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# Biotechnological Feed Additives: Alternatives to Antibiotics as Growth Promoters in Broiler Chicken Farming

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**ABSTRACT**

The restriction of antibiotic growth promoters in poultry production has grown due to concerns about antimicrobial resistance and food safety. Phytogenic and probiotic feed additives are considered promising candidates for modulating gut health, immune responses, and growth performance in broiler chickens. The present study aimed to assess the effects of dietary supplementation with the probiotic *Kluyveromyces marxianus* strain (KluActive), either alone or in combination with encapsulated hydrolyzable tannins, on innate immune responses and growth performance in antibiotic-free-fed broiler chickens. Sixty broiler chickens, each 15 days old at the start, were used in a 27-day experiment. Broiler chickens were randomly assigned to four dietary treatments, including a control group fed a standard antibiotic-free diet (G1), a group that was fed the standard diet with KluActive at 0.04% (G2), and two groups fed a combination of KluActive with encapsulated tannins at 0.04% (G3) and 0.06% (G4). Phagocytic activity, phagocytic index, phagocytic number, serum bactericidal activity, and serum lysozyme activity were assessed on days 28 and 42. Growth performance parameters, including average daily gain and average body weight gain, were recorded over 42 days. Supplementation with the probiotic and tannins enhanced selected serum innate immune parameters in broiler chickens. In particular, serum bactericidal activity was significantly increased at the higher inclusion level (0.6%), by 12.5% on day 28 and 16.7% on day 42 compared with the control group. Phagocytic activity parameters were consistently elevated in broiler chickens across all experimental groups. The best improvement in feed conversion ratio was observed in the G3 group. The current findings indicated that probiotic-tannin combinations were a promising nutritional alternative to enhance immune function and performance in antibiotic-free broiler chicken production.

**Keywords:** Growth performance, Immune response, Probiotic, Tannin, Yeast

## INTRODUCTION

The safe use of veterinary medicines, especially antibiotics in animal husbandry, is crucial for protecting the health of both animals and humans. The worldwide challenge of antibiotic resistance (AMR), driven by the overuse of antibiotics, has led many countries to implement strict legal limits on their use in animal husbandry, poultry farming, and livestock products (Tang et al., 2017).

A significant step toward aligning national regulations with European Union standards has recently been made in Ukraine. The Verkhovna Rada of Ukraine approved draft law No. 12285-d, intended to align Ukrainian veterinary and animal welfare regulations with EU standards (Verkhovna Rada of Ukraine, 2025). Recent regulatory reforms in Ukraine have imposed strict limits on antibiotic use in animal production, establishing a regulatory framework consistent with European Union standards (Matiukha et al., 2024). The restrictions on antibiotic growth promoters, limits on prophylactic use without clinical necessity, and bans on critically important antimicrobials for human medicine all reflected a comprehensive effort to address AMR. Beyond regulatory requirements, these restrictions transform production standards towards greater sustainability, enhanced biosecurity, and more responsible use of antimicrobials (Casewell et al., 2003; Abreu et al., 2023). In this changing regulatory environment, relying on antibiotics as performance boosters is unsustainable, prompting the need to develop alternative approaches to maintain productivity and protect animal health. Therefore, there is a growing focus on biotechnological feeds, especially functional additives that can influence gut microbiota, improve metabolic efficiency, and strengthen immune responses (Khan et al., 2025).

Yeast-derived feed additives, including probiotics and postbiotics, have become promising options among new supplements due to their diverse mechanisms of action, such as boosting immunity, acting as antioxidants, and supporting gut barrier health (Zamojska et al., 2021). Their application provided a well-established approach to maintaining animal performance even when antibiotics are limited. Yeast-derived feed additives play an important role in developing next-generation feeding strategies for livestock production systems. Implementing these new methods not only meets legal requirements but also supports sustainable growth in agriculture and industry, with a focus on product safety and consumer trust.

Probiotics are live microorganisms that provide health benefits to the host when given in sufficient quantities (Hill et al., 2014). Probiotics are associated with several health benefits, including improved digestion, enhanced protection against infections, and support for the immune system (Jha et al., 2020). Different effective bacterial probiotics, including *Lactobacillus*, *Bifidobacterium*, *Bacillus subtilis*, and *Enterococcus* spp., are commercially available for poultry production and are widely used to improve gut health, growth performance, and immune function (Yaqoob et al., 2022). Probiotic yeasts are increasingly used in different animal production systems (Pendon et al., 2024). In addition to bacterial probiotics, some nonpathogenic yeasts, such as *Saccharomyces cerevisiae* (*S. cerevisiae*), *Saccharomyces boulardii* (*S. boulardii*), and *Kluyveromyces marxianus* (*K. marxianus*), are used as probiotics. Yeast probiotics typically offer greater stability during feed processing and are more resistant to environmental and gastrointestinal stresses compared to bacterial probiotics. Among yeast probiotics, *K. marxianus* is considered a promising probiotic for applications in the food and feed industries (Jin et al., 2021). Furthermore, *K. marxianus* yeast has been designated as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA) and as Qualified Presumption of Safety (QPS) by the European Food Safety Authority (EFSA; Diaz-Vergara et al., 2017).

