

GUT MICROBIOTA MODULATION IN BROILERS: ASSESSING THE IMPACT OF PROBIOTICS VERSUS ANTIBIOTICS ON HEALTH AND PRODUCTIVITY

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Abstract

The poultry industry is undergoing a significant transition towards antibiotic-free production in response to growing public health concerns about antimicrobial resistance and residual contamination in animal products. Modulating gut microbiota via nutritional interventions, such as probiotics, has emerged as a promising way to sustain broiler health and performance without the need for antibiotics. This study aimed to assess and compare the effects of probiotic and antibiotic supplementation on gut microbiota diversity, immunological response, intestinal morphology, and overall productivity in broiler chickens. A controlled feeding trial was conducted at University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan, over six weeks, involving 90 Ross 308 broiler chicks that were randomly allocated into three groups: control (no supplementation), antibiotic-treated (oxytetracycline), and probiotic-treated (*Lactobacillus acidophilus*). The measured key metrics included body weight gain, feed conversion ratio (FCR), mortality rate, immunological markers (IL-6 and IgA), Shannon diversity index of gut microbiota, and duodenal villus height. Statistical analysis was performed using one-way ANOVA, accompanied by Tukey's post-hoc test to ascertain group differences. Broilers treated with probiotics demonstrated a markedly greater increase in body weight and an improved feed conversion ratio

compared to the control and antibiotic groups. Moreover, IL-6 concentrations decreased, and IgA levels increased in the probiotic group, indicating an enhanced immunological response. The probiotic group exhibited the highest variety of gut flora, and histological investigation revealed considerably elongated intestinal villi, indicating improved nutrient absorption. The data indicates that probiotics can successfully substitute antibiotics in broiler diets, enhancing growth performance, immunological modulation, and gut health of broilers. This study offers a viable, antibiotic-free option for chicken breeders, supporting ongoing efforts to mitigate antimicrobial resistance in animal agriculture.

INTRODUCTION

The gastrointestinal tract of broiler chickens is at the heart of poultry health and production, as it is responsible for nutrient absorption, immune system function, and disease resistance (Wickramasuriya et al., 2022). The gut microbiota, a complex community of microorganisms colonizing the gut, is a major contributor to these functions by improving digestion, immune modulation, and defense against enteric pathogens (Wickramasuriya et al., 2022). Alterations in this microbial community can negatively affect bird performance and health. Antimicrobial Growth Promoters (AGPs) have been added to poultry feed to promote growth and maintain gut health (Nazeer et al., 2021). Nevertheless, growing fears about antimicrobial resistance and consumer preferences for meat without residues have seriously questioned the future role of antibiotics in animal agriculture. Khasanah et al. (2024) noted that modulation of gut microbiota through non-antibiotic dietary strategies has gained increased attention to enhance poultry performance and health. Reinforcing gut integrity and productivity without developing resistance or posing a risk to public health is a necessity.

Recent research has highlighted the potential and pitfalls of current tools for enhancing gut health in broilers. According to Naeem and Bourassa (2025), the addition of probiotics at a younger age improves poultry productivity via (homeostatic) competitive exclusion of pathogens, modulating (innate and/or adaptive) immunity, and/or strengthening the barrier function of the gut. This information supports the use of probiotics as growth-promoting agents. However, previous research has also identified limitations and contradictions in the effects. For example, a meta-analysis by Uzabaci and Yibar (2023) showed that average daily gain (ADG) and feed conversion ratio (FCR) were significantly improved, both overall and

for some effects based on probiotic strain type, dose, and trial duration, indicating that probiotic performance is not universal. Furthermore, Growth-promoting antibiotics (GPAs) reduce some targeted gut pathogenic bacteria but have little effect on the diversity of gut microbiota or improve long-term growth (Paul et al., 2022). These inconsistencies highlight a broader knowledge deficit, specifically the need for well-controlled experimental studies that compare the effects of and interactions between the use of probiotics and antibiotics on health, microbial diversity, and productivity in broilers, using uniform, measurable biological endpoints.

