat samples of the experimental group was lower than in the control by 0.76%, while the dry matter content was higher. However, these differences were not statistically significant (Table 5).

Table 5
Chemical composition of 42-day-old broiler chicken meat (%, x ± SD, n = 5)

Parameters	Control group	Experimental group
Moisture	73.71 ± 1.62	72.94 ± 0.61
Total protein	21.26 ± 0.44	23.20 ± 0.46**
Fat	1.79 ± 0.23	1.46 ± 0.32
Ash	1.11 ± 0.07	1.18 ± 0.13

Notes: **P < 0.01 – relative to the control group; two groups were compared using one-way ANOVA.

In addition, administering the probiotic to the experimental group of broiler chickens, conducted in courses on the 1st to 5th, 21st to 25th, and 30th to 35th days of the study, contributed to an increase in the total protein content in the muscle tissue of the birds by 1.94%. The difference between the control and experimental groups was statistically significant. The fat content in the meat of the experimental birds was lower by 0.34% compared to the control group. A slight increase in ash content by 0.08% was observed in the experimental group, but it was not statistically significant.

The investigation of the chemical indicators of the meat was carried out on the 3rd, 5th, 7th, and 9th days of storage under chilled conditions at a temperature of 4 ± 1 °C. During the assessment of the meat's chemical indicators, it was found that on the 3rd day of storage, the pH value of the meat in the control and experimental groups likely did not differ. On the 5th day of the study, the pH values in both the control and experimental groups corresponded to those of fresh meat. By the 7th day of storage, the meat from the chickens in the control group was of questionable freshness. In the experimental group, the pH value corresponded to indicators of questionable meat freshness only by the 9th day of storage. It was found that the pH value was likely lower in the experimental group on the 9th day of meat storage (Table 6).

Table 6
Chemical and microscopic indicators of broiler chicken meat (x ± SD, n = 5)

Parameters	Storage period at 4 ± 1 °C, days	Control group	Experimental group
pH value	3	5.96 ± 0.07	5.74 ± 0.11
	5	6.04 ± 0.15	5.94 ± 0.11
	7	6.40 ± 0.19	6.16 ± 0.18
	9	6.76 ± 0.17	6.40 ± 0.19*
Acid number of fat, mg KOH	3	0.46 ± 0.15	0.48 ± 0.19
	5	0.82 ± 0.13	0.68 ± 0.21
	7	1.37 ± 0.06	0.90 ± 0.12**
	9	2.62 ± 0.25	2.18 ± 0.24
Peroxide number of fat, % iodine	3	0.003 ± 0.001	0.002 ± 0.001
	5	0.007 ± 0.002	0.006 ± 0.001
	7	0.021 ± 0.009	0.009 ± 0.004*
	9	0.046 ± 0.015	0.029 ± 0.007
Reaction with copper sulfate	3	negative (clear broth)	negative (clear broth)
	5	negative (clear broth)	negative (clear broth)
	7	dubious reaction	negative (clear broth)
	9	meat not fresh	dubious reaction
Bacterioscopy of smears-imprints	3	fresh (individual microorganisms)	fresh (individual microorganisms)
	5	fresh (not more than 10)	fresh (not more than 10)
	7	dubious (up to 30 microorganisms)	fresh (not more than 10)
	9	not fresh (more than 30)	dubious (up to 15 microorganisms)
Volatile fatty acids (VFAs)	9	10.30 ± 0.68	7.26 ± 0.60***

Notes: * P < 0.05; ** P < 0.01; *** P < 0.001 – relative to the control group; two groups were compared using one-way ANOVA.

