

The role of probiotics in the growth performance and health of poultry

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Abstract

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The present study examines the effects of Zoovit LL probiotic supplementation on growth performance, feed consumption, feed conversion, and key innate immunity parameters in broiler chickens. Specifically, the study evaluates the Alternative Pathway of Complement Activation (APCA), serum lysozyme concentrations, and beta-lysine activity. The experiment was conducted using male ROSS hybrid chicks reared on the floor for 40 days. The birds were divided into two groups: a control group, which received no probiotic supplementation, and an experimental group, which was fed a probiotic-supplemented diet. Probiotic supplementation significantly improved weight gain and feed conversion throughout the 40-day rearing period. A notable increase in APCA activity was observed in the probiotic-fed group compared to controls ($P < 0.001$), with mean values of 586.93 ± 9.26 CH50 versus 520.98 ± 6.78 CH50, respectively. Serum lysozyme levels were also significantly higher in the experimental group ($P < 0.01$), reaching 1.01 ± 0.09 $\mu\text{g/ml}$ compared to 0.61 ± 0.08 $\mu\text{g/ml}$ in controls. Although beta-lysine activity was slightly elevated in the probiotic-fed group ($36.19 \pm 2.96\%$) relative to controls ($32.57 \pm 1.61\%$), the difference was not statistically significant ($P > 0.05$). These findings suggest that Zoovit LL probiotic supplementation has immunomodulatory effects, enhancing key components of the innate immune system in broiler chickens. The observed increase in APCA and lysozyme activity highlights the potential of probiotic supplementation to strengthen immune defenses, particularly in antibiotic-free poultry production systems.

Keywords: probiotics; broiler chickens; growth performance; APCA; lysozyme; beta-lysines

Introduction

In 2006, the European Union (EU) fully banned the use of antibiotics in animal feed to reduce the risk of antibiotic resistance in pathogenic microorganisms (European Union Commission, 2006). Since then, extensive research has been conducted on alternative feed additives to improve the productivity and efficiency of livestock, including poultry.

The effects of probiotic supplementation in poultry production have been studied for decades (Nurmi and Rantala, 1973; Patterson and Burkholder, 2003; Brisbin et al. 2008;

Gadde et al., 2017; Hack et al., 2020; Shini and Bryden, 2022; Ayana and Kamutambuko, 2024). Probiotics have demonstrated beneficial effects on growth performance, gut microbiota modulation, pathogen inhibition, intestinal histological changes, immune modulation, and certain hematobiochemical parameters (Mountzouris et al., 2007; Apata, 2008; Ashayerizadeh et al., 2009; Pieniz et al., 2014; Zhang and Kim, 2014; Abdurrahman et al., 2016; Roy and Khatun, 2020; Zhang et al., 2021). The poultry industry is increasingly focusing on enhancing natural immunity parameters in broiler chickens due to concerns regarding antibiotic resist-

ance and the need for sustainable farming practices (Gadde et al., 2017).

Key components of innate immunity, such as the Alternative Pathway of Complement Activation (APCA), lysozyme, and beta-lysins, play a crucial role in protecting against pathogens, thereby reducing reliance on antimicrobial agents in intensive farming systems (El-Ratel et al., 2024). These factors are essential for maintaining poultry health and productivity, especially under high-density rearing conditions, where disease outbreaks can have significant economic consequences (Abdel-Raheem et al., 2012).

Probiotics offer a viable alternative to antibiotic growth promoters, aligning with global initiatives aimed at reducing antimicrobial use in agriculture and mitigating associated risks, such as the emergence of resistant pathogens (Gadde et al., 2017). Their use supports sustainable poultry production by promoting gut health, reducing disease incidence, and improving overall productivity (Abdel-Raheem et al., 2012).

This study investigates the effects of a specific probiotic on broiler growth performance and innate immune parameters, emphasizing its potential role in enhancing natural defense mechanisms and contributing to sustainable poultry health management.