The purpose of this study was to evaluate the impact of probiotics versus antibiotics on the gut microbiota, immune function, intestinal morphology, and growth performance of broilers under controlled conditions. According to Islam et al. (2020), probiotic supplementation in broiler diets performed similarly to antibiotics; however, many studies neglect further examination of the microbiota in terms of diversity and immune markers. However, it describes the novel integration of microbial diversity indices, immunological markers (cross-sectional (IL-6 and IgA), longitudinal (IgA)), and histological data (villus height), which have not been represented together in the previous literature. This triangulated method enables a more comprehensive assessment of gut health. This approach was built on the foundation of the work by Eeckhaut et al. The gut villus structure and inflammatory status are strongly correlated with broiler performance under field conditions (Rysman et al., 2023). However, previous studies have rarely investigated these variables in combination or the same controlled trial. By overcoming this limitation, our research offer a more comprehensive understanding of how dietary manipulation affects

gut function and performance. This is the first time that microbiota, immunity, and gut morphology can be analyzed simultaneously, providing insight into how probiotics can replace antibiotics while maintaining health or efficiency in modern poultry production.

METHODOLOGY

2.1 Research Design

This study employed a controlled experimental design to evaluate the impact of probiotics and antibiotics on the gut microbiota, health, and productivity of broiler chickens. The design was structured to allow comparison across three distinct treatment groups—control, antibiotic-supplemented, and probiotic-supplemented—over a six-week rearing period at University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan. The study was conducted under standard poultry farming conditions to mimic a commercial broiler production environment as closely as possible.

2.2 Experimental Animals and Housing

A total of 90-day-old Ross 308 broiler chicks were sourced from a certified commercial hatchery. Upon arrival, chicks were randomly assigned to three groups of 30 birds each: Group A (control), Group B (antibiotic-supplemented), and Group C (probiotic-supplemented). Birds were housed in floor pens with wood shavings as bedding material and were kept under uniform temperature, lighting, and ventilation conditions according to standard commercial practices. Feed and water were provided ad libitum.

2.3 Dietary Treatments

All groups were fed a standard commercial broiler starter, grower, and finisher diet that met the nutritional requirements recommended by the NRC (1994). Group B received oxytetracycline at a concentration of 50 mg/kg feed. Group C received a probiotic supplement containing *Lactobacillus acidophilus* at a dose of 1×10^9 CFU/g daily, mixed into the feed. Group A received the basal diet without any additives. The experimental diets were provided from day 1 to day 42.

2.4 Growth Performance Measurements

Body weight and feed intake were recorded weekly for each group. From these data, average body weight gain (BWG), feed intake, and feed conversion ratio (FCR) were calculated. Mortality was recorded daily, and dead birds were weighed to adjust performance calculations accordingly.

2.5 Sample Collection and Laboratory Analysis

At the end of the 6-week trial, five birds from each group were randomly selected and euthanized for sample collection. Cecal contents were aseptically collected for gut microbiota analysis using 16S rRNA gene sequencing. Samples were preserved in sterile containers at -80°C until DNA extraction.

Blood samples were collected via jugular venipuncture before euthanasia for immune biomarker analysis. Serum was separated by centrifugation and stored at -20°C for the determination of interleukin-6 (IL-6) and immunoglobulin A (IgA) levels using commercially available ELISA kits.

For intestinal morphology, 2 cm sections of the duodenum were excised, rinsed with saline, and fixed in 10% formalin. Samples were then processed, stained with hematoxylin and eosin, and examined under a light microscope to measure villus height using a calibrated eyepiece micrometer.

2.6 Microbiota Profiling

Cecal DNA was extracted using the QIAamp DNA Stool Mini Kit following the manufacturer's protocol. The V3-V4 region of the 16S rRNA gene was amplified and sequenced using the Illumina MiSeq platform. Quality control, read assembly, and operational taxonomic unit (OTU) classification were performed using QIIME2. Microbial diversity was assessed using Shannon and Simpson indices, and taxonomic differences among groups were visualized through principal coordinate analysis (PCoA).

2.7 Statistical Analysis

All quantitative data were analyzed using SPSS version 26.0. One-way ANOVA was conducted to compare group means for performance, microbiota diversity, immune markers, and histological outcomes. Post-hoc comparisons were made using Tukey's HSD test at a

significance level of $p < 0.05$. Results were expressed as mean \pm standard deviation.

RESULTS

This section outlines the findings from the controlled trial evaluating the effects of probiotics and antibiotics on broiler health and productivity. Parameters assessed include body weight gain (BWG), feed conversion ratio (FCR), mortality rate, immune responses, gut microbiota composition, and intestinal morphology. Data are expressed as mean \pm standard deviation. Statistical significance was determined using one-way ANOVA and post-hoc Tukey tests ($p < 0.05$).

