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EVALUATION OF EFFECTIVENESS OF PROBIOTIC FEED ADDITIVE IN EXPERIMENT ON LAYING HENS

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Abstract. *The paper represents the impact of a probiotic feed supplement on the clinical and biochemical parameters of laying hens in an amateur private farm setting for organic product production. The experiment was conducted on laying hens aged 160 days (n=200) of the Leghorn breed of egg production, floor-housed on deep litter, weighing 1500-1600 g. Before the experiment, the birds were categorised into groups of 100 based on the principle of analogues. Following a 14-day acclimatisation period, a probiotic feed additive (L. casei, L. delbrueckii, B. licheniformis, B. subtilis at a concentration of 10^6 CFU/mL each) was incorporated into the diet of the experimental group in addition to the complete diet at a dosage of 1.0 cm³ per kg of feed for 30 days. The control group of poultry got a full meal according to the standards for laying hens. Before administering the medications, and on 15th and 30th days post-initiation of probiotic feeding, the birds were slaughted under preparatory inhalation chloroform anaesthesia (n=10). Blood samples, together with tissue and organ specimens, were collected for microbiological and biochemical analyses. The results suggest that the use of probiotic feed additives did not significantly alter the quantitative composition of indicator microflora. However, there was a tendency (on the 30th day of the experiment) to increase the content of Lactobacillus spp. and Bacillus spp. by 14.6% and 8.7%, respectively; a tendency to decrease the content of potential pathogenic microorganisms was also detected - Staphylococcus spp. 19.5%, Candida spp. 15.2%, Enterococcus spp. 1.47%, E. coli 13.0%. Administration of probiotics to laying hens resulted in a significant increase in serum immunoglobulin G levels throughout the experiment, with the peak IgG concentration in the experimental group observed on the 30th day, demonstrating an increase of 11.3% ($p<0.05$). On the 30th day of the trial, immunoglobulin M and A levels rose by 14.6% and 10.2% ($p<0.05$), respectively, while circulating immune complexes increased by 26.3%. The nonspecific resistance indicators in the experimental group of hens exhibited an upward trend: the phagocytic index, bactericidal activity, and lysozyme activity rose by 11.5%, 12.0%, and 13.9%, respectively. The clinical and biochemical findings of the trial indicate that the suggested composition of probiotic bacteria is non-harmful to laying hens. The observational data demonstrate no adverse effect on egg production.*
Key words*: probiotics, microflora, resistance, antioxidant system, productivity*

The constraints of antibiotic use in poultry industry have prompted the pursuit of alternate ways to mitigate health issues. Probiotic bacterial preparations are regarded as the primary category of products for sustaining microbial homeostasis; these agents facilitate the modulation of the intestinal microbial environment to benefit the host by mitigating the adverse effects of pathogenic microorganisms (van der Klein, 2024).

Several researchers observed the beneficial impact of probiotic bacteria (*Limosilactobacillus* spp., *Enterococcus faecium, Bifidobacterium* spp.) on the equilibrium of intestinal microflora, enhancing digestion and modulating the immune system across various animal species (Rehman, 2020; RamLucken, 2020; Shehata, 2022; Zhang, 2022; Lytvynenko, 2024).

The results of studies (Abd El-Ghany, 2022) aimed at developing treatment regimens for necrotic enteritis caused by *C. pefringens* in broiler chickens have demonstrated the feasibility of using postbiotics and probiotics in combination therapy regimens.

The studies conducted by Romanovych (2019) theoretically and experimentally substantiated the potential of utilising *Saccharomyces* preparations and the BPS-44 preparation (*Bacillus subtilis*) to enhance post-vaccination immunity and antioxidant capacity in broiler chicken vaccinated against Gumboro disease.

The probiotic culture *Bacillus subtilis* DSM 32424, when administered with drinking water to laying quails, has been demonstrated to suppress the proliferation of faecal staphylococci and enhance the carotenoid content in the yolk (Ermakova, 2021; Bouchicha, 2022). It is assumed that incorporating this probiotic into the quail diet positively influences both the parent stock and their offspring.

