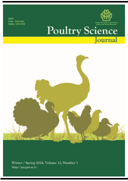

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The Influence of Different Levels of Postbiotic and Phytobiotic Combinations as Feed Additives on Growth Performance, Gut Morphology, and Faecal Bacteria In Broiler Chickens

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Abstract

The current study examined the influence of different levels of postbiotic and phytobiotic combinations on growth performance, gut morphology, and fecal bacteria in broiler chickens. 288 one-day old 308 Ross unsexual broiler chickens were allocated into 6 treatments. Each group had 4 replicates, and each replicate had 12 birds. The treatments included: basal diet without any additive (negative control,NC), basal diet + 0.01% Doxin 200 antibiotic (positive control,PC), basal diet + 0.05 thyme oil + 0.05 postbiotic (0.1% Pos+Phy), basal diet + 0.1 thyme oil + 0.1 postbiotic (0.2% Pos+Phy), basal diet + 0.15 thyme oil + 0.15 postbiotic (0.3% Pos+Phy), and basal diet + 0.2 thyme oil + 0.2 postbiotic (0.4% Pos+Phy). The results showed that broiler chickens fed 0.4% Pos+Phy significantly increased in live body weight and weight gain at 35 days compared to the NC and all other treatments. Also, feed intake increased at different levels compared to the NC and was similar to the PC. The feed conversion ratio was significantly enhanced in birds fed at 0.3% and 0.4% compared to the NC and was not significant within the PC. Generally, the gut morphology at different levels of Pos+Phy was significantly improved. The villi height and width were increased at 0.3% and 0.4% compared to the NC and PC. A higher VH: CD ratio and villus absorptive surface area were found in treatments fed at a level of 0.4%. The faecal lactic acid bacteria count increased at 0.2%, 0.3%, and 0.4% compared to the NC and PC. The *Enterobacteriaceae* count decreased at level 0.4% compared to the NC, but no significant differences compared to the PC and all other treatments. The 0.4% Pos+Phy-thyme oil is recommended as a new feed additive and growth promoter as an alternative to antibiotics in broiler.

Introduction

Global demand for broiler chickens production has seen a significant surge, accompanied by increased commercial profits. This expansion necessitates rapid economic growth and cost-effective production methods (Haque *et al*., 2020). Recently, alternative feed additives such as postbiotics and paraprobiotics have emerged as successful options. These safer derivatives of probiotic cultures have been employed to create antibiotic-free chicken products by promoting healthy gut microbiota and preventing the growth of harmful organisms, particularly during early developmental stages (Abd El-Ghany *et al*., 2022). Although probiotics are living organisms with a limited lifespan, they continue to benefit the host by releasing postbiotics, which are advantageous nutrients. Postbiotics are the metabolic by-products generated by probiotic organisms during the later stages of growth. Lactic acid bacteria are responsible for producing postbiotics, which encompass a variety of bioactive compounds such as teichoic acid, peptidoglycan-derived muropeptides, secreted proteins, surface and exopolysaccharides, bacteriocins, organic acids, short-chain lipids, carbohydrates, antimicrobial peptides, vitamins, and digestive enzymes (Moradi *et al*., 2019).

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Research by Kareem *et al*. (2016) discovered that incorporating 0.3% postbiotics and prebiotics into broiler feed significantly improved parameters like body weight, feed conversion ratio, gastrointestinal characteristics, and the expression of growth-related genes. Furthermore, adding 0.3% postbiotics to the birds' diet resulted in a notable decrease in *enterococcus* count and a significant increase in lactic acid bacteria count compared to the negative control group (Mohammed and Kareem, 2022b). Derived from *Lactiplantibacillus plantarum*, postbiotics offer promising alternatives to antibiotics and antioxidants in the poultry industry (Humam *et al*., 2021).

Farmers have long used phytobiotics to enhance chicken health. Phytobiotics, traditional food additives, are growth enhancers derived from plants, spices, and their derivatives (Sugiharto, 2016). These natural substances are gaining popularity as potential replacements for antibiotic growth promoters due to their origin, availability, safety, and lack of residual effects (Abed El-Hack *et al*., 2022).