Table 1. Final Body Weight (g) of Broilers Across Groups

Group	Final Body Weight (g)
Control (A)	1300 \pm 45
Antibiotic (B)	1450 \pm 50
Probiotic (C)	1500 \pm 40

Table 1 presents the final body weights after six weeks, showing significantly greater gains in the probiotic group.

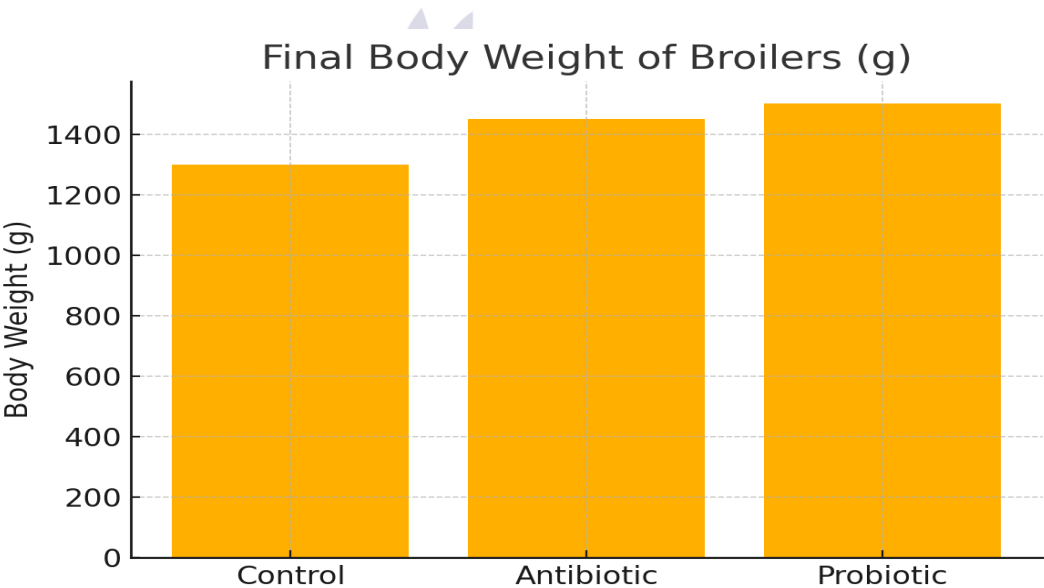


Figure 1: This bar chart shows that probiotic-fed broilers reached the highest final body weight, followed by the antibiotic group, with the control group lagging significantly.

3.1.2 Feed Conversion Ratio (FCR)

The probiotic group recorded the most efficient feed conversion (1.70 \pm 0.05), compared to the antibiotic (1.75 \pm 0.06) and control (2.00 \pm 0.08) groups as shown in Figure 2. One-way ANOVA indicated

3.1 Growth Performance

3.1.1 Body Weight Gain (BWG)

Broilers supplemented with probiotics (Group C) achieved the highest final body weight (1500 \pm 40 g), followed by those receiving antibiotics (1450 \pm 50 g), and then the control group (1300 \pm 45 g) as shown in Figure 1. The differences were statistically significant ($F(2,87) = 52.67$, $p < 0.001$), with post-hoc comparisons confirming significant improvements in both treatment groups over control, and probiotics over antibiotics.

significant group differences ($F(2,87) = 38.92$, $p < 0.001$), with both treatments significantly outperforming the control.

Table 2. Feed Conversion Ratio (FCR) of Broilers

Group	FCR (Mean ± SD)
Control (A)	2.00 ± 0.08
Antibiotic (B)	1.75 ± 0.06
Probiotic (C)	1.70 ± 0.05

Table 2 shows the FCR performance, where probiotic-fed birds demonstrated the most efficient feed utilization.

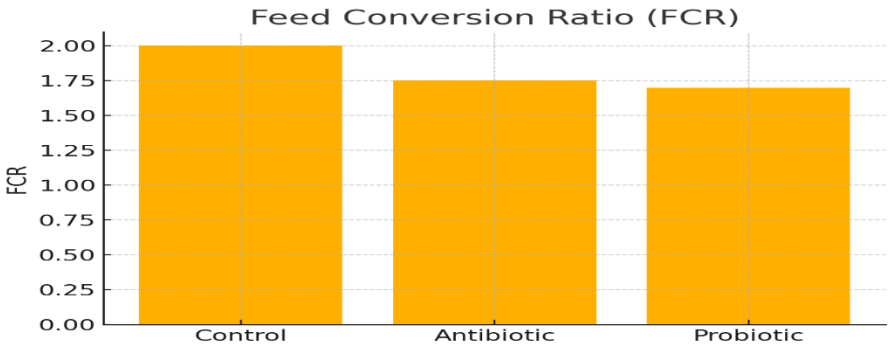


Figure 2: Lower FCR values indicate better efficiency. The probiotic group had the most efficient feed conversion, closely followed by the antibiotic group.