It was discovered that the incorporation of the probiotic strain Limosilactobacillus fermentum 2 and 3 positively influenced the feed conversion ratio, weight gain, phagocyte activity, and the proportions of T-lymphocyte subpopulations (CD8+ and CD4+CD8+), thereby affirming the immunostimulatory effect of *L. fermentum* 2 and 3 (Hudec, 2024).

Numerous research findings suggest that probiotics aid in the restoration of intestinal barrier function, enhance nutrient absorption, and diminish intestinal inflammation, among other benefits (Bernard, 2023; Cao, 2023; Goodoory, 2023; Lamari, 2021; Ban, 2021).

Scientific research (Li, 2024) supports the efficacy of *L. salivarius* ($4x10^8$ CFU/g) on the potential adverse effects of diet infected with aflatoxin B1. The researchers investigated the impact of *Lactobacillus salivarius* in a 63-day study including 300 Landes goslings, who were administered suitable dosages of aflatoxin and $4x10^8$ CFU/g *L. salivarius* as a fundamental component of the primary diet. The findings indicated that *Lactobacillus salivarius* mitigated the adverse effects of aflatoxin on gosling development, feed intake, average daily weight gain, and immunoglobulin levels; sequencing of the cecal microbiota revealed that the incorporation of *L. salivarius* increased the population of *Bacteroidetes* and *Lactococcus*. The incorporation of $4x10^8$ CFU/g of *L. salivarius*, amidst chronic aflatoxin exposure, sustained an adequate level of productivity and immune function, enhanced jejunal morphology, diminished liver inflammation, altered cecal microbial composition, and favourably influenced the growth and development of geese.

Cui (2024) demonstrate that the isolate *B. velezensis* CAU277 showed a specific prophylactic efficacy against the detrimental impact of *C. jejuni in vitro* and *in vivo.*

Our research aimed to assess the feasibility of utilising strains identified in prior studies for their capacity to generate high-density biofilms, exhibit antagonistic activity against pathogenic microorganisms and withstand the harsh conditions of the digestive tract (*L. casei – 4//1, L. delbrueckii 1/5, B. licheniformis 2/6, B. subtilis 1/21*) to sustain homeostasis in laying hens and affect their productivity.

The aim of this experimental study was to assess the efficacy of a probiotic feed additive (PFA) in laying hens within production settings and its impact on the physiology of the target avian species.

Materials and methods. The experiments were carried out at the Department of Epizoology, Microbiology, and Virology of the National University of Life and Environmental Sciences of Ukraine in Kyiv, as well as at a private farm producing "organic" products in the Vinnytsia region. The study involved 200 laying hens of the Leghorn breed, floor-housed on deep litter, aged 160 days, and weighing between 1500 and 1600 g. Before the

commencement of the experiment, the birds were categorised into groups of 100 each (n=100). Following a 14-day acclimatisation phase, the diet of the experimental group of chickens was augmented with dietary supplement probiotic feed additive (PFA) (comprising *Bacillus licheniformis 2/6, B. subtilis 1/21, L. delbrueckii 1/5, and Lactobacillus casei – 4/1*, each at a minimum concentration of 106 CFU/ml) at a dosage of 1.0 cm^3 per kg of feed for 30 days. The control group of birds was administered the dietary supplement KD by the standards for egg-laying hens.

Research on birds was conducted in compliance with the requirements of the Law of Ukraine "On the Protection of Animals from Cruelty to Animals" and Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010, on the protection of animals used for scientific purposes. purposes.

Before administration, and 15 and 30 days after the initiation of drug administration, birds were decapitated (n=10) following preliminary inhalation of chloroform anaesthesia and total exsanguination. Blood samples were collected for clinical and biochemical analyses, and post-mortem pathological examinations were conducted to obtain tissue and organ samples for microbiological investigations.