With diverse biological properties, including anti-inflammatory and antimicrobial effects, phytobiotics, also called phytochemicals or phytogenics, have recently gained traction as natural alternatives to synthetic antibiotic growth promoters (Kikusato, 2021). Beyond improving animal product quality and growth performance, phytobiotic additives can enhance digestive enzyme secretion, enhance immunity, stimulate appetite, and exhibit bactericidal, antiviral, and antioxidant properties (Krauze *et al*., 2021). Based on previous studies, it is conceivable to frame the hypothesis that varying levels of postbiotics and phytobiotics could potentially benefit both growth performance and the faecal lactic acid bacterial composition of broiler chickens. However, the synergistic effects arising from the simultaneous use of postbiotics and phytobiotics in broiler chicken diets have not been thoroughly examined. This study aims to determine the potential effects of combinations of postbiotics and phytobiotics (thyme oil) on broiler chicken growth, gut morphology, and counts of lactic acid bacteria and *Enterobacteriaceae* in faecles.

Methods and Materials

Postbiotic produce and phytobiotic provide

The stock culture of Lactobacillus plantarum (*L.plantarum*) was prepared at the Laboratory of animal resourse in the College of Agricultural Engineering Science, Salahaddin University-Erbil, Kurdistan Region, Iraq. To begin, 3 liters of MRS broth (de Man, Rogosa, and Sharpe, Microbiology, Germany) medium were prepared following the manufacturer's instructions by dissolving 52.2 g in 1 liter of distilled water. The solution was then sterilized by autoclaving for 15 minutes at 121°C to eliminate the growth of pathogenic bacteria.

This experiment used a species of probiotic lactic acid bacteria (Lactobacillus plantarum) to produce postbiotics. This strain had been previously isolated from a locally fermented dairy product and stored at -20 °C in MRS broth, which included 20% (v/v) glycerol as a cryoprotectant. The stock cultures were twice revived in MRS broth and incubated for 24 and 48 hours under static conditions at 30°C. Plate spreading was performed on the revived cultures, which were then incubated for 48 hours. A single colony was selected and inoculated into 10 mL MRS broth for 24 hours. After another 24 hours of re-culturing the colony in the same amount of broth, the culture was considered ready to be used as a fermentation inoculum. Using 2 mL of the inoculum, the bacteria were inoculated into 1000 mL of the respective media and incubated for 48 hours at 30°C under static conditions. Subsequently, centrifugation at 10,000 rotations per minute (rpm) for 15 minutes was conducted to separate the bacterial cells from the culture and obtain the postbiotics (Figure 1, Kareem *et al*., 2016). The native phytobiotic thyme oil was obtained from a company in Duhok Governorate that produces dietary supplements and essential oils under the brand name Aram Factory.

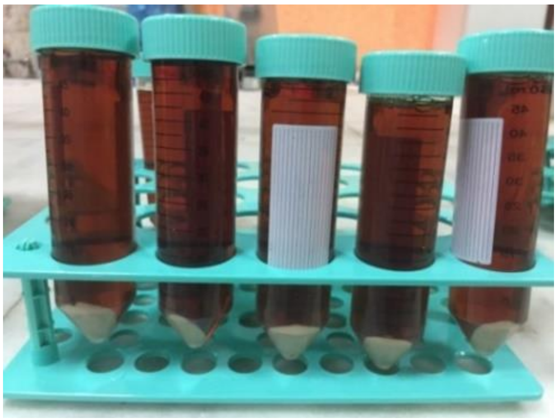

Figure 1. Postbiotics produce

Broiler chicken management and experimental design

The experiment was conducted at the Directorate of Agricultural Research farm in the Duhok government between November 11, 2022, and December 15, 2022. A total of 288 one-days old, unsexed birds from Ross 308 broiler chicks were purchased from a local poultry hatchery in Erbil. The broiler chickens were allocated into sex treatment groups. Each group had four replicates, and each replicate had twelve birds. The treatments were as follows: negative control (NC, basal diet without any additive), positive control (PC, basal diet + 0.01% Doxin200 (w/w)), 0.1%Pos+Phy (basal diet + 0.05% thyme oil + 0.05% postbiotic), 0.2%Pos+Phy (basal diet + 0.1% thyme oil + 0.1% postbiotic), 0.3% Pos+Phy (basal diet + 0.15% thyme oil + 0.15% postbiotic), and 0.4% Pos+Phy (basal diet + 0.2% thyme oil + 0.2% postbiotic(v/w)). Chicken broilers were managed according to Ross 308 Guidance. The stocking density (12 birds per 1.21m^2) allowed for the well-being of chicks. The birds were raised floor system in wire pens with dimensions of 110 cm in length, 110 cm in width, and 60 cm in height. The chicks were provided with *ad libitum* access to both feed and water. Throughout the experiment, 5 cm thick of wood shavings was used as the floor litter. The farming environment was carefully controlled; during the initial week, the temperature ranged from 33 to 32°C, gradually decreasing by 2°C each week, and by the fifth week, it was maintained at 22 to 24 °C. Humidity levels were maintained between 50% and 60%. Ventilation was adjusted as the birds aged. The lighting schedule consisted of 24 hours of continuous light during the first week, followed by 23 hours of light and 1 hour of darkness from the second week until day 35. Chicks received the starter ration from day 1 to day 10, the grower ration from day 11 to day 24, and the finisher ration from day 25 to day 35 (Table 1).

