

INFLUENCE OF DRY MICROBIAL COMPOSITION BASED ON LACTOBACILLI STRAINS ON THE PHYSIOLOGICAL AND MICROBIOLOGICAL STATUS OF ROMANIAN PHEASANTS

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↳ Supporting Information



ABSTRACT: This study presents the results of evaluating the effects of a dry microbial composition based on lactobacilli strains (*Loigolactobacillus coryniformis* and *Lactobacillus johnsonii*), isolated from the intestinal tract of pheasants, on productivity, physiological status, and gut microbiocenosis of Romanian pheasants. The experiment was conducted on 140 birds divided into four groups: one control group and three experimental groups receiving the microbial composition at inclusion levels of 0.5–1.5% of the feed mixture. The trial duration was 91 days. Survival rate, body weight dynamics, feed intake, hematological and biochemical blood parameters were assessed, along with bacteriological analysis of the cecal contents. The results demonstrated that supplementation with the microbial composition at a dose of 1.0% yielded the most pronounced effects, including an increase in survival rate up to 100%, a statistically significant increase in body weight by 7.2%, and a reduction in feed conversion ratio by 4.2% compared to the control group. Hematological and biochemical analyses revealed no signs of toxicity or adverse effects of the microbial additive on the experimental birds. Microbiological analysis of the cecal chyme showed that administration of the microbial composition induced a restructuring of the gut microbiome, characterized by an increase in beneficial microbiota, namely *Lactobacillus* spp. (1.1–2.9-fold) and *Bifidobacterium* spp. (1.4–3.6-fold), accompanied by a reduction in opportunistic microorganisms. The study shows the efficacy of the developed dry microbial composition in Romanian pheasant production, with the optimal effect, at a dosage of 1.0% of the compound feed.

Keywords: Body weight, Dry microbial composition, Feed conversion ratio, Gut microbiota, Lactobacillus.

INTRODUCTION

Modern poultry production, including the breeding of commercial and ornamental bird species such as pheasants, requires the implementation of effective feeding strategies and approaches to maintaining flock health (Pennycott et al., 2012; Gheța et al., 2020). Romanian pheasants are valued both as a resource for hunting farms and for biodiversity enrichment; therefore, improving their productivity and viability remains an important goal (Gheța et al., 2020; Yakovets et al., 2022). One of the most promising approaches is the use of probiotic feed additives based on autochthonous microbial strains, which contribute to the optimization of gastrointestinal microbiota, enhancement of natural resistance, and improved nutrient digestibility (Maquiné et al., 2024; Idowu et al., 2025).

Attentions have been paid to microbial formulations containing lactobacilli strains isolated from the intestinal tract of birds (Betancur-Hurtado et al., 2022; Lysenko et al., 2025). Being adapted to the host species, such strains may exhibit more pronounced probiotic properties compared to allochthonous cultures. Their application can not only modulate gut microbiota but also exert systemic beneficial effects on growth performance, development, and overall physiological status of poultry (Xu et al., 2022, Sirisopapong et al., 2023). A comprehensive evaluation of their effects on productivity indicators and physiological status under controlled rearing conditions is therefore required (Fathima et al., 2022).

Despite extensive research on probiotics in poultry production, the optimal dosages, efficacy, and safety of complex microbial formulations based on autochthonous lactobacilli for pheasants remain insufficiently studied.

The aim of the present study was to evaluate the effects of a dry microbial composition based on a consortium of *Lactobacillus* strains isolated from the intestinal tract of pheasants on survival rate, growth performance, feed conversion ratio, hematological and biochemical blood parameters, as well as modulation of the gut microbiome of Romanian pheasants, in order to determine its optimal dosage and assess its efficacy and safety.

MATERIALS AND METHODS

Experimental studies on the target agricultural poultry were conducted at the Center for Preclinical Research of Veterinary Medicinal Products, while selected laboratory analyses, including microbiological assays, were performed at the Department of Veterinary Medicine of the Russian State Agrarian University – Moscow Timiryazev Agricultural Academy.