Quantitative determination of microorganisms and their identification was carried out by inoculation of tenfold dilutions of cecal contents onto media. Detection of microorganisms of the genus *Lactobacillus* was carried out on MRS medium, *Bifidobacterium* – on Bifidum medium; *Staphylococcus* – on hemoagar with 7% NaCl; *Streptococcus* – on Garrot medium; *Enterococcus* – on enterococcus agar; fungi of the genus *Candida* – on Sabouraud medium; *E. coli* – on Endo medium, respectively. The number of microorganisms in 1.0 g was expressed as colony-forming units (CFU/g).

To assess the impact of the analysed PFA samples by the clinical and biochemical markers, blood samples were collected from poultry before and during drug administration. To evaluate the detoxification capabilities of the liver and kidneys, the concentrations of creatinine, glucose, uric acid, total proteins, and their albumin and globulin fractions were measured, alongside the enzymatic activity levels of aspartate-aminotransferase (AST, KF 2.6.1.1), alanine-aminotransferase (ALT, KF 2.6.1.2), alkaline phosphatase (AP, KF 3.1.3.1), and γ-glutamyltranspeptidase (GGT, KF 2.3.2.2) using an automatic biochemical analyser LabLine-010 (Austria) with reagent test kits produced by CORMAY (Poland). The quantities of erythrocytes and leukocytes, as well as the total haemoglobin content, were assessed utilising the methodologies outlined (Vlizlo, 2012). The bactericidal activity of blood serum (BABS) was assessed using the *Escherichia coli* strain 2017/4 tx2) from the National University of Life and Environmental Sciences (NULES) collection as the test microorganism (Vlizlo, 2012). The bactericidal and lysozyme activity of blood serum (LABS), neutrophil phagocytic activity, and phagocytic index were examined utilising the methodologies outlined by Vlizlo (2012).

The concentration of circulating immune complexes of medium molecular weight (CIC) was assessed using the Grinevich (1981) technique. Protein complexes of antigen-antibody were precipitated using PEG-6000, and seromucoids (Sm) were analysed spectrophotometrically by measuring the difference in optical density at 260 nm and 280 nm, as detailed in the study by Men'shikov (1987). The degree of lipid peroxidation (LPO) was assessed by measuring the generation of diene conjugates (DC) and TBA-active compounds in heptane-isopropanol extracts using the method developed by Gavrylova and Mishkorudna (1985). The degree of oxidative modification of proteins (OMB) in membrane fractions was assessed by the generation of carbonyl derivatives of neutral (NH) and basic (OH) nature, as per Archakov and Mykhosoev (1998). Aldehyde- and ketoderivatives of neutral character were measured at a wavelength of 370 nm, whereas those of basic character were measured at 430 nm, as outlined in the study by Arachkov and Mykhosoev (1998). The condition of the antioxidant system (AOS) was assessed using the enzymatic activity of catalase (KF 1.11.1.6), utilising H_2O_2 spectrophotometrically at a wavelength of 410 nm (Korolyuk M. A., 1988). The

total antioxidant activity (AOA) of lipids isolated from blood plasma was assessed as outlined in the study by Klebanov (1988).

The obtained results of the study and the differences between them were subjected to a one-way analysis of variance (ANOVA), (t-test), according to the Dunnett criterion with an error rate of 0.05 (P<0.033*; 0.002**; 0.001***), using GraphPad Prism 8.3.0 for Windows.

Results and discussion. No changes in the general condition of the experimental chickens were detected during the experiment. The chickens were mobile, took food and water well, and had a characteristic appearance for the breed: the comb and wattles were shiny, bright red, the beak was yellowish, the plumage was white, smooth and shiny, dense, and fit well to the body surface. On the 28th day of the experiment, when the chickens of the experimental and control groups were dissected, no changes in the internal organs under the influence of the probiotic were noted.