Table 1: Ingredients and composition of experimental basal diet (day 1–35 days).

Ingredient (%)	Starter diet (1-10 days of age)	Grower diet (11-24 days of age)	Finisher diet (25-35 days of age)
Broiler concentrates (5%) [A]	5	5	3
Corn	45	50	49
Soybean meal (48%) [B]	31	27.4	23
Wheat	14.1	12	19.2
Vegetable oil	2.3	3	3.2
Limestone	1.3	1.3	1.4
Di-calcium phosphate	0.6	0.6	0.6
Salt	0.2	0.2	0.2
DL-Methionine	0.17	0.17	0.17
L-Lysine	0.13	0.13	0.13
Threonine	0.1	0.1	0.1
Antitoxin	0.05	0.05	0.00
Anticoccidial	0.05	0.05	0.00
Composition%	100	100	100
NIR analyses of diets (%):			
ME (kcal/ kg)	2922	2986	3050
Crude protein (%)	22.33	21.44	20.14
Crude fat (%)	3.3	4.36	5.17
Crude fiber (%)	2.58	2.69	2.84
Ash (%)	8.63	7.15	5.66
Ca (%)	0.93	0.98	1.07
P (available) (%)	0.58	0.6	0.6

A- 5%Broiler concentrate means the ratio mixing to feed, that contains: 40%Cp, 2.3%CF, 4.5%CF , 3.5 Lysine digestible, 3.4 Methionine dig., 4.1 Meth.+Cystine dig., 0.53 Tryptophan dig., Vitamin and mineral mix supplied/kg of diet: 12000 IU Vit A , 2200 IU Vit D3 , 10 mg Vit E , 2 mg Vit K3 , 1 mg Vit B1, 4 mg Vit B2, 1.5 mg Vit B6, 10 mg Vit B12, 20 mg Niacin, 10 mg Pantothenic acid, 1 mg Folic acid, 50 mg Biotin, 10 mg Copper, 1 mg Iodine, 30 mg Iron, 55 mg Manganese, 50 mg Zinc, and 0.1 mg Selenium. B- 48% ratio of crude protein.

Gut Morphology

The histomorphology of the guts was measured as described in the method used by Humam *et al*., 2019. Measurement for histomorphology was conducted at the Vin Privet Hospital laboratory in Duhok City. Following the slaughter of the broiler chickens, samples of the intestinal tract were extracted from eight chickens subjected to each experimental treatment for subsequent analysis. A segment approximately 5–6 cm in length was taken from the jejunum, spanning from the entrance of the bile ducts to Meckel's diverticulum, as depicted in (Figure 2).

Subsequently, the segments were excised to around 3mm and then immersed in a 10% neutral buffered formalin solution. After an overnight incubation in plastic cassettes, the samples were fixed. Following that, the intestinal samples underwent dehydration using a tissue processing device (Leica ASP 3000, Tokyo, Japan) and were subsequently encased in paraffin wax (Leica EG 1160, Tokyo, Japan). The intestinal specimens were then trimmed to a thickness of 30 µm, with subsequent sections of 4 µm being sliced and affixed onto slides on a hot plate set at 60°C. Following this, the slides were subjected to hematoxylin and eosin staining, after which they were mounted and examined using light microscopes. The final phase of the trial encompassed an evaluation of jejunal histomorphology. Using Dinocapture 2.0 software version 1.4.0.B, the histological sections were examined employing a low-power microscope (10x) to measure various parameters, including villus height, villus width, crypt width, and the ratio of villus height to crypt depth. Further, the mean absorptive surface area (ASA) was also calculated with the help of the following formula by Kisielinski *et al*., (2002):

$$ASA\ (mm)^2 = [\ (\text{villus width} \times \text{villus length}) + (\text{villus width}/2 + \text{crypt width}/2)^2 - (\text{villus width}/2)^2]\ /\ [(\text{villus width}/2\ +\ \text{crypt width})/2)^2\ ]$$

Figure 2. Villus morphometric measurements: 1-villus height; 2- crypt depth; 3- villus width.