The acid number of fat from fresh chilled chicken carcasses should not exceed 1 mg KOH, peroxide number – correspondingly 0.01% iodine. On the 3rd and 5th day of storage, no statistically significant difference between the groups was found; the arithmetic means of peroxide number of fat and acid number corresponded to fresh meat. On the 7th day of meat storage, during the determination of the acid number of fat, a statistically significant lower value was found in the experimental group compared to the control; the amount of potassium hydroxide used for titration was less than 1 mg. Thus, the meat of the control group belongs to the category of dubious freshness according to the acid number, and that of the experimental group – to fresh meat. Similar indicators were obtained for the study of peroxide number of fat on the 7th day of storage. On the 9th day, an increase in the acid number of fat was found in the control group, with the average value corresponding to non-fresh meat. In the experimental group, the acid number fluctuated within the range characteristic of meat of dubious freshness. However, no statistically significant difference between the groups was established. The peroxide number of fat in the experimental group on the 7th day of storage was likely lower compared to the control and corresponded to the indicator of fresh meat.

The reaction of the extract from the chicken meat with copper sulfate on the 3rd and 5th days of storage of chilled carcasses of the control and experimental groups did not change, the broth remained clear, indicating that the meat was fresh. On the 7th day of meat storage, the broth from the chicken meat of the control group was cloudy, and the filtrate from the meat of the experimental group corresponded to the indicators of fresh meat. On the 9th day, the broth from the chicken meat of the control group acquired a gel-like state, indicating that the meat was not fresh. The broth from the chicken meat of the experimental group corresponded to the indicators of meat of dubious freshness.

In the microscopic examination of imprints from deep layers of muscle tissue of broiler chickens of the control and experimental groups on the 3rd day, single microorganisms were found – mainly cocci, and no signs of tissue destruction were found. On the 5th day of storage of broiler chicken meat, the number of microorganisms increased: in the control group, up to 10 microorganisms were found in the field of view, in the experimental group - up to 3–5 monococci and individual rods. No traces indicated the breakdown of muscle tissue in the control and experimental groups. As a result of the studies, it was established that on the 5th day of storage, the chicken meat was fresh and suitable for consumption. On the 7th day, in the meat of the control group, according to the microscopy of imprints, 15–25 cocci and occasionally rods were found in the field of view. In the experimental group, a smaller number of microorganisms – up to 10 – were registered under the microscope. On the 9th day, imprints from the chicken meat of the control group revealed the disappearance of striations of muscle fibers and a significant number of microorganisms in the field of view (more than 30). Thus, the meat was not fresh. In the experimental group, a less pronounced striation of muscles was registered, and up to 15 microorganisms were found in the field of view of the microscope. Such meat is considered of dubious freshness.

The content of volatile fatty acids in the meat of broiler chickens of the control group on average is 10.3 mg NaOH, and in the experimental group – 7.26 mg NaOH. The difference between the groups is statistically significant. Meat is considered of dubious freshness if it contains volatile fatty acids from 4.51 to 9 mg sodium hydroxide, and above 9 mg – not fresh. Thus, the meat of broiler chickens in the experimental group on the 9th day of storage was fresh, indicating an extension of its storage period.

Discussion

During the research on the clinical condition of broiler chickens, specifically their behavior, body temperature, pulse rate, and respiratory rate, no significant differences were observed between the control and experimental groups. This indicates the absence of negative effects of the probiotic supplement on the clinical condition of the birds.

The live weight indicators of broiler chickens were analyzed at different stages of the study. In the early stages of the research, no statistically significant differences in body weight were found between the control and experimental groups. The increase in body weight gains of broiler chickens in the experimental group on the 28th and 42nd day of the study indicates a positive effect of the probiotic supplement on the biochemical processes in the birds' bodies, which in turn ensures efficient digestion of nutrients in the diet and positively influences morphological indicators of the carcass. Similar studies have been conducted by other researchers who have noted the positive impact of probiotic preparations on poultry weight gain (Aljohara et al., 2023; Poberezhets et al., 2023). Nath et al. (2023) report that probiotic preparations based on lactic acid bacteria not only increase poultry weight gains but also reduce the mortality rate compared to the control group that did not receive the supplement. This is consistent with our results of a likely increase in absolute and average daily weight gains in the experimental group of birds. The likely increase in live weight in the experimental group of chickens compared to the control may be associated with the ability of probiotic cultures to produce enzymes. Amylase, protease, and lipase enhance the speed of digesting nutrient components of the diet, such as starch, fat, and protein (Rehman et al., 2020). Thus, probiotic supplements positively affect the intestinal biocenosis of poultry (Ye et al., 2021), improve nutrient absorption, and increase productivity.