It should be noted that 559 eggs were obtained from the control group of chickens during the observation period (30 days), and 686 from the experimental group, respectively, which was 22.7% more than in the control group. According to the results of the veterinary and sanitary assessment of eggs from chickens of the experimental and control groups by DSTU 5028:2008 "Chicken eggs for food. Technical conditions", it was established that their quality indicators met the requirements: the egg shell was smooth, strong, and without damage; the yolk was yellow, evenly colored, of elastic consistency; the protein was clean, transparent, viscous, without signs of spoilage; the smell was characteristic of fresh eggs. According to the results of weighing, the eggs were divided into several categories. In the control group, 7.0% of eggs had a mass from 35 to 44.9 g (category "small"); 47.5% of eggs were classified as "first" category; 45.5% of eggs were classified as "higher" category (weight from 63 to 72.9 g). In the experimental group, 55.7% of eggs were classified as the "first" category; 37.6% – as "higher" and 6.7% of eggs were classified as the "selected" category (weight 73 g and more), respectively. That is, based on the results of observation of experimental poultry, it is possible to conclude that adding probiotic supplements to the feeding had a positive effect on the quantitative and qualitative indicators of egg production.

In bacteriological studies of the droppings/rectal content of chickens, the goal was to determine changes in the content of indicator bacteria (Table 1).

The results obtained indicate that the use of probiotic feed additives did not significantly change the quantitative composition of the indicator microflora. However, on the 15th and 30th day of the experiment, an increase in the content of *Lactobacillus* spp. and *Bacillus* spp. by 14.6% and 8.7% ($p<0.05$), respectively, was detected in the chickens of the experimental group, as well as a tendency towards an increase in the number of *Bifidobacterium* spp. (2.3%) and a decrease in the content of potential pathogenic microorganisms - *Staphylococcus* spp. (19.5%; ($p<0.05$)), *Candida* spp. (15.2%; ($p<0.05$)), *Enterococcus* spp. (1.5%), *E. coli* (13.0%; ($p<0.05$)), respectively.

In poultry, the indices of immunocompetent protein fractions were assessed (Table 2): in the blood of the control group hens, the level of γ-globulins ranged from (35.8±1.30%) to (36.7±0.80%), whereas in the experimental group, this parameter was marginally elevated at (36.1±0.55%). Significant alterations in γ-globulin levels were noted in the blood of laying hens in the experimental group on the 15th and 30th days of the study, indicating an elevated degree of immunological reactivity in the avian subjects. The concentration of β-globulins in avian blood varied within physiological limits. The primary role of β-globulins is to transport iron to sites of utilisation or storage organs (liver, spleen); hence, the stability of this signal underscores the innocuousness of the experimental PFA.

The lack of an elevation in seromucoids (α-fetoproteins - immunosuppressive proteins) in the blood serum throughout the experiment suggested that no immunosuppressive responses developed in the chickens during the administration of the experimental PFA. The aggregate quantity of immunoglobulins and their proportional composition in laying hens with

PFA are presented in Table 3: following the administration of the probiotic, the immunoglobulin levels markedly elevated on the 30th day of the experiment, exhibiting an increase of 11.3% ($p<0.05$) in comparison to the control group of chickens.

Table 1

Concentration of indicator bacteria (*Lactobacillus* spp., *Bifidobacterium* spp., *Enterococcus* spp., *E. coli, Candida* spp.) in the chicken droppings during 30-day exposure to PFA (CFU/g; n=10; (p<0.05) – versus control)

Groups	Period of sampling after start of probiotic supplement, days		
	Before supplement	15	30
Lactobacillus spp. x10^7			
Experiment	7.32 + 0.2	**7.96 + 0.24***	**8.33 + 0.21***
Control	7.06 + 0.21	7.19 + 0.23	7.27 + 0.24
Bacillus spp. x10^7			
Experiment	7.13 + 0.23	**7.86 + 0.21***	**7.96 + 0.2***
Control	7.03 + 0.21	7.31 + 0.23	7.32 + 0.24
Bifidobacterium spp. x10^6			
Experiment	7.47 + 0.23	7.55 + 0.21	**7.66 + 0.23**
Control	7.37 + 0.25	7.43 + 0.22	7.49 + 0.22
Staphylococcus spp. x10^5			
Experiment	5.11 + 0.13	5.03 + 0.11	4.28 + 0.24*
Control	5.04 + 0.2	5.21 + 0.21	5.32 + 0.15
Enterococcus spp. x10^5			
Experiment	4.46 + 0.19	5.58 + 0.20	5.34 + 0.18
Control	4.45 + 0.19	5.46 + 0.18	5.42 + 0.18
E. coli x10^6			
Experiment	5.33 + 0.20	4.74 + 0.22	4.69 + 0.19*
Control	5.35 + 0.15	5.36 + 0.19	5.39 + 0.18
Candida spp.x10^3			
Experiment	4.35 + 0.1	4.10 + 0.2	3.80 + 0.19*
Control	4.39 + 0.2	4.44 + 0.16	4.48 + 0.17

