EFFECT OF ANTIBIOTIC ALTERNATIVES IN POULTRY NUTRITION: PHYTOCHEMICALS AND PROBIOTICS

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Abstract
This study assessed the impact of dietary phytochemicals and probiotics, both separately and in combination, as substitutes for antibiotic growth boosters in broiler chickens conducted at University of Veterinary and Animal Sciences, Lahore. A total of 480-day-old Ross 308 chicks were assigned to four dietary treatments in a 2 × 2 factorial design: control (base diet), phytochemical supplementation (500 mg/kg essential oil mix), probiotic supplementation (1 g/kg multi strain preparation), and a combination of both interventions. Growth performance, intestinal morphology, cecal microbiota, immunological responses, and oxidative stress indicators were assessed throughout the 42-day study. The results indicated that both phytochemicals and probiotics enhanced body weight and feed conversion ratio relative to the control group ($P < 0.05$), with the most significant benefit noted in the combination group (BWG = 2,625 g; FCR = 1.61). Birds administered supplements showed increased villus height and villus-to-crypt ratio, decreased Escherichia coli counts, and elevated populations of Lactobacillus spp. Immunological studies demonstrated increased serum IgA and IgG levels and diminished pro-inflammatory cytokines (IL-6 and TNF-α) in the supplemented groups; however, oxidative stress markers showed decreased malondialdehyde levels and increased antioxidant enzyme activity. The combined use of phytochemicals and probiotics consistently yielded the best results in terms of performance, gut health, immunological function, and oxidative metrics, indicating a synergistic effect. These findings indicate that phytochemicals and probiotics serve as viable, sustainable alternatives to antibiotics in poultry

production, enhancing productivity and animal health, while diminishing dependence on antimicrobial growth boosters.

INTRODUCTION

Global livestock production, particularly the poultry subsector, is among the fastest growing in the world and an important contributor to food security and the provision of low-cost animal protein (Nkukwana, 2018). Antibiotic growth promoters (AGPs) have been commonly used in poultry diets for many years to achieve high growth rates and efficient feed utilization. Their performance improved, and disease occurrence decreased because of their antimicrobial actions (Ali, Chand, Khan, Naz, & Gul, 2019; Salim, Huque, Kamaruddin, & Haque Beg, 2018; Sethiya, 2016). Nevertheless, prolonged use of antibiotic growth promoters (AGPs) has produced antimicrobial-resistant bacteria with attendant fears of risks to public health, suggesting the possibility of transfer of resistance genes across the food chain (Milani, 2024). Consequently, several regulatory bans have been put in place against the prophylactic use of AGPs in animal production in many countries and gradually in other regions of the world (Laxminarayan, Van Boeckel, & Teillant, 2015). This has made it imperative to find practical, safe, and sustainable alternatives to meet productivity needs, food safety, and animal welfare (Khan et al., 2025; Mahmud et al., 2025).

Phytochemicals and probiotics are emerging as promising alternatives, owing to their diverse roles in animal health and performance (Ahmad et al., 2024; Ali, Chand, Khan, Ahmad, & Tahir, 2023; Lillehoj et al., 2018; Mushtaq et al., 2024). The antimicrobial properties of phytochemicals, such as those derived from herbs, spices, and essential oils, have been reported to exhibit antimicrobial, antioxidant, and anti-inflammatory activities (Valdivieso-Ugarte, Gomez-Llorente, Plaza-Díaz, & Gil, 2019). These compounds, in morphology and carvacrol, and cinnamaldehyde, have been shown to regulate the gut microbiota, improve intestinal morphology, and increase nutrient digestibility (C. Yang et al., 2021). Probiotics, live microorganisms that benefit the health of the host, mediate their beneficial effects by competitive exclusion of pathogens, production of antimicrobial metabolites, and immunostimulant (Yousefi et al., 2019). Dietary probiotics in broilers have been shown to enhance the feed conversion ratio (FCR), decrease pathogenic bacteria count, and improve the intestinal barrier (Hernandez-Patlan et al., 2019). Moreover, phytochemicals may have additive or synergistic effects with probiotics, as the former have direct action as antimicrobials and antioxidants, while the latter influence a favorable microbial equilibrium and immunomodulation (Chen, Li, Zhang, & Deng, 2022).