The object of the present study was a dry microbial preparation consisting of a consortium of two lyophilized lactobacilli strains, (*Loigolactobacillus coryniformis* VKM B-3724D and *Lactobacillus johnsonii* VKM B-3725D), combined in a 1:1 ratio. Both strains were originally isolated from the intestinal tract of pheasants and were uniformly dispersed throughout a prebiotic matrix composed of plant-derived polysaccharides. The biological model consisted of domesticated Romanian pheasants (Figure 1).

Ethical consideration

All experimental procedures involving vertebrate animals were approved by the Bioethics Commission of the Russian State Agrarian University – Moscow Timiryazev Agricultural Academy (Order No. 549 dated September 30, 2021; Protocol No. 65 dated November 14, 2025). All animal experiment was carried out in accordance with Directive 2010/63/EU (2010).



Figure 1 - Rearing of pheasants under laboratory conditions.

The experimental design is presented in Table 1. The duration of the experiment was 91 days. The experimental procedures in poultry were conducted in accordance with the guidelines developed by the All-Russian Research Institute of Poultry Farming (Egorov et al., 2013). The clinical status of birds in control and experimental groups was assessed daily by visual inspection, including evaluation of behavior, feather condition, feed intake, and drinking activity.

Body weight gain was monitored weekly by individual weighing using laboratory scales. Total weight gain over the experimental period was calculated as the difference between the initial body weight of day-old pheasants and their final body weight at 91 days of age. In cases of mortality, necropsy was performed to determine the cause of death, and survival rate was calculated for the entire experimental period. Feed intake was recorded daily, and feed conversion ratio (FCR) was calculated at the end of the experiment as the amount of compound feed consumed per 1.0 kg of body weight gain.

On day 91, morphological and biochemical blood analyses were performed. Biochemical parameters of blood serum were determined using an automated biochemical analyzer (Zybio EXC200) with veterinary reagent kits (DiaVetTest). The analysis of selected representatives of the intestinal microbiome in the cecal contents of pheasants was conducted at the end of the rearing period in accordance with established methodological guidelines (Ministry of Agriculture of the Russian Federation, 2004).

Table 1 - Experimental design for evaluating the effects of a dry microbial composition on Romanian pheasants

Group	Number of birds	Feeding and watering conditions
Control	35	Compound feed (CF) + drinking water
Experimental 1	35	CF + microbial composition at 0.5% of feed weight + drinking water
Experimental 2	35	CF + microbial composition at 1.0% of feed weight + drinking water
Experimental 3	35	CF + microbial composition at 1.5% of feed weight + drinking water

Statistical analysis

The obtained data were processed using standard methods of mathematical statistics applied in biology and medicine, employing Microsoft Office Excel 2019 software. Statistical significance was assessed using Student's *t*-test, and differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The results on survival rate, body weight, weight gain, feed intake, and feed conversion ratio (FCR) in pheasants are presented in Table 2 and Figure 2.

At the end of the experiment, the survival rate was 85.7% in the control group, 94.3% in experimental group 1, 100.0% in experimental group 2, and 97.1% in experimental group 3. Compared to the control, survival rate increased by 8.6%, 14.3%, and 11.4%, respectively. At the beginning of the experiment (day 1), the body weight of chicks was comparable across all groups. From day 7 onward, pheasants in experimental groups 2 and 3 showed significantly higher body weight compared to the control (by 4.4% and 5.7%, respectively; $P \leq 0.05$). Starting from day 14, all experimental groups demonstrated a statistically significant increase in body weight relative to the control. This trend persisted throughout the entire experimental period.

By day 21, body weight exceeded the control by 8.7%, 13.8%, and 20.1% in experimental groups 1, 2, and 3, respectively ($P \leq 0.001$). Similar patterns were observed at subsequent time points (days 28–84), with consistently higher values in all experimental groups. By the end of the experiment (day 91), the body weight in the control group reached 972.50 g, whereas it was significantly higher in experimental group 1 (998.17 g; +2.6%), experimental group 2 (1042.08 g; +7.2%), and experimental group 3 (1026.10 g; +5.5%) ($P \leq 0.001$). The average body weight gain over the experimental period was 950.21 g in the control group, compared to 975.32 g, 1019.56 g, and 1004.61 g in experimental groups 1, 2, and 3, respectively. This corresponds to an increase of 2.64%, 7.29%, and 5.72% relative to the control.