In addition to probiotics, safe alternatives to antibiotics include tannins, polyphenolic compounds that precipitate proteins. These are secondary metabolites found in plants, seeds, bark, wood, leaves, and fruit skins, serving as defense mechanisms against predators. In addition to probiotics, other promising and safe alternatives to antibiotics include phytobiotics, among which tannin-rich plant extracts are of particular interest. A relevant example is chestnut (*Castanea sativa*) extract, known for its high content of hydrolyzable tannins, polyphenolic compounds that can precipitate proteins and are classified as secondary plant metabolites. Tannins are widely distributed in plants, including seeds, bark, wood, leaves, and fruit skins, where they act as natural defenses against herbivores and pathogens (Mueller-Harvey, 2006; Redondo et al., 2014; Fraga-Corral et al., 2021). High levels of tannins are known to have antinutritional effects in monogastric animals, as they can decrease feed intake, nutrient digestibility, and growth performance in chickens (Garcia et al., 2004). In poultry production, tannins have recently attracted significant interest as a substitute for antibiotic growth promoters, owing to their antimicrobial, antioxidant, and anti-inflammatory effects (Daglia, 2012; Brus et al., 2018). However, the effects of tannins on the growth performance and gut ecosystem of chickens remain inconsistent, and their mode of action is unclear. Therefore, a comprehensive understanding of the chemical properties and biological effects of tannins is crucial to optimizing their application as feed supplements in poultry. Additionally, it is important to develop evidence-based guidelines for their safe inclusion levels, both as individual compounds and within feed additive complexes. A combination of biologically active ingredients, including probiotics and phytobiotics such as tannins, can function synergistically in antibiotic-free diets, enhancing each other’s beneficial effects and ultimately improving productivity while maintaining high-quality performance (Gadde et al., 2017).

Supplementation of tannins in combination with other bioactive compounds may be more beneficial for poultry than the use of tannins alone. Tannins can form complexes with proteins or polysaccharides, potentially decreasing their ability to bind with endogenous and dietary proteins, as well as metal ions. In addition, different bioactive compounds may exhibit synergistic antimicrobial effects against both Gram-negative and Gram-positive bacteria. Bioactive compounds can influence the gut through different mechanisms, potentially improving overall gut health and function in a complementary manner (Redondo et al., 2014; Tonda et al., 2018). Finally, using multiple bioactive compounds could lower the risk of antimicrobial resistance because pathogenic bacteria are less able to adapt to diverse, combined modes of action. Prior studies had already established that probiotics in antibiotic-free diets positively affect productivity and immune parameters (Matiukha et al., 2024). The present study aimed to assess whether combining tannins with the yeast-based probiotic (*K. marxianus*) is beneficial in poultry nutrition by evaluating its effects on the immune system and productivity of broiler chickens fed antibiotic-free diets.

## MATERIALS AND METHODS

### Ethical approval

The present study was conducted in strict accordance with established ethical guidelines for animal research and welfare. The experimental protocol was approved by the Institute of Animal Biology NAAS (approval number 04/01/2025) and adhered to the guidelines established by the World Organisation for Animal Health (OIE) to ensure the welfare of animals used for scientific purposes.

### Experimental design and animals

The study involved 60 clinically healthy Ross 308 broiler chickens, from day 15 to day 42 of age, under controlled conditions. Chickens were randomly assigned to four groups (15 chickens each) following the principle of analogs. From day 1 to day 14 of age, broiler chickens were reared under standard commercial conditions on a private poultry farm. On day 15, chickens were transferred to an experimental facility for the trial. During days 15-17, chickens remained on the same diets and feeding schedule as at the initial farm to reduce stress-related effects during the transfer and adaptation period. Starting from day 17 of age, following the adaptation period, the chickens were allocated to different experimental feeding treatments. Broiler chickens were housed within a commercial-type experimental facility under controlled environmental conditions. Chickens were housed in battery cages at a stocking density of five chickens per cage, providing approximately 0.05 m² per chicken. Feed and water were provided *ad libitum* throughout the experimental period. Feed intake was recorded to calculate feed conversion parameters. Environmental conditions, including temperature, ventilation, and lighting program, were maintained in accordance with Ross 308 management guidelines. No antibiotics were used during the experimental trial. All chickens were clinically healthy and vaccinated in accordance with the standard farm vaccination protocol prior to the experiment.