A likely increase in the total protein content in the muscle tissue of the experimental group of chickens receiving the probiotic "TIMM-P" was noted. Similar studies have been conducted on broiler chickens using probiotic supplements that include *Bifidobacterium* and *Lactobacillus*, as well as *Bacillus* and *Streptococcus* (Suryadi et al., 2019). The obtained data are consistent with the results of our study. The increased protein content in the meat of broiler chickens fed with probiotics can be explained by the influence of lactic acid bacteria. These microorganisms can survive in the digestive tract, adhere to the intestinal wall, and produce digestive enzymes – proteases. Enzymes break down chemical bonds in nutrients, making their molecules smaller and facilitating their absorption and assimilation, particularly proteins. Scientists note that probiotic supplements fed to broiler chickens increase the synthesis of essential amino acids in muscle tissue, specifically lysine, histidine, arginine, threonine, valine, methionine, and leucine. There is also improvement in the absorption of minerals such as Ca, P, Mg, Cu under the influence of probiotic preparations (Poberezhets et al., 2021).

During the determination of chemical indicators of meat, it was found that the concentration of hydrogen ions in the muscle tissue begins to increase during the storage of poultry carcasses. As meat matures due to the action of autolytic enzymes, the pH shifts towards acidity. A pH value of 6.2 is characteristic of fresh matured meat. However, during prolonged storage, meat spoilage begins. Microbial enzymes cause profound changes in proteins with an accumulation of alkaline degradation products, leading to an increase in pH. It should be noted that the pH of meat depends on many factors, such as the condition of the poultry before slaughter, the chemical composition of the meat, etc. In most cases, the meat of different freshness cannot be distinguished based solely on pH, so this study uses pH measurement in combination with other methods. Indices such as the acid and peroxide values of fat on the 7th day of storage indicated questionable freshness of meat in the control group. Meanwhile, the meat in the experimental group met the requirements for fresh meat, indicating a positive effect of the probiotic preparation in extending the shelf life of carcasses up to 7 days. The results of these studies are also supported by the reaction with copper sulfate and bacterioscopy of smears on the 7th and 9th day of poultry carcass storage. To confirm the results on the 9th day of storage of broiler chicken meat, an additional study was conducted to determine the content of volatile fatty acids using an improved device. It was found that the meat of the control group on the 9th day of storage was not fresh, while that of the experimental group was fresh, which corroborates the results of chemical and microscopic studies.

Conclusions

The practicality and effectiveness of using the new probiotic complex preparation "TIMM-P" in the poultry industry, which is rapidly developping and requires effective and safe therapeutic and preventive measures, have been theoretically justified and experimentally confirmed. The use of the probiotic "TIMM-P," added to the water during poultry watering, positively influences the growth dynamics of live weight, evidenced by a probable increase in absolute and average daily weight gains of broiler chickens. It has been established that the use of probiotic preparation contributes to an increase in the mass fraction of total protein in the meat of broiler chickens, as indicated by studies of its chemical composition. Results from organoleptic, chemical, and microscopic studies indicate the safety of the new probiotic preparation for the organisms of broiler chickens and suggest its potential use in poultry farming to enhance productivity. Additionally, the meat from the experimental poultry remains fresh for a longer period, extending the shelf life of chilled carcasses for consumption, which is economically advantageous.

For a more in-depth understanding of the probiotic's impact on the organism of broiler chickens, it would be promising to conduct further study of the microbiota of the digestive tract.

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