The average feed intake per bird was 7431 g in the control group and 7549 g, 7645 g, and 7812 g in experimental groups 1, 2, and 3, respectively, exceeding the control values by 1.59%, 2.88%, and 5.13%. Feed conversion ratio (FCR) was 7.82 kg/kg in the control group and decreased to 7.74, 7.49, and 7.77 kg/kg in experimental groups 1, 2, and 3, respectively, corresponding to improvements of 1.02%, 4.22%, and 0.64%.

The results of hematological and biochemical blood analyses (Table 3) indicated no adverse effects of the microbial composition on the physiological status of pheasants. Morphological blood parameters remained within ranges comparable to the control group, with no statistically significant deviations. The erythrocyte count in the control group was $2.49 \times 10^{12}/L$, while in the experimental groups it increased by 4.0–8.0%, reaching a maximum of $2.69 \times 10^{12}/L$ in experimental group 3. Hemoglobin concentration ranged from 92.07 g/L in the control group to 94.61 g/L in experimental group 3 (+2.8%). Leukocyte levels increased slightly from $17.69 \times 10^9/L$ in the control group to $18.12 \times 10^9/L$ in experimental group 3 (+0.9–2.4%). Hematocrit values varied between 33.81% and 35.24%, with no statistically significant differences between groups. These findings are in agreement with Wang et al. (2023) on Lingnan yellow chicken which increases specific immunoglobulin level after supplementation of *Lactobacillus plantarum*. Also, present findings are in agreement with Akter et al. (2023) reports for catfish which increases in blood cell, white blood cell and lymphocyte counts were observed in *Lactobacillus acidophilus* supplemented group.

The analysis of the leukocyte differential revealed no pathological changes. The proportion of lymphocytes in the control group was 65.16%, while in the experimental groups it ranged from 65.94% to 66.75%, showing a slight increase compared to the control. The monocyte content decreased from 9.74% in the control group to 8.63–8.87% in the experimental groups. The proportion of heterophils remained consistent across all groups (24.58–25.20%). Eosinophils and basophils were not detected in any samples.

The biochemical profile of blood serum also showed no statistically significant differences between groups. Alanine aminotransferase (ALT) activity was 11.76 U/L in the control group and ranged from 11.69 to 13.28 U/L in the experimental groups. Aspartate aminotransferase (AST) activity varied between 177.14 and 182.08 U/L, remaining close to the control value (178.48 U/L). Alkaline phosphatase levels were comparable across all groups (2470.91–2493.10 U/L). Serum cholesterol concentration slightly decreased in the experimental groups (2.99–3.03 mmol/L) compared to the control (3.10 mmol/L). A similar trend was observed for glucose, which decreased from 17.96 mmol/L in the control group to 17.63–17.70 mmol/L in the experimental groups. In contrast, total protein content increased from 29.76 g/L in

the control group to 30.24–31.19 g/L in the experimental groups, with the highest value recorded in experimental group 3. Total bilirubin levels ranged from 6.66 to 7.13 μmol/L, remaining within the control range. In the study of Eglite et al. (2023) serum total cholesterol decreased following supplementation of *Lactobacillus farciminis* and *Lactobacillus rhamnosus*. Present finding on pheasants is in agreement with Eglite et al. (2023) report.

Mineral metabolism parameters were also comparable across groups, with no statistically significant differences. Calcium levels ranged from 2.34 to 2.48 mmol/L, while phosphorus concentrations increased slightly from 1.94 mmol/L in the control group to 2.01–2.05 mmol/L in the experimental groups. Overall, the use of the dry microbial composition did not adversely affect hematological or biochemical blood parameters in pheasants. The observed trends indicate a normal physiological status of the birds. The results of the intestinal microbiota analysis in pheasants are presented in Figure 3.