Faecal lactic acid bacteria (LAB), *Enterobacteriacaeae* (ENT) count, and pH determination

At 35 days of age, fresh faecal droppings were aseptically collected from all groups of broiler chickens. Using sterile plastic boxes, four samples of feces were collected for each treatment group and stored at -20 °C before laboratory analysis. To determine fecal lactic acid bacteria (LAB) and *Enterobacteriacaeae*, (ENT) populations, we followed the method outlined by Goh *et al*. (2020). Faecal samples were left at room temperature for 1 hour after performing a 10-fold dilution (w/v) in sterile peptone water. Subsequently, 10-fold serial dilutions (v/v) were carried out. We used MRS-agar (*Lactobacillus*-Agar MRS) for LAB counts, and the plates were incubated in anaerobic jars at 30°C for 48 hours.

For the determination of Enterobacteriacaeae, incubation was performed aerobically for 24 hours at 37°C after spreading and counting them on EMB–Agar (Eosin-methyleneblue Lactose Sucrose Agar, Merck, KgaA, and Darmstadt). Colony forming unit (CFU) values were expressed as the base 10 logarithm of CFU (log CFU) per gram. Calculation of the colonies (1-250) was conducted using the equation CFU = total colonies count (up to 250 colonies) x 10, with triplicate samples.

Approximately 9 ml of deionized distilled water was used to homogenize 1 gram of fecal sample in a universal tube to determine the pH value of fecal samples. We measured the pH value of feces using a Mettler-Toledo pH meter with a glass electrode (Mettler-Toledo LTD, England). The pH meter was calibrated with buffer solutions at pH 4 and 7 before measuring the pH of the fecal samples (Choe *et al*. 2012).

Statistical analysis

Statistical analysis was conducted using SAS package 9.1 software using a completely randomized design procedure (CRD) model (SAS Institute, 2012). Duncan's multiple range test was used to compare the significant differences between the treatment means at a probability level of ($P \leq 0.05$).

Results

Growth Performance

The influence of different levels of feed additives on final growth performance is presented in Table 2. The live body weight and total weight gain significantly ($P \leq 0.05$) increased with increased levels of a combination of postbiotic and phytobiotic from 0.1% to 0.4% Pos+Phy in broiler chicks' dietary supplements. In the last week (35 days) of the experiment, the average WG of birds in T6 that were fed a 0.4% Pos+Phy supplementation diet reached 2209.23 g/bird, and it had the higher weight compared to all treatment groups. However, the average WG of birds fed a level of 0.3% Pos+Phy of the combination of postbiotic and phytobiotic reached 2133.80 g/bird, which was significantly similar to the average of birds fed antibiotic (positive control), and it had the higher weight compared to that of birds fed supplement diet in levels 0.1%, 0.2% Pos+Phy, and negative control. There was a directly proportional relationship between increasing the level of feed additives and increasing body weight. The broiler chicken's cumulative feed consumption from 1 to 35 days of age increased significantly in all treatment groups fed different levels (0.1%, 0.2%, 0.3%, and 0.4% Pos+Phy) of experimental diet supplement compared to bird groups in the positive control that were fed antibiotics. Additionally, there were significant differences ($P \leq 0.05$) in the birds in FCR among all experimental treatment groups in the final weeks (5th). The cumulative FCR of broiler chickens fed levels 0.3% Pos+Phy, 0.4 Pos+Phy, and positive control were significantly improved compared to that of birds fed levels 0.1% Pos+Phy, 0.2% Pos+Phy, and negative control.

Table 2. Effect of different levels of postbiotic and phytobiotic combination on growth performance of broilers (at 35 days)