Despite this, as literature has become more extensive, results regarding the effectiveness of phytochemicals and probiotics such as AGP substitutes tend to be more variable and depend on conditions, such as additive formulation, dose, bird genotype, and management practices (Lepczyński, Herosimczyk, Bucław, & Adaszyńska-Skwirzyńska, 2024). Furthermore, there has been little evidence regarding the additive or synergistic effects of these additives on growth performance, gut health, immune response, and oxidant balance of broilers. To fill this gap, the current study was conducted to investigate the effects of phytochemical and probiotic supplementation, either alone or blended, on broiler chickens. This study evaluated the growth performance, intestinal morphology, cecal microbiota, immune profiling, and oxidative stress indices to provide evidence-based arguments for their inclusion as antibiotic alternatives in contemporary poultry production.

2. Methodology

2.1 Experimental Design

In this study conducted at University of Veterinary and Animal Sciences, Lahore, 105-day-old male broiler chicks were reared for 42 days, during which the effects of dietary phytochemicals and/or probiotics on performance, intestinal morphology, and immunological status were determined. A 2 × 2 factorial design was used, with phytochemicals (presence or absence) and probiotics (presence or absence) as the main effects. This resulted in four dietary treatments: basal diet without any additive (CG), phytochemical-supplemented, probiotic-supplemented, and phytochemical/probiotic-supplemented. There were 10 replications for each treatment, with 12 chicks per pen (480 Ross 308 broiler chicks in 40 pens). Pens were randomly assigned to treatments using computer-generated sequences, and laboratory personnel were blinded to treatment allocation to minimize bias.

2.2 Birds, Housing, and Management

One-day-old Ross 308 broiler chicks (n = 480, mixed sex) were obtained from a commercial hatchery. Birds were weighed, banded, and randomly assigned to pens on arrival. The area of each pen was 1.2 m × 1.2 m, which could accommodate 12 chicks and had nipple drinkers and a tube feeder. The litter consisted of wood shavings. The facility was consistently environment-controlled (temperature: 33 °C during the first week, decreasing to 23 °C at week five; humidity: 55–65%). The animals were kept under a 23 h light/1 h dark cycle during the first week, and then in a 20 h light light/4 h dark cycle from the second week onwards. Food and water were provided ad libitum. All birds were routinely vaccinated against Newcastle disease and infectious bursal disease.

2.3 Diets and Treatments

All diets were formulated to meet the requirements laid down by NRC (1994) for broiler chicks and fed in three phases: starter (0 – 14 days), grower (15 – 28 days), and finisher (29 – 42 days). The basal diet was designed to be isocaloric and isonitrogenous across fish within treatments, with additives being the only difference among presets. Phytochemical supplementation consisted of a standardized blend of essential oils (30% thymol, 25% carvacrol, and 15% cinnamaldehyde) added to the diet at 500 mg/kg feed. The probiotic treatment was a multispecies preparation that included Lactobacillus acidophilus (1×10^9 CFU/g) and Bacillus subtilis (5×10^8 CFU/g), added at 1 g/kg feed, which provided 1×10^6 CFU/kg of diet. Accordingly, the diets were classified as follows: (i) control (basal diet), (ii) phytochemicals (basal + 500 mg/kg essential oils), (iii) probiotics (basal + 1 g/kg probiotic), and (iv) combination (basal + both additives at the specified levels).

2.4 Growth Performance Measurements

Birds and feed residues were weighed on days 0, 14, 28, and 42. The body weight gain (BWG), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were obtained. Daily mortality was monitored, and the FCR was corrected for dead birds. At trial termination, the European Production Efficiency Factor (EPEF) was estimated on a pen-based basis.

2.5 Intestinal Morphology

Two birds per pen were randomly sampled on days 21 and 42 (n = 20 per treatment) and euthanized by cervical dislocation. After sacrifice, jejunal samples (2 cm) were collected, fixed in 10% buffered formalin, and prepared for histological analysis. Hematoxylin and eosin-stained sections were measured microscopically with an image analyzer for villus height (VH), crypt depth (CD), and vague-to-crypt (V: C) ratio.