Figure 2 - Weighing of pheasants using laboratory scales

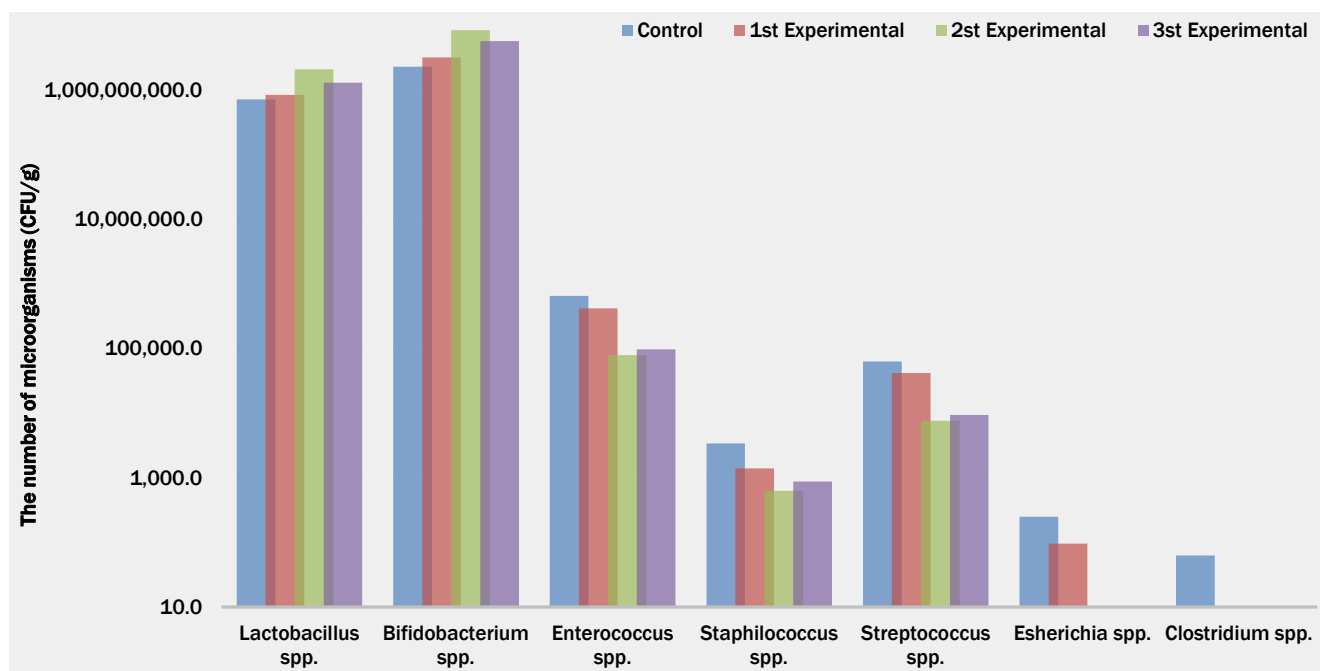
Table 2 - Survival rate, body weight dynamics, weight gain, and feed utilization in Romanian pheasants (n = 35, M ± m)

Parameter	Control	Experimental 1	Experimental 2	Experimental 3
Survival rate, %	85.7	94.3	100.0	97.1
Relative to control, %	-	+8.6	+14.3	+11.4
Body weight dynamics, g (M ± m)				
Day 1	22.29±0.33	22.85±0.33	22.52±0.32	21.49±0.32
Day 7	39.24±0.49	39.98±0.51	40.97±0.48*	41.46±0.50**
Day 14	85.43±0.56	87.74±0.46**	92.41±0.93***	95.67±0.90***
Day 21	133.72±1.16	145.31±1.25***	152.14±1.48***	160.59±1.35***
Day 28	192.08±2.08	209.13±2.24***	217.06±1.80***	224.58±2.20***
Day 35	335.59±2.33	343.49±2.17*	354.09±2.14***	353.42±2.21***
Day 42	432.38±1.68	438.64±1.63*	450.16±1.57***	457.70±1.63***
Day 49	512.47±1.83	525.86±1.88***	537.90±2.54***	546.87±2.64***
Day 56	614.12±2.16	621.58±2.49*	647.76±2.02***	657.11±2.71***
Day 63	700.36±3.47	711.56±2.89*	731.90±3.00***	739.13±2.32***
Day 70	760.13±3.30	771.30±3.21*	786.87±2.42***	793.64±3.31***
Day 77	807.70±3.77	824.12±3.05*	850.19±3.53***	862.61±4.12***
Day 84	905.71±3.13	925.72±3.22***	948.25±3.48***	954.11±3.94***
Day 91	972.50±4.15	998.17±4.79***	1042.08±5.36***	1026.10±6.25***
Body weight gain over the rearing period (Day 0–91)				
Average per bird, g	950.21	975.32	1019.56	1004.61
Relative to control, %	-	+2.64	+7.29	+5.72
Feed intake over the rearing period (Day 0–91)				
Average per bird, g	7431	7549	7645	7812
Relative to control, %	-	+1.59	+2.88	+5.13
FCR, kg/kg	7.82	7.74	7.49	7.77
Relative to control, %	-	-1.02	-4.22	-0.64