Treatment[A]	Initial body weight (g)	Live body weight (g)	Total weight gain (g)	Feed intake (g)	Feed conversion ratio
Negative Control	39.38	1982.48[d]	1943.10[d]	3437.73[a]	1.77[a]
Positive Control	39.05	2163.53[b]	2121.48[b]	3241.98[b]	1.51[d]
0.1%Pos+Phy	38.92	2047.78[cd]	2008.85d[c]	3418.60[a]	1.70[ab]
0.2%Pos+Phy	39.03	2083.68[c]	2044.60[c]	3368.15[a]	1.64[bc]
0.3%Pos+Phy	38.88	2172.68[b]	2133.80[b]	3367.63[a]	1.57[cd]
0.4%Pos+Phy	39.23	2248.33[a]	2209.23[a]	3466.03[a]	1.56[cd]
P-value	0.767	0.0001	0.0001	0.014	0.0002
SEM [B]	0.095	20.349	20.380	22.315	0.021

a-d meanings with different superscripts within columns are significantly different ($P \leq 0.05$).
[A] negative control (NC, basal diet without any additive), positive control (PC, basal diet + 0.01% doxin200(w/w)), 0.1%Pos+Phy (basal diet + 0.05% thyme oil + 0.05% postbiotic), 0.2%Pos+Phy (basal diet + 0.1% thyme oil + 0.1% postbiotic), 0.3% Pos+Phy (basal diet + 0.15% thyme oil + 0.15% postbiotic), and 0.4% Pos+Phy (basal diet + 0.2% thyme oil + 0.2% postbiotic(v/w)).
[B] *SEM*, standard error of the means (pooled).

Gut Morphology

Table 3 shows the results of the effects of different levels of postbiotic and phytobiotic combinations on jejunum parts morphology of gut parameters, which include villi height, villi width, crypt depth, villi height: crypt depth (VH: CD)ratio, and absorptive surface area in broiler chickens. Data analyses found that villi height was significantly increased compared to levels 0.1% Pst+Phy ,0.2% Pst+Phy and positive control. No significant differences ($P \geq 0.5$) across bird's group 0.1% pos+Phy, 0.2% Pos+Phy and birds fed antibiotic (PC). Villi width had significantly increased in experimental treatment groups 0.3 Pos+Phy compared all other treatment group. No significant differences ($P \geq 0.5$) on villi width between birds fed 0.4 Pos+Phy and 0.1% Pos+Phy. It was also observed that crypt depth was significantly improved ($P \leq 0.05$) in all experiment treatment groups (0.1, 0.2, 0.3, and 0.4 Pos+Phy) of the experimental diet compared to the negative control, and there were no significant differences ($P \geq 0.05$) compared to the positive control. No significant differences in crypt depth were found among all experimental treatment groups (0.1, 0.2, 0.3, and 0.4 Pos+Phy) fed different levels of the experimental diet compared to the positive control that was fed antibiotics. No significant differences were observed in the ratio of VH: CD and the absorptive villus surface area among the experiment treatments, which they fed at diet levels of 0.1, 0.2, and 0.3 Pos+Phy compared to the positive control. However, a higher ratio of VH: CD and the absorptive villus surface area were recorded in broiler chickens' villus at 0.4 Pos+Phy compared to all other experimental treatment groups.

Faecal lactic acid bacteria (LAB), *Enterobacteriaceae* (ENT), and pH in broiler chickens

The effect of different levels of postbiotic and phytobiotic combinations on lactic acid bacteria and Enterobacteriaceae population count and pH value in broiler chickens' faecals at age 35 days is shown in Table 4. The data analysis results demonstrated that various levels of 0.1, 0.2, 0.3, and 0.4% of Pos+Phy significantly increased ($P \leq 0.05$) the LAB population in all experimental treatment groups compared to the negative control and positive control. No significant differences (P ≥ 0.05) were shown among 0.1% Pos+Phy , positive control, and negative control. The lowest ENT population was recorded in birds fed 0.4 Pos+Phy, and there were no significant differences (P ≥ 0.05) across 0.4 Pos+Phy, 0.3 Pos+Phy, 0.2 Pos+Phy, and positive control. In addition, no significant differences (P ≥ 0.05) in faecal pH values were shown among all experimental treatment groups compared to the negative control and positive control; a lower pH (4.8) was recorded at 0.4 % Pos+Phy.

Table 3. Effect of different levels of postbiotic and phytobiotic combination on jejunum parts morphology in broilers

Treatments [A]	Villi height (um)	Villi width (um)	Crypt depth (um)	VH: CD Ratio	absorptive villus surface area (um)2
Negative Control	1129[c]	130[d]	173[b]	6.5[c]	7100[c]
Positive Control	1237[b]	135[d]	175[ab]	7.2[bc]	7800[bc]
0.1%Pos+Phy	1220[b]	154[bc]	182[ab]	6.8[bc]	7500[bc]
0.2%Pos+Phy	1273[b]	146[cd]	188[a]	6.8[bc]	7500[bc]
0.3%Pos+Phy	1365[a]	197[a]	188[a]	7.3[b]	7900[b]
0.4%Pos+Phy	1389[a]	162[b]	174[ab]	8.4[a]	9100[a]
P-value	0.0001	0.0001	0.045	0.0001	0.033
SEM [B]	12.953	2.99	1.978	0.117	115

a-d meanings with different superscripts within columns are significantly different (P≤0.05).