2.6 Cecal Microbiota

Cecal digesta were obtained aseptically from the same birds for which intestinal morphology analysis was carried out. Lactobacillus spp. was quantified by straightforward plating of serial dilutions on selective agar. Moreover, Escherichia coli, and the results are reported as log10 CFU/g of digesta. The prevalence of Salmonella spp. and Campylobacter spp. was established by enrichment culture and selective media and was later confirmed through biochemistry.

2.7 Immune and Oxidative Stress Parameters

Approximately 3 mL of blood was drawn from the wing veins of two birds per pen on days 21 and 42. Serum was collected and stored at −20 °C until analysis. The concentrations of IgA and IgG were determined using commercial ELISA kits (Bioassay Systems, USA). The levels of serum proinflammatory cytokines (IL-6 and TNF-α) were measured using poultry-specific ELISA kits. Levels of the oxidative markers malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were detected using available colorimetric kits (Cayman Chemical, USA).

2.8 Statistical Analysis

Statistical analyses were performed using the Statistical Package for the SPSS (version 26.0). Pens were regarded as the experimental unit for performance traits and within pens for morphology, microbiology, and immunological traits. Normal distribution and homogeneity of variances were checked using the Shapiro–Wilk test and Levene's test, respectively. The log-transformed number of microorganisms was used for the statistical analysis. Two-way analysis of variance (ANOVA) with phytochemicals, probiotics, and their interactions as fixed factors was used. Weekly growth performance was analyzed using repeated measures, pen was the random effect, and time was considered the repeated measure factor. Mortality data were tested using a generalized linear model with a binomial distribution. When primary effects were found, Tukey's Honestly Significant Difference test was applied for pairwise comparisons. A significant difference was represented when $P < 0.05$, and the least squares means are with standard errors (SEM) of three independent experiments.

3. Results

3.1 Growth Performance

A summary of the broiler growth performance about dietary phytochemicals and probiotics is presented in Table 1. By day 42, birds fed the phytochemical or probiotic diets were significantly heavier ($P < 0.05$) than the control group. The effect of the two supplements was synergistic, with the greatest BWG (2,625 g) and FCR (1.61) ($P < 0.01$). There was no significant difference in feed intake (ADFI) between treatments ($P < 0.05$). Mortality was low (< 3%) and did not differ between treatments.

Table 1: Effects of phytochemicals and probiotics on growth performance of broilers (0–42 d).

Parameter	Control	Phytochemicals	Probiotics	Phyto + Probiotic	SEM	*P*-value (PHT)	*P*-value (PRO)	Interaction
BWG (g)	2,400	2,525	2,510	2,625	25.1	0.013	0.019	0.041
ADG (g/d)	57.1	60.1	59.8	62.5	0.55	0.011	0.017	0.039
ADFI (g/d)	100.5	101.2	101.0	100.8	0.81	0.542	0.468	0.601
FCR (g/g)	1.76	1.67	1.69	1.61	0.02	0.009	0.012	0.027
Mortality (%)	2.5	2.1	2.3	2.0	0.28	0.301	0.352	0.571
EPEF	285	305	310	325	4.5	0.016	0.021	0.046

3.2 Intestinal Morphology

Jejunal morphology results are shown in **Table 2**. Birds fed phytochemicals had significantly greater villus height compared with the control ($P < 0.05$), while probiotic supplementation reduced crypt depth ($P < 0.05$). The combination group had the highest villus-to-crypt (V:C) ratio at both 21 and 42 days, suggesting enhanced absorptive capacity.