Table 2

The level of immunocompetent protein fractions in the blood serum of laying hens during 30-day exposure to PFA (M±m; n=10; (p<0.05) – versus control)

Period of sampling *day)	γ-globulins, %		β-globulins, %		seromucoids, mg/cm^3	
	control	experiment	control	experiment	control	experiment
before administ-ration	35.8±1.30	36.1±0.55	10.6±0.50	11.1±0.31	2.20±0.12	2.21±0.08
15	36.2±0.78	**40.4±0.67***	11.1±0.27	11.9±0.33	2.30±0.22	2.36±0.11
30	36.7±0.80	**41.7±1.09*****	11.3±0.35	**12.9±0.53***	2.42±0.16	2.38±0.17

The administration of PFA resulted in a sustained elevation in the bloodstream of laying hens of immunoglobulin G levels throughout the experiment, peaking on the 30th day in the experimental group, surpassing control values by 11.3% ($p<0.05$). The levels of immunoglobulins M and A increased by 14.6% and 10.2% ($p<0.05$), respectively, on the 30th day of the experiment; the concentration of circulating immune complexes of average molecular weight rose by 26.3% ($p<0.05$) compared to the control group.

Table 3

Total content of immunoglobulins and their fractions in the blood serum of laying hens during 30-day exposure to PFA (M±m; n=10; (p<0,05) – versus control)

Period of sampling (day)	Total amount Ig, g/dm^3		Ig G, g/dm^3		Ig M, g/dm^3		Ig A, g/dm^3		CIC, mg/cm^3	
	control	experiment	control	experiment	control	experiment	control	experiment	control	experiment
before administ-ration	21.55± 1.23	23.36± 1.00	16.23± 0.34	17.31± 0.81	2.14± 0.04	2.27± 0.11	2.85± 0.17	3.03± 0.13	0.16± 0.08	0.18± 0.05
15	23.12± 1.43	25.22± 1.22	17.33± 0.58	18.82± 0.73	2.27± 0.16	**2.47± 0.13***	3.12± 0.12	3.29± 0.12	0.19± 0.09	**0.21± 0.01***
30	23.65± 1.33	**26.34± 1.43***	17.73± 0.78	**19.74± 0.45***	2.39± 0.22	**2.74± 0.13****	3.15± 0.13	**3.47± 0.15***	0.19± 0.03	**0.24± 0.02***

Table 4 illustrates our findings about the markers of nonspecific resistance in the bodies of experimental hens. Consequently, in the blood of hens from the experimental group, the phagocytic index exhibited an average rise of 11.5% (p<0.05), while serum bacterial activity and serum lysozyme activity rose by 12.0% and 13.9% (p<0.05), respectively, compared to the control group.