[A] negative control (NC, basal diet without any additive), positive control (PC, basal diet + 0.01% doxin200(w/w)), 0.1%Pos+Phy (basal diet + 0.05% thyme oil + 0.05% postbiotic), 0.2%Pos+Phy (basal diet + 0.1% thyme oil + 0.1% postbiotic), 0.3% Pos+Phy (basal diet + 0.15% thyme oil + 0.15% postbiotic), and 0.4% Pos+Phy (basal diet + 0.2% thyme oil + 0.2% postbiotic(v/w)).

[B] *SEM*, standard error of the means (pooled).

Table 4. Effect of different levels of postbiotic and phytobiotic combination on Lactic acid bacteria counts, *Enterobacteriaceae* counts, and pH in broiler chickens fecal at 35 days (log CFU/g)

Treatments [A]	*lactic acid bacteria* LAB	*Enterobacteriaceae* ENT	pH
Negative Control	3.0[b]	2.6[a]	5.4
Positive Control	2.8[b]	1.9[b]	5.4
0.1%Pos+Phy	4.2[ab]	2.7[a]	5.7
0.2%Pos+Phy	5.3[a]	2.5[ab]	5.2
0.3%Pos+Phy	5.5[a]	2.4[ab]	5.2
0.4%Pos+Phy	4.7[a]	2.0[b]	4.8
P-value	0.011	0.054	0.680
SEM [B]	0.294	0.096	0.151

a-b meanings with different superscripts within columns are significantly different (P≤0.05)..

[A] negative control (NC, basal diet without any additive), positive control (PC, basal diet + 0.01% doxin200(w/w)), 0.1%Pos+Phy (basal diet + 0.05% thyme oil + 0.05% postbiotic), 0.2%Pos+Phy (basal diet + 0.1% thyme oil + 0.1% postbiotic), 0.3% Pos+Phy (basal diet + 0.15% thyme oil + 0.15% postbiotic), and 0.4% Pos+Phy (basal diet + 0.2% thyme oil + 0.2% postbiotic(v/w))

[B] *SEM*, standard error of the mean (pooled).

Discussion

Growth Performance

The results of the present study demonstrate that different levels of postbiotics and phytobiotics in broiler chickens significantly improved growth performance, including LBW, WG, and FCR, especially at the high levels of 0.3% and 0.4% Pos+Phy in the experimental broiler chicken diet. These findings are similar to those of Ferdous *et al.* (2019) and Hussein *et al.* (2020), who found that the average live body weight, weight gain, and feed consumption significantly increased in bird groups when they received dietary supplements containing combinations of phytobiotics and probiotics. Additionally, the feed conversion ratio significantly decreased compared to the control group. Similar results were reported by Humam *et al.* (2019 and 2021), who noted that birds fed 0.3% postbiotics from different strain diets exhibited significantly higher average weight gain and feed intake, along with a lower FCR, compared to the treatment group of birds that received antibiotics and a basal diet.

Furthermore, the results correlate with Kareem *et al.* (2016), who demonstrated that birds fed a combination of various strains of 0.3% postbiotics and 0.8%–1.0% prebiotics (inulin) had significantly higher average final live body weight and total weight gain compared to other treatments. Additionally, their feed conversion ratio was lower compared to birds fed a basal diet in the control group, although it was similar to birds in other treatments fed antibiotics and another strain of postbiotics. However, Kareem's (2020) results agree with our current study regarding increased live body weight and body weight gain in quail birds presented with a dietary supplement containing 0.4% postbiotics. Nevertheless, their study disagrees with ours regarding significant differences in FCR and FI when different postbiotic levels (0.2%, 0.4%, and 0.6%) were compared to the control. Kareem *et al*. (2021) also concur with our results, showing that live body weight, cumulative weight gain, total feed intake, and feed conversion ratio significantly improved in broiler chickens fed at levels of 0.3% and 0.45% (postbiotics + prebiotic inulin) compared to other treatments with levels of 0.15% and 0.6%, as well as negative and positive control treatments.