Materials and Methods

Animals, experimental design, and diets

This study was conducted between September 12 and November 22, 2024, at the Experimental Facility of the Agricultural Institute – Stara Zagora. The experiment involved 120 one-day-old male broiler chickens of the ROSS hybrid, reared on a floor-based system for a duration of 40 days. The birds were randomly assigned to two experimental groups, each consisting of three subgroups with 20 birds per subgroup, following the scheme below:

- Group I (Control) – received a standard compound feed without probiotics.
- Group II (Experimental) – received a standard compound feed supplemented with the probiotic Zoovit LL.

All birds were fed balanced, isoenergetic and isocaloric compound feeds formulated according to the nutritional requirements of the ROSS hybrid. Optimal microclimatic conditions were maintained uniformly across all groups (Table 1). Feed and water were provided *ad libitum*.

The study monitored body weight, feed intake, and feed conversion ratio (FCR). Individual body weight was recorded on days 1, 14, 28, and 40 using a precision scale with an accuracy of 1 g. Weighing was performed in the morning, following a consistent sequence for all groups. Based on

these measurements, absolute weight gain was calculated for each period. Feed intake was measured over three distinct periods:

- Day 1 to Day 14
- Day 15 to Day 28
- Day 29 to Day 40

The feed conversion ratio (FCR) was determined by relating total feed consumption to weight gain over the corresponding periods.

Table 1. Composition and nutritional value of compound feeds

Ingredients (%)	Compound feed		
	Starter 1–14 day	Grower 15–28 day	Finisher 28–40 day
Wheat	25.440	22.890	28.000
Corn	30.000	36.000	35.920
Soybean meal	35.000	31.000	24.000
Sunflower meal	3.000	3.000	3.000
Sunflower oil	3.000	4.000	6.000
DL-methionine	0.170	0.130	0.080
Lysine-98%	0.240	0.130	0.090
Optizim	0.150	0.150	0.150
Salt	0.200	0.200	0.200
Limestone	0.500	0.440	0.490
Dicalcium phosphate	2.100	1.860	1.870
Vitamin premix	0.200	0.200	0.200
Nutritional value:			
Metabolizable energy (kcal/kg)	2.914	3.018	2.982
Crude protein, %	22.05	20.54	18.02
Crude fiber, %	4.35	4.11	3.74
Lysine, %	1.44	1.23	1.01
Methionine, %	0.51	0.46	0.37
Calcium, %	1.02	0.90	0.90
Phosphorus, available, %	0.50	0.45	0.45

Source: Authors' own elaboration

Probiotic supplementation

The probiotic supplement used in this study, Zoovit LL, was produced by LB Lact BAS Laboratory. The composition of the probiotic included the following bacterial strains, with their respective concentrations:

- *Lactobacillus delbrueckii subsp. bulgaricus* – 3.5×10^7 CFU/mL
 - *Lactobacillus rhamnosus* – 2.1×10^7 CFU/mL
 - *Lactobacillus casei* – 1.8×10^7 CFU/mL
 - *Streptococcus thermophilus* – 2.9×10^8 CFU/mL
- Additionally, probiotic yeast was also included:
- *Kluyveromyces lactis* – 3.0×10^5 CFU/mL

The probiotic supplement was added daily to the feed of the experimental group for a duration of 40 days at a dose of 250 g per 100 kg of feed, as recommended by the manufacturer. Neither the control nor the experimental group received antibiotics or any other pharmaceutical treatments throughout the trial period.

Blood sampling

Blood samples were collected from broiler chickens using plain vacutainers without anticoagulant, ensuring a sample volume of approximately 3 ml per bird. The selected site for venipuncture was the wing vein (*vena cutanea ulnaris*). Immediately after collection, samples were placed in cool bags to maintain their integrity during transportation to the laboratory. Upon arrival, blood samples were centrifuged at 3000 revolutions per minute (rpm) to separate the serum from cellular components, facilitating the subsequent analysis of immune parameters.