Note: Differences compared to the control group are statistically significant at * – P≤0.05, ** – P≤0.01, *** – P≤0.001.

Table 3 - Hematological and biochemical parameters of Romanian pheasants (n = 10, M ± m)

Parameter	Control	Experimental 1	Experimental 2	Experimental 3
Hematological parameters				
Erythrocytes, $\times 10^{12}/L$	2.49±0.07	2.59±0.08	2.66±0.09	2.69±0.08
Hemoglobin, g/L	92.07±0.92	92.47±1.19	94.40±0.78	94.61±0.88
Leukocytes, $\times 10^9/L$	17.69±0.21	17.88±0.15	17.85±0.16	18.12±0.15
Hematocrit, %	34.17±0.91	33.81±0.53	35.24±0.57	35.04±0.73
Lymphocytes	65.16±0.69	66.23±0.96	66.75±0.64	65.94±1.08
Monocytes	9.74±0.96	8.63±1.35	8.67±0.85	8.87±1.06
Heterophils	25.20±0.67	25.14±0.79	24.58±0.85	25.19±0.65
Eosinophils	0	0	0	0
Basophils	0	0	0	0
Biochemical parameters				
ALT, U/L	11.76±0.56	13.28±0.53	11.69±0.32	12.40±0.47
AST, U/L	178.48±3.33	181.11±2.43	177.14±3.48	182.08±3.01
Alkaline phosphatase, U/L	2491.57±58.19	2470.91±62.56	2493.10±60.15	2480.60±61.20
Cholesterol, mmol/L	3.10±0.12	3.03±0.13	3.01±0.11	2.99±0.16
Glucose, mmol/L	17.96±0.23	17.66±0.29	17.70±0.18	17.63±0.14
Total protein, g/L	29.76±0.59	30.24±0.58	30.86±0.84	31.19±0.55
Total bilirubin, $\mu\text{mol}/L$	6.83±0.23	6.66±0.21	6.94±0.20	7.13±0.19
Calcium, mmol/L	2.36±0.07	2.47±0.07	2.34±0.09	2.48±0.06
Phosphorus, mmol/L	1.94±0.05	2.04±0.03	2.05±0.05	2.01±0.03

**Figure 3 - Microbiological analysis of cecal contents in Romanian pheasants (n = 5, M ± m)**

The results of the microbiological analysis of cecal contents demonstrated a pronounced effect of the microbial composition on both the quantitative and qualitative structure of the intestinal microbiome. Similar findings are reported for other bird species such as Guinea Fowls (Galosi et al., 2021) and Shaoming duck (Sun et al., 2022). In all experimental groups, a significant increase in indigenous microbiota was observed. The abundance of *Lactobacillus* spp. increased by 1.1-fold in experimental group 1 ($P \leq 0.01$), 2.9-fold in experimental group 2 ($P \leq 0.001$), and 1.8-fold in experimental group 3 ($P \leq 0.001$) compared to the control group. Similarly, *Bifidobacterium* spp. increased by 1.4-fold ($P \leq 0.05$), 3.6-fold ($P \leq 0.001$), and 2.4-fold ($P \leq 0.001$) in experimental groups 1, 2, and 3, respectively.