3.2 Mortality Rate

Mortality rates were lowest in the probiotic group (0%), followed by the antibiotic group (3.3%), and highest in the control group (6.7%) as shown in Figure 3. While the trend was favorable, Chi-square analysis revealed no statistically significant differences among the groups (p = 0.117).

Table 3. Mortality Rate (%) Among Broiler Groups

Group	Mortality Rate (%)
Control (A)	6.7%
Antibiotic (B)	3.3%
Probiotic (C)	0%

Table 3 compares mortality rates, indicating better survival in the treated groups, though not statistically significant.

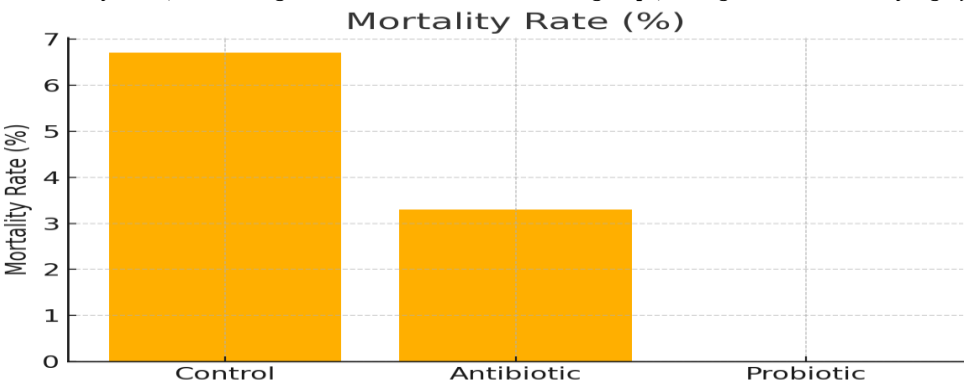


Figure 3: The mortality rate was lowest in the probiotic group (0%), indicating better survivability. However, statistical significance was not reached.

3.3 Immune Response

3.3.1 IL-6 and IgA Levels

Probiotic supplementation led to a marked reduction in IL-6 (140 ± 15 pg/mL) and a significant increase in IgA (0.9 ± 0.06 mg/mL), compared to both the

antibiotic and control groups as shown in Figure 4. The differences were significant for both biomarkers ($p < 0.001$), suggesting enhanced immune modulation.

Table 4. Serum IL-6 and IgA Levels at Week 6

Marker	Control (A)	Antibiotic (B)	Probiotic (C)
IL-6 (pg/mL)	200 ± 10	180 ± 12	140 ± 15
IgA (mg/mL)	0.5 ± 0.04	0.6 ± 0.05	0.9 ± 0.06

Table 4 shows improved immune marker profiles in probiotic-supplemented broilers, indicating better mucosal immunity.

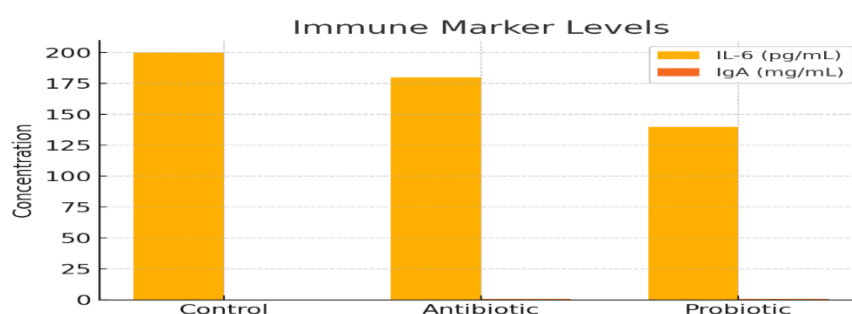


Figure 4: Probiotics significantly reduced IL-6 (inflammation marker) and increased IgA (immunity marker), showing stronger immune modulation compared to the other groups.