### Location and veterinary supervision

The experiment was conducted at a commercial poultry farm in the Lviv region, Ukraine. All procedures, such as weekly health checks, body weight measurements, and chicken handling, were performed under the supervision of a licensed veterinary specialist to ensure animal welfare and data reliability.

### Diets and experimental treatments

The first group, serving as the control (G1), received a standard, nutritionally balanced diet specifically formulated for broiler chickens at each phase; prestarter, starter, grower, and finisher. The experimental groups received the same basal diet supplemented according to the experimental design (Table 1). The nutrient profile of the basal diet remained unchanged across all experimental groups, as the addition of experimental supplements had no impact. The second group was given a standard diet supplemented with 0.04% KluActive (SD + 0.04% KluActive, G2).

**Table 1.** Nutritional composition of the diets for broiler chickens (as-fed basis)

| Nutrient/parameter | Starter phase (0-10 days) | Grower phase (11-29 days) | Finisher phase (30-42 days) |
|---|---|---|---|
| Metabolizable energy (ME, kcal/kg) | 2950 | 3050 | 3150 |
| Crude protein (%) | 22.5 | 20.0 | 18.0 |
| Crude fat (%) | 5.0 | 5.5 | 6.0 |
| Crude fiber (%) | 3.5 | 4.0 | 4.5 |
| Crude ash (%) | 6.5 | 6.0 | 5.8 |
| Lysine (%) | 1.30 | 1.10 | 0.95 |
| Methionine + cystine (%) | 0.95 | 0.85 | 0.75 |
| Calcium (Ca, %) | 1.00 | 0.90 | 0.85 |
| Available phosphorus (P) (%) | 0.50 | 0.45 | 0.42 |
| Sodium (Na, %) | 0.18 | 0.17 | 0.16 |

Values are provided according to the feed manufacturer's specifications

KluActive is an encapsulated probiotic yeast (*K. marxianus*) that was isolated, identified, and evaluated for its technological stability and probiotic potential at the R&D center (Biotechnology Company Enzyme Group in Lviv, Ukraine). The third group was provided with a standard diet supplemented with a combination of KluActive and encapsulated tannins (CHT) at a level of 0.04% (SD + KluActive + CHT 0.04%, G3), and the fourth group received the basal diet supplemented with a combination of KluActive and tannins (SD + KluActive + CHT 0.06%, G4). The phytobiotics component, in the form of encapsulated hydrolyzable tannins, was obtained as a commercially available product from a feed additive supplier.

### KluActive production

The encapsulation of the commercial probiotic product was performed at the manufacturer's industrial production facilities (Enzyme Group, Lviv, Ukraine). A specially developed fluidized-bed drying technology enabled the production of biocapsulated probiotic yeast, which exhibited high resistance to technological stresses. The granules were approximately 0.5-2 mm in size and contained a viable yeast cell count exceeding $1.0 \times 10^9$ CFU/g, using the strain *K. marxianus*. The technology guaranteed product stability under thermal processing conditions (up to 60˚C without loss of biological activity), as well as resistance to acidic pH and viability in the gastrointestinal tract. The technology and formulation protocol were developed by the producer.

### Blood sampling and biochemical analysis

Blood samples were collected from 10 chickens in each experimental group on days 28 and 42 of the study. Sampling was conducted in accordance with established avian blood collection protocols, ensuring aseptic conditions and minimizing stress to the birds. Blood was obtained from the brachial (wing) vein using sterile needles (23-25 G) and syringes. The puncture site was disinfected with 70% ethanol prior to venipuncture. Blood samples were collected into plain tubes without anticoagulants and left to clot at room temperature. The serum was then separated by centrifugation at 3000 rpm for 10-15 minutes and stored at −20°C until biochemical analysis in a certified laboratory. Serum bactericidal activity (SBA) was assessed using an established microbiological assay that detects the inhibition of *Escherichia coli* growth, adapted for poultry studies as described by Vlizlo et al. (2012). Serum lysozyme activity (SLA) was measured using a turbidimetric method with *Micrococcus lysodeikticus* as the substrate, based on the technique outlined by Shugar (1952). To assess the phagocytic immune response, leukocyte phagocytic function was evaluated using standard microscopic techniques. To assess the phagocytic immune response, leukocyte phagocytic function was evaluated using standard microscopic techniques. Phagocytic activity (PA, %) indicated the number of neutrophils actively involved in ingesting test microorganisms. The phagocytic index (PI) measured the average number of microorganisms each neutrophil ingested, while the phagocytic number (PN) demonstrated the total number of microorganisms ingested per 100 neutrophils. These parameters were determined using established hematological methods for leukocyte functional assessment, as described by Harmon et al. (1998). Phagocytosis assays were conducted using heat-inactivated bacterial cultures, following established veterinary immunology protocols for poultry. The selected immune parameters served as indicators of nonspecific (innate) immunity, which was essential for maintaining health and optimal performance in broiler chickens.