The improvement in growth performance could be attributed to the bioactive compounds in phytobiotics, such as thymol and carvacrol found in thyme oil. These compounds are known to stimulate appetite and the secretion of digestive enzymes. Additionally, postbiotics contain various nutrients, including protein compounds, organic acids, peptides, vitamins, and short-chain fatty acids, that will probably lead to greater feed utilization, nutritional bioavailability, increased gastrointestinal motility, accelerated production of digestive enzymes, and a variety of positive actions, including anti-inflammatory, antispasmodic, and antioxidant benefits (Gholami-Ahangaran *et al*., 2022). Furthermore, the presence of several typical nutrients in postbiotics, including vitamin B12, vitamin K, folic acid, amino acids, lipopolysaccharides, enzymes, short-chain fatty acids, bacterial lysates, and cell-free supernatants, could play a role in preventing respiratory tract infections and exhibiting antibacterial, anti-inflammatory, antiproliferative, and antioxidant activities, thereby promoting bird health and subsequently improving growth performance (Thorakkattu *et al*., 2022). Additionally, the enhanced growth performance observed in birds fed a diet combination of postbiotics and inulin could be attributed to the bacteriocins, organic acids, and vitamins in the postbiotics (Kareem *et al*., 2021).

Phytobiotics (thyme oil) can significantly impact bird growth performance, particularly under stressful environmental conditions, by improving intestinal health, increasing the production of digestive enzymes, and enhancing dietary digestion and absorption (Hosseini *et al*., 2013). According to Hussein *et al*. (2020), another cause for the growth increase may be the altering of microbial populations and changes in nutrient absorption carried on by postbiotics' changing gut pH. Additionally, postbiotics from *L.plantarum* exhibit both bactericidal and bacteriostatic properties, which could inhibit the proliferation of harmful bacteria in the gut.

Gut Morphology

The results of the present study demonstrated that feed additives containing a combination of postbiotic and phytobiotics at different levels added to the bird's diet, especially levels 0.3 and 0.4%, caused enlargement in the villus length, villus width, crypt width, ratio of villi height to crypt width, and absorptive villus surface area in broiler chickens. Danladi *et al*. (2022) agree with the current study finding showing that the ratio of VH: CD and villi length was increased, while they disagree with us on the crypt depth that was decreased in the jejunum parts of birds that received a diet supplement with 0.2% postbiotic RI11 compared to other treatments. In addition, Humam *et al*. (2019) found the same results as ours: villi height, crypt depth, and villi height were increased when fed birds with a different strain of 0.3% postbiotic (RI11, RSS, UL4 *Lactobacillus plantarum* strain) compared to the negative and positive controls. In agreement with the current study findings, Pipaliya *et al*. (2022) reported that supplementing with a combination of essential oils (thymol and carvacrol) and probiotics significantly increased the bird's jejunal histology in terms of villus height, villus width, VH: CD ratio, and intestinal absorptive surface area and improved growth performance in comparison to the control. Peng *et al*. (2016) disagree with our finding when they observe no significant differences in villus length, villi width, crypt depth, or the ratio of villi length to crypt depth of small intestinal m Mohammed orphology (duodenum, illume) in birds feeding a diet with *Lactobacillus plantarum* compared to controls. Mohammed and Kareem (2022b) agree with our findings, which demonstrated that birds given diets containing 0.3% postbiotic significantly increased villi height, crypt depth, and VH: CD ratio in the ileum part of the small intestine compared to negative and positive controls. The small intestine is the longest part of the chicken gastrointestinal system, responsible for nutrient absorption through its three parts: the duodenum, which is specific for absorbing glucose and vitamins; the jejunum, which absorbs amino acids; and the ileum, which absorbs fatty acids (Simon *et al*. 2019).