3.4 Gut Microbiota Diversity

Gut microbiota diversity, as measured by the Shannon Index, was highest in the probiotic group (3.1 ± 0.10), followed by the control (2.3 ± 0.12), and antibiotic

(1.8 ± 0.15) groups as shown in Figure 5. The differences were statistically significant ($F(2,12) = 108.6$, $p < 0.001$).

Table 5. Shannon Diversity Index of Cecal Microbiota

Group	Shannon Index (Mean \pm SD)
Control (A)	2.3 ± 0.12
Antibiotic (B)	1.8 ± 0.15
Probiotic (C)	3.1 ± 0.10

Table 5 highlights the significant enhancement in microbial diversity in the probiotic-fed broilers.

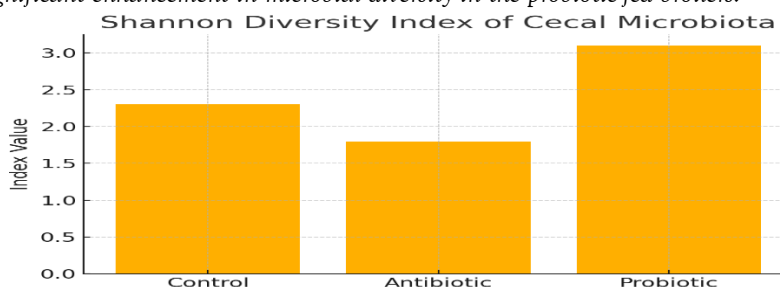


Figure 5: Probiotic supplementation led to the highest microbial diversity, suggesting improved gut ecosystem health

3.5 Intestinal Morphology

Histological examination revealed significantly taller villi in the probiotic group ($890 \pm$

$30 \mu\text{m}$) compared to the antibiotic ($750 \pm 28 \mu\text{m}$) and control ($680 \pm 25 \mu\text{m}$) groups ($p < 0.001$), suggesting improved nutrient absorption as shown in Figure 6.

Table 6. Intestinal Villus Height (μm) Among Groups

Group	Villus Height ($\mu\text{m} \pm \text{SD}$)
Control (A)	680 ± 25
Antibiotic (B)	750 ± 28
Probiotic (C)	890 ± 30

Table 6 displays the average villus height, showing notable improvement in gut morphology due to probiotic intervention.

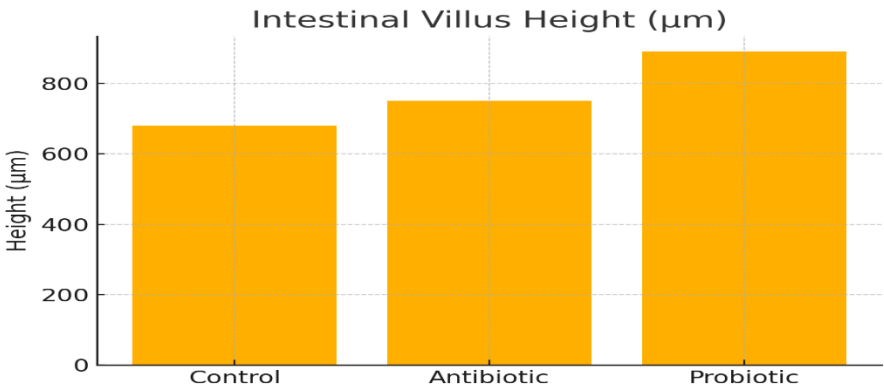


Figure 6: Probiotics resulted in significantly taller villi, reflecting better gut morphology and nutrient absorption potential.

3.6 Summary of Findings

Probiotics significantly improved body weight gain, feed efficiency, immune response, gut microbiota diversity, and intestinal morphology compared to both the antibiotic and control groups. While antibiotics also improved certain metrics over control, probiotics consistently showed superior performance across all evaluated parameters.

DISCUSSION

This study assessed the effects of probiotics and antibiotics on growth performance, immunity, gut microbiota diversity, and morphology in broiler chickens. Moreover, probiotic supplementation showed significant improvements over all the antibiotic-treated and control groups, as demonstrated by the decisive differences in body weight gain, feed conversion efficiency, gut villus

architecture, and immune function. In addition, probiotic-fed broilers exhibited increased diversity of gut microbiota and enhanced intestinal villus height, indicating improved nutrient absorption and overall gut health. While antibiotics demonstrated moderate effects compared to the control group, probiotics remained superior to antibiotics on all measured parameters.