Table 4

The indices of non-specific resistance in the blood of laying hens during 30-day exposure to PFA (M±m, n=10; (p<0.05) – versus control)

Period of sampling (day)	Phagocytic activity, %		Phagocytic index, units		Lisozyme activity (LABS), %		Bactericidal activity (BABS), %	
	control	experiment	control	experiment	control	experiment	control	experiment
before administration	27.62± 0.81	27.98± 0.75	1.55± 0.08	1.60± 0.07	18.53± 0.44	18.95± 0.65	49.19± 0.48	50.06± 0.55
15	28.13± 0.72	28.48± 0.88	1.58± 0.05	1.69± 0.10	18.91± 0.50	**21.11± 0.49***	50.11± 0.42	**52.79± 0.99***
30	27.95± 0.76	28.85± 0.64	1.56± 0.06	1.74± 0.09	19.02± 0.48	**21.68± 0.70***	49.38± 0.39	**55.32± 0.60*****

The erythrocyte and leukocyte levels in the blood of hens administered PFA with their food ration remained within the control values throughout the trial (Table 5). The sole exception was the elevation in total haemoglobin levels in the blood of hens in the experimental group, averaging 18.5% (p<0.05) compared to the control values of the indicator.

Table 6 presents the results of studies of the protein profile and nonspecific resistance status in the serum of experimental poultry during 30-day exposure to PFA. Thus, in chickens of the experimental group under the influence of PFA, an increase in the level of total proteins was recorded, starting from the 14th day after the start of the supplement, which on average amounted to 8.4% (p<0.05) respectively relative to the control values of this indicator.

The growth of total protein levels in the blood serum of the experimental group of chickens is correlated with a rise in the total globulin count, while the albumin fraction remains at the control (physiological) level. Consequently, the experimental group of hens exhibited an average rise of 8.1% (p<0.05) in total globulin content compared to the control group. The observed rise in total globulin levels following 30 days of probiotic supplementation in the diet remained within physiological norms for this parameter.

The results, along with the data on the dynamics of haematological markers and alterations in the proteinogram, compellingly demonstrate the adaptogenic effect of experimental PFA on nonspecific resistance and protein metabolism in experimental chicken.

The findings obtained show that the application of PFA enhanced both cellular and humoral components of immunological reactivity in experimental laying hens.

Table 5

Contencentrations of erythrocytes, leukocytes and total hemoglobin in the blood of laying hens during 30-day exposure to PFA (M±m; n=10; (p<0.05) – versus control)

Group	Period of study, days		
	Before administration	15	30
Erythrocytes, 10^{12}/dm^3			
Control	4.08±0.14	4.14±0.10	3.99±0.26
Experiment		4.24±0.12	3.76±0.05
Leukocytes, 10^9/dm^3			
Control	21.38±1.07	22.16±2.12	23.28±3.42
Experiment		19.95±2.74	20.20±1.56
Total hemoglobin, g/dm^3			
Control	91.32±4.28	92.09±5.30	91.52±4.41
Experiment		99.24±3.61	**108.48±3.25****

Table 6

The indices of protein profile and non-specific resistance in the blood serum of laying hens during 30-day exposure to PFA (M±m; n=10; (p<0.05) – versus control)

Period of sampling, days	Total protein, g/dm^3		Albumins, g/dm^3		Total globulins, g/dm^3	
	control	experiment	control	experiment	control	experiment
Before administration	57.90± 3.03	59.60± 2.90	16.23± 0.24	16.27± 0.56	41.67±1.05	43.33±1.00
15	58.68± 2.48	**63.35± 2.27**	15.63± 1.26	17.00± 0.82	43.05±1.47	**46.35±1.25**
30	59.50± 1.25	**65.10± 1.55***	16.03± 1.22	18.10± 1.05	43.47±1.01	**47.00±1.25***

The liver is known to directly or indirectly engage in all metabolic processes of the body, linking portal and systemic circulation, thereby playing a crucial role in metabolic regulation and rendering the body particularly susceptible to endogenous and exogenous factors. Table 7 represents the findings of investigations on the primary biochemical markers in the blood serum of poultry, reflecting the functional status of the liver and kidneys in experimental hens. It was shown that following a 30-day dietary consumption of PFA, no statistically significant variations in the levels of glucose, uric acid, and creatinine were seen in the blood serum of chickens.