Alternative pathway of complement activation

The assessment of the alternative pathway of complement activation (APCA) was conducted according to the method described by Sotirov et al. (2021). Each serum sample was initially diluted by mixing 100 µl of serum with 350 µl of veronal-veronal Na buffer, resulting in final concentrations of 146 mM NaCl, 1.8 mM 5.5-diethylbarbituric acid sodium salt, 3.2 mM 5.5-diethylbarbituric acid, 1 mM EGTA, and 0.8 mM MgCl₂. Serial dilutions of the prepared serum were made in U-bottomed plates (Flow Laboratories, UK) in seven steps, including serum-to-buffer ratios of 8/45, 7/45, 6/45, 5/45, 4/45, 3/45, and 2/45. Next, 50 µl of buffer and 100 µl of a 1% rabbit erythrocyte suspension were added to each well. The plates were incubated for 1 hour at 37°C, followed by centrifugation at 150 G for 3 minutes at room temperature (23°C). After centrifugation, 150 µl of the supernatant was transferred to flat-bottomed plates, and optical density was measured at 540 nm using a “Sumal-PE2” ELISA reader (Karl Zeiss, Germany).

Blood serum lysozyme

Lysozyme concentrations in blood serum were determined using the method described by Sotirov et al. (2011), with modifications to enhance precision. A 2% agarose solution was prepared in phosphate buffer (0.07 M NaH₂PO₄ and NaH₂PO₄) and mixed with a suspension of a 24-hour culture of *Micrococcus lysodeikticus* at 67°C. This mixture was poured into 14-cm Petri dishes and allowed to solidify at room temperature. Once solidified, 5-mm wells were created in the agarose using a specialized device. A volume of 50 µl of undiluted serum from each sample was pipetted into

individual wells. Additionally, eight standard lysozyme dilutions (ranging from 0.025 to 3.125 µg/ml) were prepared and loaded into separate wells for reference. The plates were incubated at 37°C for 20 hours to allow lysozyme activity to manifest. The diameters of the lytic zones were then measured, and lysozyme concentrations were calculated using specialized software developed at Trakia University, ensuring precise quantification.

Beta-lysine activity

Beta-lysine activity was measured using a spectrophotometric method, as described by Karakolev and Nikolov (2015), with improvements for accuracy. Flat-bottomed plates were used for optimal optical density measurement. A prepared spore suspension of *Bacillus subtilis* ATCC 6633 was used for the assay. To establish controls, 80 µl of saline solution and 80 µl of spore suspension were added to four initial wells. Experimental sera were then added to additional wells, with each well receiving 80 µl of serum and 80 µl of spore suspension. The plates were homogenized using a plate shaker and pre-incubation optical density readings were taken at 630 nm using a BioTek L80 spectrophotometer. The plates were subsequently incubated at 37°C for 2 hours, followed by a second optical density reading. Beta-lysine activity was calculated using the formula:

$$\% \text{ of lysis} = (\text{OD1} - \text{OD2}) / \text{OD1} \times 100$$

where: OD1 represents the optical density before incubation and OD2 represents the optical density after incubation. Since control optical densities remained stable, changes in sample optical densities were used to determine the percentage of lysis.

Statistical analyses

The data on the productivity indicators were statistically processed with the IBM SPSS Statistics 19 software, and the application of the Independent Samples t-test for comparison of two independent groups. A check was performed for homogeneity of variances and normal distribution of the individual variables. When rejecting the null hypothesis, a non-parametric analogue of the Independent Samples t-test was performed – the Mann-Whitney test. Differences at $P < 0.05$ are considered statistically significant, and a trend towards significance is assumed at $P < 0.10$. Prior to statistical analysis, the dataset of the innate immunity parameters, was evaluated for normality using the Shapiro-Wilk test and for homogeneity of variances using the Levene test. Descriptive statistics, including mean values $\pm$ standard error, were calculated for each variable. A two-way analysis of variance (ANOVA) was conducted using the General Linear Model

procedure to assess the effects of different water types as the main factor. Post-hoc comparisons were performed using Bonferroni adjustment to correct for multiple testing. A significance threshold of $P < 0.05$ was used. All statistical analyses were performed using SPSS version 14.01 to ensure rigorous data interpretation.