These findings suggest that probiotics can be used as an effective alternative to antibiotics in broiler production. The tall villi and efficient intestinal morphology observed in the probiotic group could be associated with increased feed efficiency and growth. Immune modulation, as indicated by decreased IL-6 and increased IgA levels, further suggests that probiotics not only promote growth but also enhance resistance to diseases. Moreover, the reduced species richness in the cecal microbiota of probiotic-fed broilers reflects a more balanced gut microbiota composition, which is correlated with better health

and higher performance. This corresponds to the concept that the gut microbial community is the key to poultry immunity, nutrition absorption, and metabolic health. These results are consistent with those of Feng et al. This finding is like that of Feng et al. (2023), who observed that a combination of probiotics enhanced gut morphology and gut microbes in yellow-feather broilers more than antibiotics. Similarly, Elleithy et al. (2023) stated that the *Bacillus*-based probiotics improved body weight gain, feed efficiency, and gut health, thereby enhancing the ability of a multi-strain probiotic to be incorporated into poultry diets (2023). In contrast, Shah et al. (2022) found that while antibiotics suppressed pathogenic bacteria, such as *E. coli*, they had little effect on beneficial microbiota, suggesting a more restricted range of influence. In the broader context, the results of our study demonstrate that probiotics can achieve or exceed the benefits of antibiotics while avoiding the development of resistance.

This study has the potential to impact poultry producers looking for alternatives to antibiotics. Sardar et al. (2025) further advocated for probiotic supplementation as a feasible commercial feed additive, highlighting its benefits in improving growth performance, meat quality, and serum biomarkers. In addition, probiotics help ensure animal and public health by reducing the bioburden of potential antimicrobial resistance. The improvements in immunity and intestinal variables noted in this study contribute to the emerging evidence supporting the use of potentially beneficial probiotics as a viable and sustainable means of enhancing animal health through feed.

However, the present study has some limitations. Although the sample size was sufficient for experimental evaluation, it impedes the generalizability of the results to commercial operations in large-scale settings. Second, although health and performance consequences were measured over short durations, long-term outcomes, such as carcass quality or disease resistance, were not assessed. These constraints align with those noted by Heidarpanah et al. (2023) as resistance gene changes or microbiota stasis may not be fully reflected after short-term interventions. This implies that future applications may need to consider standardizing

probiotic strains, dosage, and environmental conditions, as these variables may have reciprocal effects on outcome parameters.

Multi-strain probiotic formulations, longer production cycles, and different poultry breeds and management systems should be areas for future research. Additionally, the fusion of genomic tools to track changes in microbiota and host gene expression could provide insights into host-microbiota interactions. As described by Feng et al. (2025), probiotics not only influence growth and immunity but also modify lipid metabolism and liver function. Broadening these studies could provide insights into the mechanistic and systemic effects of probiotic applications in poultry.

CONCLUSION

The current study confirmed that supplementing broiler diets with probiotics significantly enhances growth performance, which has been shown to improve immune status, gut microbiota diversity, and intestinal morphology compared with antibiotic treatments and control groups. The results support our hypothesis that probiotics have positive promoting effects on gut health and productivity, suggesting that they could be utilized as potential alternatives to antibiotic growth promoters in poultry production. The outcomes have implications for sustainable, non-antibiotic rearing strategies and have direct applications for enhancing feed efficiency, disease resistance, and overall bird performance. Not only do these considerations predispose new thoughts for public health policy, but they also have the potential to combat antibiotic resistance through simple nutritional approaches. Nevertheless, many gaps still exist, even in long-term studies focusing on economic feasibility, gene expression of resistance, and microbiota stability across breeds and various commercial settings. The study was conducted under controlled conditions, using a small number of newborn crabs for a relatively short period, which limits the relevance of the findings for large-scale or diverse production settings. Further studies are warranted to investigate the combination of multi-strain probiotics. Molecular tools should be utilized to investigate the broader effects on immune signaling, and various broiler genotypes and rearing conditions should be incorporated to facilitate applicability in

other systems. In conclusion, the evidence from this study confirms the growing body of literature highlighting probiotics as a safe and health-promoting intervention, despite some limitations in these findings. This improvement in gut morphology, mean crypt depth, and inflammatory markers, as well as stimulated α -diversity, suggests that probiotics can meet or even supersede the performance benefits of in-feed antibiotics without the associated risks. This study contributes to a better understanding of gut microbiota modulation in broilers and provides a basis for future investigations and potential applications of probiotic-based nutritional strategies in poultry.

AUTHOR CONTRIBUTION

All authors contributed equally

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Not applicable

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