Table 7

Content of glucose, ureic acid, and creatinine in the blood serum of laying hens during 30-day exposure to PFA (M±m; n=10; (p<0.05) – versus control)

Group of poultry	Period of study, days		
	Before administration	15	30
Glucose, mM/dm^3			
Control	5.77±0.23	5.76±0.24	5.79±0.41
Experiment		5.69±0.34	5.80±0.32
Ureic acid, μM/dm^3			
Control	302.06±10.12	296.22±15.20	290.62±10.55
Experiment		304.32±13.88	298.08±8.70
Creatinine, μM/dm^3			
Control	113.02±10.26	106.74±8.06	111.80±7.10
Experiment		116.22±7.13	119.45±9.96

The results of studies of changes in activity of the main hepatospecific enzymes in the blood serum of experimental poultry are presented in Table 8.

Table 8

Activity of hepatospecific enzymes in the blood serum of laying hens during 30-day exposure to PFA (M±m; n=10; (p<0.05) – versus control)

Period of sampling (days)	ALT activity, mM/h dm^3		AST activity, mM/h dm^3		AP activity, nM/sec dm^3	
	control	experiment	control	experiment	control	experiment
Before administ-ration	0.38±0.02	0.38±0.13	3.67±0.08	3.71±0.13	2924.8±183.7	2926.5±301.2
15	0.40±0.04	0.41±0.17	3.64±0.13	3.55±0.17	3028.5±227.5	2728.3±211.6
30	0.37±0.01	0.40±0.14	3.54±0.12	3.57±0.32	3126.4±192.5	3031.8±173.2

The results indicate that the functional status markers of the liver and kidneys in birds, both before and during the administration of PFA, did not statistically vary from the control group and remained within physiological limits.

The levels of lipid peroxidation (LPO) in the blood serum of laying hens throughout the experiment are presented in Table 9. It was shown that prolonged administration of PFA did not result in substantial alterations in the intensity of lipid peroxidation processes, as evidenced by the levels of its products in the blood plasma of experimental hens. The results suggest that the levels of lipoperoxidation products, MDA and DC, in the blood of the experimental group of chickens, did not substantially differ from the control values.

The overproduction of membrane-altering hazardous byproducts from lipid peroxidation indicates an imbalance in the enzymatic and non-enzymatic components of the antioxidant system (AOS), which is crucial for the regulation and prognosis of cell membrane defence. The markers reflecting the condition of the enzymatic connection and overall AOS in the bodies of experimental laying hens were examined (Table 10).

Table 9

Intensity of lipid peroxidation in the blood serum of laying hens during 30-day exposure to PFA (M±m; n=10; (p<0,05) – versus control)

Period of sampling (days)	Intensity of LPO, products of lipid peroxidation			
	DC, μM/dm^3		MDA, ΔD	
	control	experiment	control	experiment
Before administration	26.06±1.23	24.92±2.16	5.92±0.42	5.86±0.26
15	24.88±2.32	25.68±2.40	6.00±0.37	6.23±0.48
30	25.58±1.18	24.74±1.42	6.22±0.60	6.18±0.42

The alterations in the condition of antioxidant resource levels in the natural pool, as shown by the total AOA metric in the bodies of experimental hens, were more significant due to the impact of PFA. From the 15th to the 30th day of the experiment, a rise in total AOA activity was observed, attributed to an increase in the AOA indicator by an average of 17.5% and 19.6% (p<0.05), alongside a reduction in catalase activity, which decreased by an average of 20.1% and 22.4% (p<0.05) compared to control values. The findings concerning the alterations in LPO/AOA indicators in experimental poultry demonstrate the regulatory influence of the probiotic supplement on LPO intensity levels and suggest the activation of adaptive and compensating mechanisms in the physiology of laying hens.

Based on the results of the clinical and biochemical experiments on laying hens, it can be stated that the proposed composition of probiotic bacteria is harmLess and its modulating effect on the state of metabolic and immune reactions in the body of the target bird does not cause side effects.