Results

Growth performance

The statistically processed data on broiler body weight from the experiment are presented in Table 2. No significant differences in live weight were observed between the control and experimental groups from day 1 to day 28 of the study. However, during this period, the birds receiving the probiotic-supplemented feed exhibited a slight advantage. On day 28, the average body weight of the probiotic group was 1075.58 g, compared to 1058.24 g in the control group, though this difference was not statistically significant ($P > 0.05$).

By the end of the experiment, at 40 days of age, broilers in the experimental group showed a significantly higher final body weight of 2313.48 g, compared to 2130.84 g in the control group ($P < 0.001$). Mortality was recorded exclusively in the control group throughout the trial. On day 28, two birds from the control group (out of 60) were lost, resulting in a 3.33% mortality rate. In contrast, no mortality was observed in the probiotic-supplemented group.

Table 2. Body weight development (g) of the broilers (Mean±SEM)

Age in day/Group	Control	Experimental
1 day	45.95±0.51	45.82±0.41
14 day	292.77±7.20	284.97±5.94
28 day	1058.24±26.89	1075.58±19.23
40 day	2130.84±39.47	2313.48±32.51***

SEM, standard error of the mean

*** – $P < 0.001$

Source: Authors' own elaboration

Data on absolute weight gain, feed intake, and feed conversion ratio (FCR) per bird are summarized in Table 3. Minor differences in these parameters were observed between the two groups from day 1 to day 28. Although not statistically significant, feed intake was consistently higher in the control group across all three feeding phases – starter, grower, and finisher.

As shown in Table 3, during the final phase (days 28 to 40), probiotic supplementation had a positive effect on weight gain. Broilers fed Zoovit LL had a significantly higher relative weight gain (1237.90 g) compared to the control group (1072.60 g) ($P < 0.001$).

Over the entire 40-day period, the absolute weight gain in the experimental group was 177.99 g higher than in the control group ($P < 0.001$).

Table 3. Weight gain, feed consumption and feed conversion of the broilers (average for the period for 1 chicken) (Mean±SEM)

Items	Group	
	Control	Experimental
1–14 day		
Weight gain, g	246.82±7.112	239.15±5.82
Consumption, g	370.35±7.37	342.41±17.95
Feed conversion ratio, g/g	1.50±0.05	1.44±0.13
15–28 day		
Weight gain, g	764.17±21.22	790.62±14.99
Consumption, g	2140.14±6156	2120.70±81.06
Feed conversion ratio, g/g	2.83±0.27	2.69±0.15
29–40 day		
Weight gain, g	1072.60±20.56	1237.90±19.30***
Consumption, g	2124.73±117.46	2109.70±49.40
Feed conversion ratio, g/g	1.98±0.13	1.70±0.038
1–40 day		
Weight gain, g	2084.38±39.41	2262.37±32.14***
Consumption, g	4635.20±141.19	4572.81±33.83
Feed conversion ratio, g/g	2.23±0.14	2.02±0.04

SEM, standard error of the mean

*** – $P < 0.001$

Source: Authors' own elaboration

Immunity parameters

The statistically processed data for the three investigated natural immune parameters are presented on Table 4. Results indicate that APCA activity in the experimental group was significantly higher ($P < 0.001$) compared to the control group (586.93±9.26 CH50 vs. 520.98±6.78 CH50, respectively). Serum lysozyme levels were also significantly elevated in the experimental group ($P < 0.01$) compared to the control group (1.01±0.09 µg/ml vs. 0.61±0.08 µg/ml, respectively). Although beta-lysine levels were slightly higher in the experimental group, the difference was not statistically significant ($P > 0.05$; 36.19±2.96% vs. 32.57±1.61%, respectively).