### Productive performance measures

Productive performance parameters were recorded throughout the experimental period in compliance with established poultry production methodologies. Average daily gain (ADG, g/day), calculated from individual or group body weight values during the experimental period, absolute body weight gain (ABWG, g) indicated the total increase in body weight during the experimental period, feed intake (FI, g/bird) was the total feed consumed per chicken, feed conversion ratio (FCR, kg feed/kg gain) was calculated as the ratio of feed intake to body weight gain, and feed efficiency was measured as body weight gained per unit of feed consumed. All measurements were performed under controlled housing and feeding conditions to minimize environmental variability.

### Statistical analysis

Data were analyzed utilizing one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (Tukey's HSD) post hoc test to identify pairwise differences between groups. All statistical analyses were conducted using Microsoft Excel (Microsoft Office Professional Plus, Microsoft Corporation, USA) with the Analysis ToolPak add-in. Differences between groups were deemed statistically significant at a p-value less than 5% ($p < 0.05$). The present results were presented as mean ± standard deviation (SD).

## RESULTS

### Humoral and cellular innate immunity parameters

The effects of dietary supplementation with KluActive alone or in combination with CHT on phagocytic activity, phagocytic index, and phagocytic number in broiler chickens are presented in Figure 1.

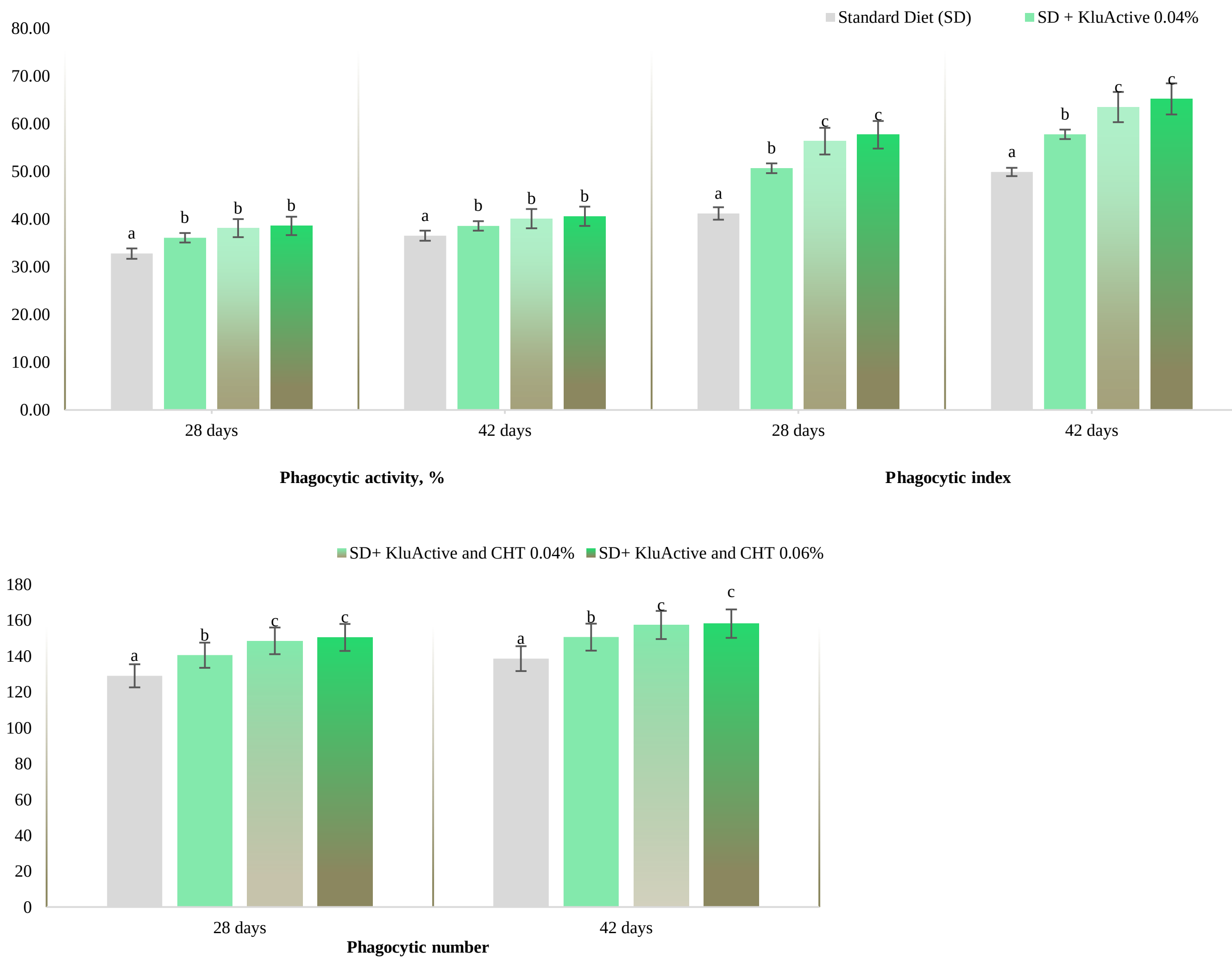