A key outcome of the application of the dry microbial composition was a statistically significant reduction in opportunistic microorganisms. This effect is likely associated not only with the probiotic activity of the lactobacilli strains included in the formulation but also with the increased abundance of beneficial microbiota (*Lactobacillus* spp. and *Bifidobacterium* spp.), which are known to produce biologically active compounds with antagonistic activity against opportunistic and pathogenic microorganisms in the gastrointestinal tract.

Specifically, the abundance of *Enterococcus* spp. decreased by 1.5-fold in experimental group 1 ($P \leq 0.01$), 8.2-fold in experimental group 2 ($P \leq 0.001$), and 6.7-fold in experimental group 3 ($P \leq 0.001$) compared to the control. A significant reduction in *Staphylococcus* spp. was also observed: from $3.4 \pm 0.1 \times 10^3$ CFU/g in the control group to $1.4 \pm 0.2 \times 10^3$ CFU/g, $6.3 \pm 0.3 \times 10^2$ CFU/g, and $8.8 \pm 0.2 \times 10^2$ CFU/g in experimental groups 1, 2, and 3, respectively, corresponding to decreases of 2.4-fold ($p \leq 0.05$), 5.4-fold, and 3.8-fold ($P \leq 0.001$). The level of *Streptococcus* spp. in the cecal chyme of the control group ($6.3 \pm 0.4 \times 10^4$ CFU/g) was significantly higher than in the experimental groups by 1.5-fold ($P \leq 0.05$), 8.2-fold, and 6.7-fold ($P \leq 0.001$), respectively. The findings are in agreement with Perera et al. (2024) for preventing microbial infection in birds (by supplemented special probiotic), and also in agreement with Saeed et al. (2025) for evaluating new Lactiplantibacillus based nutritional strategies. Notably, complete elimination of certain opportunistic and pathogenic microorganisms, including *Escherichia* spp. and *Clostridium* spp., was observed in experimental groups 2 and 3. In the control group, these microorganisms were detected, while in experimental group 1, *Escherichia* spp. were present but at levels 2.6-fold lower than in the control ($P \leq 0.05$). Representatives of *Pseudomonas* spp. were not detected in any of the groups.

CONCLUSION AND RECOMMENDATION

The results of the present study demonstrate that the inclusion of a dry microbial composition in the diet of Romanian pheasants exerts a positive effect on productivity, physiological status, and modulation of the intestinal microbiota. The most pronounced effects were observed in the group receiving the microbial additive at a dosage of 1.0% of compound feed (experimental group 2), where survival rate reached 100%, final body weight increased by 7.2% ($P \leq 0.001$), body weight gain increased by 7.3%, and feed conversion ratio improved by 4.2% compared to the control. Additionally, the abundance of beneficial microbiota, particularly *Lactobacillus* spp. and *Bifidobacterium* spp., increased by 2.9- and 3.6-fold, respectively ($P \leq 0.001$). Hematological and biochemical blood parameters in all experimental groups remained within the physiological range and did not differ significantly from the control values, confirming the safety and good tolerability of the microbial composition. Overall, the developed dry microbial composition is recommended for practical application in Romanian pheasant production at a dosage of 1.0% of compound feed, as it ensures improved production performance with reduced feed costs.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

A.V. Luneva, Y.A. Lysenko, and E.Y. Marchenko contributed to the research, data analysis, and manuscript writing.

Conceptualization, data curation and funding acquisition: A.V. Luneva.

Formal analysis: Y.A. Lysenko, E.Y. Marchenko.

Methodology: Y.A. Lysenko, E.Y. Marchenko.

Resources, Software and Visualization: E.Y. Marchenko.

Supervision: A.V. Luneva, Y.A. Lysenko.

Writing – original draft: A.V. Luneva, Y.A. Lysenko, E.Y. Marchenko.

Investigation, Project administration, Validation, Writing – review & editing: Y.A. Lysenko.

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Competing interests

The authors declare no competing interests in this research and publication.

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