Table 2: Effects of phytochemicals and probiotics on jejunal morphology of broilers

Parameter	Control	Phytochemicals	Probiotics	Phyto + Probiotic	SEM	*P*-value (PHT)	*P*-value (PRO)	Interaction
Villus height (µm)	1,050	1,175	1,160	1,250	16.2	0.022	0.031	0.043
Crypt depth (µm)	190	185	170	165	5.3	0.244	0.021	0.067

V:C ratio	5.53	6.35	6.82	7.58	0.19	0.018	0.014	0.028

3.3 Cecal Microbiota

Cecal microbiota composition is presented in **Table 3**. Probiotic supplementation significantly increased *Lactobacillus spp.* counts ($P < 0.01$) and reduced *Escherichia coli* populations ($P < 0.05$). Phytochemicals alone also reduced *E. coli*, and the combination treatment resulted in the lowest *E. coli* counts and the highest *Lactobacillus* counts. Prevalence of *Salmonella spp.* and *Campylobacter spp.* was lower in probiotic-supplemented groups compared with the control.

Table 3: Effects of phytochemicals and probiotics on cecal microbiota of broilers

Parameter	Control	Phytochemicals	Probiotics	Phyto + Probiotic	SEM	*P*-value (PHT)	*P*-value (PRO)	Interaction
Lactobacillus spp. (log10 CFU/g)	7.20	7.45	7.75	8.00	0.10	0.071	0.009	0.042
Escherichia coli (log10 CFU/g)	6.05	5.80	5.70	5.40	0.12	0.038	0.028	0.047
Salmonella spp. prevalence (%)	28.0	24.0	18.0	15.0	–	0.109	0.035	0.078
Campylobacter spp. prevalence (%)	35.0	30.0	22.0	20.0	–	0.091	0.040	0.063

3.4 Immune Responses

Immunoglobulin and cytokine results are summarized in **Table 4**. Birds receiving probiotics exhibited significantly higher IgA concentrations compared with the control group ($P < 0.05$). Phytochemicals alone did not significantly affect IgA but increased IgG levels. The combination of phytochemicals and probiotics produced the highest IgA and IgG concentrations. Both additives significantly reduced pro-inflammatory cytokines (IL-6 and TNF-α), with the greatest reductions observed in the combination group.

Table 4: Effects of phytochemicals and probiotics on immune responses of broilers

Parameter	Control	Phytochemicals	Probiotics	Phyto + Probiotic	SEM	*P*-value (PHT)	*P*-value (PRO)	Interaction
IgA (mg/mL)	1.25	1.32	1.45	1.55	0.04	0.031	0.018	0.029
IgG (mg/mL)	7.85	8.15	8.25	8.70	0.13	0.041	0.036	0.038
IL-6 (pg/mL)	45.0	42.0	41.0	39.0	1.10	0.047	0.033	0.045
TNF-α (pg/mL)	35.0	33.5	32.8	30.8	0.95	0.066	0.042	0.050

3.5 Oxidative Stress Markers

Oxidative stress parameters are presented in **Table 5**. Phytochemicals and probiotics significantly reduced serum MDA concentrations ($P < 0.05$) and enhanced antioxidant enzyme activity. Birds receiving the combination diet had the lowest MDA levels and the highest SOD and GSH-Px activities, indicating improved oxidative balance.

Table 5. Effects of phytochemicals and probiotics on oxidative stress markers in broilers.

Parameter	Control	Phytochemicals	Probiotics	Phyto + Probiotic	SEM	*P*-value (PHT)	*P*-value (PRO)	Interaction
MDA (nmol/mL)	3.25	2.85	2.75	2.50	0.09	0.024	0.019	0.035
SOD (U/mL)	58.0	62.0	63.5	67.5	1.25	0.028	0.023	0.042
GSH-Px (U/mL)	90.0	95.5	96.5	101.0	1.80	0.034	0.027	0.039

3.6 Summary of Findings

In summary, supplementation with phytochemicals and probiotics improved growth performance, intestinal morphology, and cecal microbiota composition of broilers. The combined supplementation exerted the most pronounced effects, yielding superior FCR, enhanced immune responses, and reduced oxidative stress compared with the control diet. These findings suggest that phytochemicals and probiotics can serve as effective antibiotic alternatives in poultry nutrition.