**Figure 1.** Changes in phagocytic activity, phagocytic index, and phagocytic number of neutrophils in broiler chickens at 28 and 42 days of age. Values are expressed as (mean ± SD). [a,b,c] Means with different superscript letters within the day of study indicate a significant difference ($p < 0.05$).

At 28 days of age, broiler chickens fed the standard diet (G1) demonstrated the lowest phagocytic activity, phagocytic index, and phagocytic number. Broiler chickens in G2 demonstrated significantly higher phagocytic activity than the control group ($p < 0.05$). Broiler chickens in G4 exhibited the highest average phagocytic activity; however, this difference was not statistically significant compared to the G2 and G3 groups ($p > 0.05$). In contrast, phagocytic index and phagocytic number increased progressively across treatment groups. Broiler chickens in G3 and G4 groups exhibited significantly higher levels of these parameters than those in the control and G2 groups ($p < 0.05$). Specifically, the number of phagocytes increased significantly in G2, G3, and G4 groups by 8.9%, 15.1%, and 16.7%, respectively, compared to the control group ($p < 0.05$). Similarly, the phagocytic index increased significantly across all supplemented groups, from 23.02% to 40.06%, exceeding the control value ($p < 0.05$).

At 42 days of age, a similar pattern was observed. Phagocytic activity in the G2 group remained significantly higher than in the control group ($p < 0.05$), while the inclusion of encapsulated tannins in combination with the probiotic resulted in additional increases. The phagocytic index and phagocytic number demonstrated the same trend, with significantly higher values in G3 and G4 groups than in G1 and G2 groups ($p < 0.05$). Furthermore, chickens in the G4 group demonstrated significantly higher phagocytic responses than those in the G3 group, indicating a dose-dependent effect ($p < 0.05$).

The effects of dietary supplementation with *K. marxianus* alone or in combination with encapsulated tannins on SBA and SLA in broiler chickens are shown in Figure 2.