Table 10

Activity of the indices of antioxidant system in blood serum of laying hens during 30-day exposure to PFA (M±m; n=10; (p<0.05) – versus control)

Group of poultry	Period of study, days		
	Before administration	15	30
Catalase activity, nM H2O2/sec mg of protein			
Control	45.28±1.81	44.90±3.33	45.19±2.62
Experiment	45.32±1.74	**35.48±2.52***	**35.07±2.05***
Total AOA, % inhibition			
Control	48.5±2.8	49.7±4.3	48.1±3.7
Experiment	48.3±2.4	**58.4±3.0***	**57.5±2.3***

Conclusion. Analysis of bacteriological studies of the rectal contents of laying hens revealed that the administration of a probiotic feed additive (*Bacillus licheniformis* 2/6, *B. subtilis* 1/21, *L. delbrueckii* 1/5, and *Lactobacillus casei*) did not significantly alter the quantitative composition of the indicator microflora and did not adversely impact egg productivity and quality. Analysis of the clinical and biochemical study data indicates that PFA does not adversely affect the homeostasis of the bird. Conversely, the 30-day incorporation of PFA into the feeding regimen of laying hens induces modulation of metabolic and immune responses in the avian subjects, evidenced by the enhancement of cellular and humoral immune reactivity indicators, total antioxidant activity, and protein profile.

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ОЦІНКА ЕФЕКТИВНОСТІ ПРОБІОТИЧНОЇ КОРМОВОЇ ДОБАВКИ В ЕКСПЕРИМЕНТІ НА КУРЯХ-НЕСУЧКАХ

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***Резюме**. У статті представлено вплив пробіотичної кормової добавки на клініко-біохімічні показники курей-несучок в умовах аматорського приватного господарства з виробництва органічної продукції. Дослід проводили на курях-несучках віком 160 діб (n=200) породи леггорн яєчного напряму продуктивності, що утримувалися на підлозі на глибокій підстилці, масою 1500-1600 г. Перед початком досліду птицю було розподілено на групи по 100 голів за принципом аналогів. Після 14-денного періоду акліматизації до раціону дослідної групи додатково до повнораціонного комбікорму вводили пробіотичну добавку (L. casei, L. delbrueckii, B. licheniformis, B. subtilis у концентрації 106 КУО/мл кожна) у дозі 1,0 см³ на кг корму впродовж 30 днів. Контрольна група птиці отримувала повнораціонний комбікорм за нормами для курей-несучок. Перед введенням препаратів, а також на 15-ту та 30-ту добу після початку згодовування пробіотиків птицю забивали під підготовчим інгаляційним хлороформним наркозом (n=10). Зразки крові, а також зразки тканин та органів відбирали для мікробіологічного та біохімічного аналізів. Отримані результати свідчать про те, що застосування пробіотичних кормових добавок суттєво не змінило кількісний склад індикаторної мікрофлори. Проте спостерігалася тенденція (на 30-ту добу досліду) до збільшення вмісту Lactobacillus spp. та Bacillus spp. на 14,6% та 8,7% відповідно; виявлено також тенденцію до зменшення вмісту потенційно патогенних мікроорганізмів - Staphylococcus spp. на 19,5%, Candida spp. на 15,2%, Enterococcus spp. на 1,47%, E. coli на 13,0%. Застосування пробіотиків курям-несучкам призвело до значного підвищення рівня імуноглобуліну G у сироватці крові протягом усього досліду, причому пік концентрації IgG у дослідній групі спостерігався на 30-ту добу, продемонструвавши зростання на 11,3% (p<0,05). На 30-й день дослідження рівень імуноглобулінів M та A зріс на 14,6% та 10,2% (p<0,05) відповідно, а циркулюючих імунних комплексів - на 26,3%. Показники неспецифічної резистентності в дослідній групі курей мали тенденцію до зростання: фагоцитарний індекс, бактерицидна активність та активність лізоциму зросли на 11,5%, 12,0% та 13,9% відповідно. Клінічні та біохімічні результати дослідження свідчать про те, що запропонована композиція пробіотичних бактерій не є шкідливою для курей-несучок. Дані спостережень свідчать про відсутність негативного впливу на несучість.*

Ключові слова: пробіотики, мікрофлора, резистентність, антиоксидантна система, продуктивність

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