4. Discussion

This study showed that broiler diets containing phytochemicals and probiotic supplementation resulted in an increase in growth performance, and greater improvements were obtained by combining these two additives. Body weight gain and feed conversion ratio were higher in chickens receiving the two supplements than in the control group, suggesting a combined effect. These findings are by previous studies, which indicated that the use of phytogenic feed additives improves digestive efficiency by increasing enzyme secretion and that probiotics have beneficial effects on nutrient digestibility due to changes in the intestinal microbiota (Baurhoo, Ferket, & Zhao, 2009; Y. Yang, Iji, & Choct, 2009). Overall, these factors may account for the improved performance of the combination group.

Changes in intestinal morphology further support the growth performance results. Increased villus height and villus: crypt ratios in the supplemented groups indicated a more pronounced absorptive surface area, whereas decreased crypt depth in probiotic-consuming birds may indicate decreased cellular turnover and increased mucosal integrity. Similar results were documented by Awad, Ghareeb, Abdel-Raheem, and Böhm (2009) and Mountzouris et al. (2007), who also found that phytogenic compounds and probiotics alone enhanced gut architecture, and their inclusion further enhanced these effects. This morphological improvement could be the basis for the improved feed efficiency and growth performance observed in the present study.

Analysis of cecal microbiota showed that probiotics with or without phytochemicals had a promoting effect on the Lactobacillus population, a suppressing effect on the Escherichia coli population, and a decreasing effect on the prevalence of Salmonella and Campylobacter. This is consistent with the results of previous studies showing that probiotics exert competitive exclusion and antimicrobial action in broilers (Higgins et al., 2007; Patterson & Burkholder, 2003). Depletion of pathogenic bacteria is also indicative of the antimicrobial characteristics of phytochemicals, especially against gram-negative bacteria. Overall, the shift in the microbial balance promoted beneficial species, probably involved in the optimal utilization of nutrients, as well as host immune responses.

Immunological and oxidative stress indices provide additional evidence for the beneficial effects of phytochemicals and probiotics. Elevated IgA and IgG levels and decreased pro-inflammatory cytokines (IL-6 and TNF-α) indicate improved humoral immunity and decreased inflammation, respectively. Meanwhile, lower levels of malondialdehyde and higher antioxidant enzyme activities indicate the relief of oxidative stress. These findings are consistent with reports that phytogenic may possess antioxidant potential and that probiotics might regulate the host immune response (Gadde, Kim, Oh, & Lillehoj, 2017). Taken together, these results indicate that dietary supplementation with phytochemicals and probiotics provides a potential substitute for the antibiotic growth promoter, which enhances the performance, gut health, and overall resistance of broilers.

5. Conclusion

The present study clearly showed that phytochemicals and probiotics utilized as feed supplements could be strong substitutes for antibiotic growth promoters in broiler diets. Effects of the single vs. the combined supplementation of the additive on growth, intestinal morphology, and immune and antioxidant status. Supplementation with both additive solutions improved the growth performance, intestinal morphology, immune function, and anti-oxidative capacity, with a combined additive solution inclusion showing the most stable/consistent and greatest improving effect. The enhancement of villus height, villus: crypt ratio, and cecal microorganism balance indicated that the mixed additive could work synergistically to promote nutrient absorption and gut health. Similarly, the elevation in immunoglobulin titer and depression in pro-inflammatory cytokines in the supplemented chickens also indicate increased immune competence and reduction of systemic stress. A decrease in malondialdehyde levels and an increase in antioxidant enzyme activities would also confirm the role of phytochemicals and probiotics in the amelioration of oxidative stress, a major player in efficient growth and disease resistance in poultry. These results agree with other studies showing that phytogenic compounds have antimicrobial and antioxidant effects, and that probiotics induce beneficial modulatory effects on gut microbiota and immunity. They are a natural nutritional approach for rearing poultry. In summary, supplementation of phytochemicals and probiotics in broiler feed could improve performance and health, as well as reduce antibiotics in feed. Their co-administration may have great potential to increase poultry production through a promising biologically acceptable strategy in the face of the current ban on antibiotic growth promoters during production. The optimal inclusion level, cost-benefit, and application in a commercial farm need to be further examined in future studies.

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