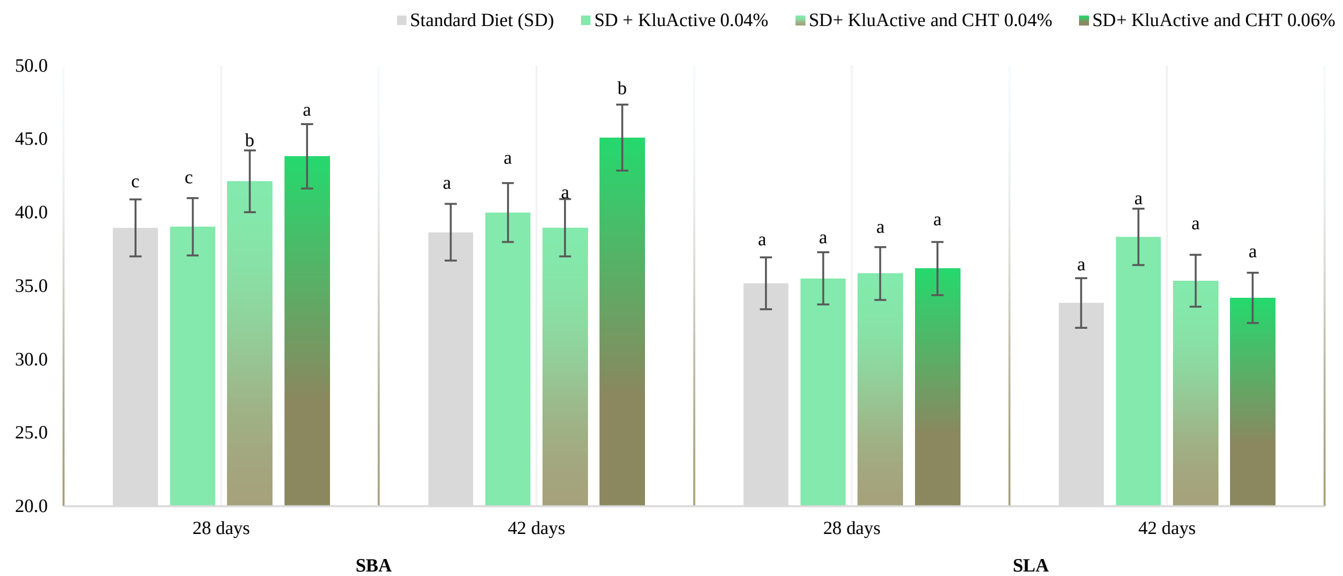


**Figure 2**. Lysozyme and bactericidal activities of blood serum in broiler chickens at 28 and 42 days of age. Values are expressed as (mean ± SD). [a,b,c] Means different superscript letters within the same day of study indicate a significant difference ($p < 0.05$).

At 28 days of age, SBA was the lowest in broiler chickens fed the standard diet and increased progressively with dietary supplementation. A slight numerical increase in SBA was observed in the G2 group (39.015 ± 0.992) compared with the control group (38.93 ± 0.515); however, this difference was not statistically significant ($p > 0.05$). The highest value was observed in the G4 group compared with the control group, indicating a dose-dependent response ($p < 0.05$). In G4 group, SBA was 12.5% higher than in the control group, and in the G3 group, SBA was 8.2% higher. At day 42, broiler chickens supplemented with KluActive and encapsulated tannins at the higher inclusion level (G4 group) exhibited significantly higher SBA than the control and the other G2 and G3 groups, respectively ($p < 0.05$). Serum bactericidal activity (SBA) was significantly higher in the G4 group, increasing by 12.5% on day 28 and 16.7% on day 42 compared with the corresponding values in the serum of birds from the control group ($p < 0.05$).

Serum lysozyme activity revealed minor differences among treatments at 28 days of age. However, at 42 days, SLA was significantly higher in the G1 group than in the control group ($p < 0.05$). In the blood serum of chickens from G3 and G4 groups, SLA values were lower than those in the G2 group but still exceeded those in the control group. Overall, dietary supplementation with *K. marxianus* was associated with improvements in phagocytic parameters and bactericidal activity in broiler chickens. The combined use of the probiotic and encapsulated tannins generally resulted in higher values than the control, with a tendency toward greater responses at the higher inclusion level (0.06%). Lysozyme activity increased mainly in the G2 group, while values in the G3 and G4 groups persisted at levels equal to or exceeding those of the control.

### Productive performance

Supplementation of the antibiotic-free diet with all three additives improved growth performance, feed utilization, and feed conversion efficiency, although the responses differed by additive type and dosage (Table 2). Dietary supplementation with experimental additives positively affected the growth performance of broiler chickens. Analysis of ADG demonstrated higher values across all experimental groups than in the control group. Broiler chickens in G2 and G3 groups reached ADG values of 98.35 and 100.45 g/day, respectively, while the control group had an ADG of 88.49 g/day. Similarly, the final body weight of broiler chickens increased by 11.14% in the G2 group, 13.52% in the G3 group, and 4.85% in the G4 group compared to the control group. Evaluation of feed efficiency parameters further indicated improved feed utilization in all supplemented groups. The lowest FCR was observed in the G3 group, at 1.5, compared to 1.67 in the control group. These findings indicate that combining probiotic and tannin additives may enhance nutrient utilization efficiency and growth performance in broiler chickens fed antibiotic-free diets.

**Table 2.** Growth performance of broiler chickens fed experimental diets (days 15-42 of age)

| Treatment group | Average daily gain (g/day) | Absolute body weight gain (g) | Change versus control (%) | Feed intake (g/bird/period) | Feed conversion ratio | Feed efficiency (%) |
|---|---|---|---|---|---|---|
| Standard diet (SD) | 88.49 | 2300.67 | - | 3850 | 1.67 | 59.76 |
| SD + 0.04% KluActive | 98.35 | 2557.00 | 11.14 | 3960 | 1.55 | 64.57 |
| SD + KluActive + 0.04% CHT | 100.45 | 2611.67 | 13.52 | 3925 | 1.50 | 66.54 |
| SD + KluActive + 0.06% CHT | 92.78 | 2412.33 | 4.85 | 3905 | 1.62 | 61.78 |