Table 4. Influence of Probiotic Zoovit LL to innate immunity parameters of the broiler chickens. (Mean±SEM)

Items/ Group	Control	Experimental
APCA(CH50)	520.98±6.78	586.93±9.26***
Lisozyme(µg/ml)	0.61±0.08	1.01±0.09**
Beta-lysines (%)	32.57±1.61	36.19±2.96

SEM, standard error of the mean

*** – $P < 0.001$; ** – $P < 0.01$

Source: Authors' own elaboration

Discussion

The current experiment demonstrated a positive effect of probiotic supplementation on live weight and weight gain in broilers. Throughout the entire experimental period, from day 1 to day 40, the processed data on absolute daily weight gain showed a consistent advantage for the experimental group compared to the control group. However, probiotic inclusion had no statistically significant effect on feed intake across the different feeding phases or on the feed conversion ratio (FCR) in this study.

Numerous researchers have reported the beneficial effects of probiotic supplementation on body weight and feed efficiency. Sri-Harimurti et al. (2013) found that a probiotic mixture containing *Lactobacillus murinus* Ar3, *Streptococcus thermophilus* Kd2, and *Pediococcus* significantly improved live weight. Similarly, Lukanov et al. (2018) investigated the impact of a combination of *Lactobacillus* spp. probiotic and garlic powder on male broilers and observed that probiotic supplementation enhanced weight gain. Other authors, including Abaza et al. (2008), Midilli et al. (2008), and Ignatova et al. (2009), have also reported positive effects of probiotic additives on poultry performance. The mechanism of action of probiotic microorganisms involves inhibiting pathogenic activity through nutrient competition, producing antimicrobial compounds (such as volatile fatty acids and pH reduction), competing for binding sites on the intestinal epithelium, stimulating immune system function, and ultimately enhancing productivity (Pan and Yu, 2014).

The present study evaluated the impact of Probiotic Zoovit LL supplementation on innate immune parameters in broiler chickens, specifically focusing on the alternative pathway of complement activation (APCA), serum lysozyme levels, and beta-lysine activity. The findings revealed significant enhancements in both APCA activity and serum lysozyme concentrations in the experimental group compared to the control group, while beta-lysine activity showed a non-significant increase.

The observed increase in APCA activity in the experimental group suggests that Probiotic Zoovit LL may potentiate the complement system's alternative pathway, a crucial component of innate immunity responsible for the rapid identification and neutralization of pathogens. This enhancement aligns with previous research indicating that probiotic supplementation can modulate immune responses in poultry. For instance, Ahmat et al. (2021) reported that *Bacillus amyloliquefaciens* supplementation alleviated immunological stress in broilers, leading to improved immune function.

In terms of serum lysozyme levels, the experimental group exhibited a significant increase compared to the control group. Lysozyme is an essential enzyme in avian innate immunity, contributing to the degradation of bacterial cell walls and thus providing a first line of defence against infections. This finding is consistent with Abdel-Latif et al. (2017), who demonstrated that dietary lysozyme supplementation enhanced growth performance and gut health in broiler chickens by modulating gut microbiota and upregulating genes involved in gut antioxidant status and nonspecific immunity.

Although beta-lysine activity was higher in the experimental group, the difference was not statistically significant. Beta-lysins are cationic proteins with bactericidal properties, playing a role in the innate immune response. The lack of significant change in beta-lysine activity may suggest that Probiotic Zoovit LL's immunomodulatory effects are more pronounced in other components of the innate immune system, such as the complement pathway and lysozyme production.

Comparing these results with similar studies, it is evident that probiotic supplementation can have varying effects on different immune parameters in poultry. For example, Sadeghi et al. (2015) observed that environmental conditions significantly influenced the efficacy of probiotic strains in modulating immune responses in broilers. Additionally, Yitbarek et al. (2012) found that multi-strain probiotics provided immune modulation by upregulating cytokine expression in the ileum of chickens, indicating a complex interaction between probiotics and the host immune system.

Conclusion

In conclusion, supplementation with Zoovit LL in broiler chickens appears to enhance growth performance and specific innate immune parameters, notably APCA activity and serum lysozyme levels. These findings contribute to the growing body of evidence supporting the immunomodulatory potential of probiotics in poultry production. Further research is warranted to elucidate the mechanisms underlying these effects and to determine the optimal conditions for probiotic efficacy.

Conflicts of interest

The authors have no conflicts of interest to declare.

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