CHT: Chestnut hydrolyzable tannins

## DISCUSSION

The present study demonstrated that supplementation of antibiotic-free broiler diets with *K. marxianus*, either alone or in combination with CHT, improved growth performance and certain innate immune parameters in real-world production settings. Previous studies have separately demonstrated the beneficial effects of probiotics and tannin-based phytogenic additives in broiler chickens, including improvements in growth performance, gut health, and immune responses (Shehata et al., 2022; Ranjan et al., 2023). In the present study, intestinal barrier function, microbial ecology, and systemic immune responses were not directly evaluated. Hydrolyzed tannins, characterized by their lower molecular weight and greater bioavailability than condensed tannins, have been indicated to modulate local immune responses by regulating cytokine expression, enhancing epithelial barrier integrity, and exerting antioxidant and antimicrobial effects. Consequently, these actions help reduce immune pressure associated with enteric pathogens (Guo et al., 2023). Encapsulation technology can further enhance these effects by increasing tannin stability and enabling targeted release throughout the gastrointestinal tract, which reduces potential antinutritional interactions while maintaining biological activity (Choi and Kim, 2020).

The present results demonstrated that probiotic yeast supplementation stimulated the phagocytic component of innate immune defense, as well as SLA and SBA in broiler chickens at the end of the experimental period. A more substantial impact on phagocytic activity was observed following supplementation with the combined additive comprising probiotic and phytobiotic components. In particular, SBA remained elevated throughout the study, with the highest values recorded in broiler chickens receiving the higher inclusion level of the combined additive. These findings generally align with the findings of Akhoundzadeh et al. (2025), who studied the immunomodulatory effects of probiotics and tannins in poultry and other animals.

Concurrently, *K. marxianus*, a probiotic yeast, demonstrated immunomodulatory potential, including stimulation of humoral immunity, enhancement of immune organ development, and modulation of gut microbiota composition in broiler chickens, similar to conventional *Saccharomyces*-based products (Wang et al., 2017; Rassmidatta et al., 2024). The components of the yeast cell wall and metabolites of *K. marxianus* have the potential to activate innate immune pathways and support the function of gut-associated lymphoid tissue (GALT), thereby enhancing immune competence without inducing excessive inflammatory activation (Maccaferri et al., 2012). The combined use of hydrolyzable tannins and probiotic *K. marxianus* may result in complementary and reinforcing effects. Probiotic yeasts stabilize intestinal morphology and microbial balance, while tannins reduce oxidative and microbial stress, collectively promoting a more balanced and efficient immune response (Romanin et al., 2016; Wang et al., 2017; Akhoundzadeh et al., 2025). The synergistic interactions were particularly relevant in antibiotic-free broiler production systems, where supporting immune resilience and gut health was vital to maintaining performance during commercial rearing (Yuan et al., 2023).

The markers of phagocyte-mediated innate immunity were notably increased by the addition of the experimental feed additives, which is especially important since the diets were entirely antibiotic-free throughout the rearing period. Different studies have investigated the role of yeasts and their derivatives in animal and poultry nutrition (Abd El-Ghany et al., 2015; Shurson, 2018; Perricone et al., 2022). Yeasts and their byproducts are widely used as a partial or complete substitute for excessive antibiotic use in animal production systems. In addition, due to their valuable composition and highly digestible protein content, yeast-derived byproducts are frequently incorporated into poultry diets as an alternative to conventional protein sources such as soybean meal and fish meal. However, yeast products should not be regarded solely as protein sources, as their primary value lies in their rich content of bioactive compounds, which can positively influence animal health, immunity, and gut function (Bilal et al., 2021; Abd El-Ghany, 2025). While direct evidence on the immunomodulatory activity of *K. marxianus* was limited, findings from *Saccharomyces cerevisiae* may offer relevant mechanistic insights, as both yeasts have similar cell wall compositions rich in mannan oligosaccharides (MOSs) and β-glucans (Gheisari and Kholeghipour, 2006). Furthermore, the cell wall of *K. marxianus* was characterized by a substantial content of structural polysaccharides, including mannans and β-glucans, which are known for their immunomodulatory and prebiotic properties (Perpetuini et al., 2019; Tang et al., 2022). Therefore, similar immunological mechanisms might play an important role in the beneficial effects of *K. marxianus* in animal nutrition.

Previous studies demonstrated that dietary supplementation with hydrolyzable tannins (0.15-0.30%) or organic acids (0.20-0.40%) improved FCR and intestinal health in broiler chickens, suggesting that these additives may positively modulate nutrient utilization and gut functionality (Khorasgani et al., 2025). These effects may partially explain the beneficial responses observed following *K. marxianus* supplementation in the present study. Tannin sources and animal species may influence the immunoregulatory effects on gut health in broiler chickens (Guo et al., 2023). In the present study, supplementation of antibiotic-free diets with the probiotic KluActive (G1) improved FCR by 7.19% compared with the control group. Likewise, supplementation with the probiotic–tannin product improved FCR by 10.18% at the lower dose (G2) and by 2.99% at the higher dose (G3), indicating a non-linear dose-response relationship.

The observed improvements in productive performance were consistent with previous studies indicating that probiotic supplements improved broiler nutrition and that phytogenic compounds such as tannins promoted growth (Redondo et al., 2014). Supplementing *K. marxianus* at a high dose (0.25%) proved to be more effective for improving feed efficiency and intestinal health in broiler chickens, whereas innate immunity was optimized at a medium dose (0.1%; Wang et al., 2017). *In vitro* studies using intestinal epithelial cell culture systems have demonstrated that *K. marxianus* may exert immunomodulatory effects and support intestinal barrier integrity, as evidenced by modulation of pro-inflammatory cytokines and preservation of tight junction proteins (Kourelis et al., 2010; Romanin et al., 2010; Maccaferri et al., 2012). Supplementation with tannins and probiotics enhanced growth performance in broiler chickens compared with the antibiotic and control groups. No remarkable differences were observed in the retention of dry matter, ether extract, or phosphorus among broiler chickens fed diets supplemented with tannins, probiotics, or antibiotics, except for calcium digestibility, as reported by Pramod Ranjan et al. (2023). Present results indicated that adding tannins and probiotics to the diet could improve productive performance traits such as body weight gain, FCR, and performance index, consistent with findings by Ranjan et al. (2023). Similarly, Wang et al. (2017) found that increasing the dietary *K. marxianus* level consistently lowered FCR during the grower phase and overall rearing period in broiler chickens, demonstrating enhanced feed efficiency, especially at higher supplementation levels (0.2% and 0.25%).

## CONCLUSION

The yeast probiotic containing *Kluyveromyces marxianus* (KluActive) at a dose of 0.04% demonstrated positive effects on innate immune parameters and enhanced feed conversion ratio by 7.2%. Supplementing the diet with KluActive at 0.04% and chestnut hydrolyzable tannins (CHT) at 0.04% resulted in a feed conversion ratio of 1.50, representing a 10.2% improvement over the control group. Supplementing the diet with KluActive alone was associated with increased serum lysozyme activity and improved phagocytic activity. In the group receiving KluActive with CHT at 0.04%, lysozyme activity demonstrated only a slight increase, remaining close to the control level. Conversely, supplementing the diet of broiler chickens with a higher level of CHT at 0.06% primarily enhanced serum bactericidal activity and phagocytic capacity, while its impact on lysozyme activity was less significant. The current results indicated that KluActive and CHT differentially influenced selected nonspecific immune parameters in broiler chickens. Dietary supplementation with KluActive improved growth performance in broiler chickens, particularly when combined with CHT at a 0.04% inclusion level. Chickens received a combination of KluActive and CHT at 0.04%, resulting in improved productive performance and feed efficiency, along with a reduced feed conversion ratio. Increasing the concentrations of KluActive and CHT to 0.06% slightly reduced the growth-promoting effects, although the results remained generally superior to those of the control group. The present findings indicated that combined supplementation with KluActive and CHT may improve productive performance and selected immune-related parameters in broiler chickens. Future studies should include a broader assessment of blood biochemical indicators and should validate the results obtained under commercial production conditions. Additionally, large-scale trials on commercial poultry farms are needed to evaluate the real-world effectiveness and cost-efficiency of adding these supplements to broiler diets.

## DECLARATIONS

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### Authors' contributions

All authors contributed to data analysis and the writing of the manuscript. All authors contributed to the study conception, experimental design, data collection, analysis, and manuscript preparation. Iryna Matiukha was responsible for study organization, data collection during the experiment, result analysis, and manuscript preparation. Olena Krasovska was responsible for yeast strain collection and identification. Oleh Vishchur contributed to the study organization and preparation of the results. Natalia Broda performed blood parameter analysis and statistical processing of the results. Daria Mudrak provided veterinary supervision of the experiment. Maria Masiuk contributed to the analysis of blood parameters and productive performance indicators. The manuscript was reviewed and edited by Victor Tomchuk and Andriy Voltornistyi. All authors have reviewed and approved the final edition of the manuscript and have given their permission for this article to be published.

### Availability of data and materials

All materials required to complete the current study are available at the Institute of Animal Biology NAAS.

### Competing interests

The authors disclosed no conflicts of interest related to the present study.

### Ethical considerations

The authors affirm that this study is original and has not been previously submitted for publication elsewhere. The authors also declared that the information contained within this publication is accurate and has not been modified. No AI tools were employed in conducting and preparing this study. The authors have reviewed all content and take full responsibility for the accuracy, originality, and integrity of the final manuscript, from submission through revision to publication.

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