In the current study, the reasons for the alteration in the morphology of the gut, represented by an increase in the villi length and villi width, as well as the increase in the surface area of absorption and the VH: CD ratio, may be due to the synergistic effects of postbiotic and phytobiotics combinations that can be useful for stimulating beneficial bacteria and improving the health of the gut. As we know, postbiotic activity is based on the bioactive compounds generated during fermentation, such as microbial metabolites, organic acids, proteins, carbohydrates, lipids, vitamins, cell wall components, or other complex molecules that may be used to promote health (Salminen & Szajewska, 2022). In addition, thymol and carvacrol, present in thyme, increase digestive enzyme activities by increasing the activities of amylase, protease, and lipase enzymes (Hashemipour *et al*., 2013). Also, the improved intestinal morphology can be attributed to the alleviating effects of thymol volatile oils on bacterial toxins, which are known to have negative effects on intestinal morphology, as we know, thyme oil properties, in general, are antimicrobials (Heydarian *et al*., 2020). Longer villi are associated with a healthy digestive system, while shorter villi and deep crypts have been associated with toxins or tissue renewal (Itzá-Ortiz *et al*., 2019). However, because the small intestine plays a crucial role in the digestion and absorption of nutrients, any modifications to its functionality may have an impact on other organs and systems (Sarker *et al*., 2021). A decrease in the VH: CD ratio is associated with a decrease in the absorption capacity of the intestine (Shang *et al*., 2018). In broiler chickens, a reduced villus area at a young age might result in a body receiving fewer necessary nutrients, which can slow growth and produce subpar performance. So, there is a beneficial link between the absorptive surface and nutritional status (Simon *et al*., 2019). However, various factors can impact or damage the gut microbiota, such as antibiotics, feed antinutritional factors, pesticides, poisonous chemicals, and bacterial toxins that can cause intoxication or localized inflammation (Shehata *et al*., 2022).

Lactic acid bacteria (LAB), *Enterobacteriaceae* (ENT), and pH of faecal in broiler chickens

Our findings showed that the LAB population was significantly increased in the treatment group fed high levels 0.3% and 0.4% of postbiotics and phytobiotics compared to the negative and positive controls, significantly decreased the *ENT* count compared to the positive control, and showed no significant differences compared to the negative control. While no significant differences in the pH value occurred in bird faecal that fed different levels of the postbiotics and phytobiotics combination compared to negative and positive controls, This finding is consistent with those of Kareem *et al*. (2016), who observed that dietary containing 0.3% postbiotics (RI11, RG14) significantly increased the faecal LAB and reduced the faecal *ENT* count in broiler chickens compared to the negative and positive controls, while regarding the faecal pH value, they disagreed with our findings when they showed the pH was decreased in all treatments fed 0.3% postbiotics (RI11, RG14) and 0.8–1% inulin. Also, Loh *et al*. (2014) agree with our finding, who observed that faecal bacteria count LAB was increased and *ENT* decreased in layer chickens when they were fed 0.6% of different postbiotic metabolite combinations produced by *L.plantarum* compared to a control. Also, the same researcher, Loh *et al*. (2010), found that the *ENT* count was reduced and the *lactic acid bacteria* were increased in broiler chicken-fed supplements with different levels of metabolite combinations by *lactobacillus Plantarum*, especially at levels of 0.2% and 0.4%. Previous research by Humam *et al*. (2019) reported similar findings when the 0.3% postbiotic RI11 group recorded significantly higher *Lactobacillus* counts and lower *Enterobacteriacea*e and *E. coli* counts, and in contrast with our finding, the caecal pH was decreased compared to the negative control and positive control treatment groups. In addition, Mohammed and Kareem (2022a) found the same results as ours: a high population of digesta LAB and a lower population of *Enterobacteriaceae* in broiler gut when they used 0.3% postbiotics as feed additives in dietary compared to negative and positive controls.

Our interpretation of these results is that the decreased *ENT* population and increased LAB might be due to the organic acids such as formic acids, lactic acids, citric acids, malic acids, tartaric acids, and phosphoric acids that are present in postbiotics and can enter the cell wall and cut off the cellular functions of the microbial population (Goh *et al*., 2020). Also, the reason may attribute the decrease in *Enterobacteriaceae bacteria* to the antimicrobial activity of the thyme essential oil compounds thymol and carvacrol against pathogenic bacteria, which led to determining their colony's population (Doneria *et al*., 2020). In addition, the current study findings indicate that the faecal pH value is within the normal range. However, it is not statistically significant and is so low that it creates a suitable environment for the activity of beneficial lactic acid bacteria, as lactic acid bacteria are able to grow well in relatively low pH environments. They can resist the condition better than the population of pathogenic bacteria *Enterobacteriaceae* (Kareem *et al*., 2017). Peng *et al*. (2016) Furthermore, it was discovered that lactic acid bacteria metabolize carbohydrates to create large amounts of lactic acid, which lowers the pH of their habitat in the gastrointestinal system and inhibits the growth of other bacteria.

Conclusions

The study demonstrated that adding different levels of a combination postbiotics and phytobiotics had beneficial effects on total body weight, feed conversion ratio, gut morphology, and faecal bacteria in broiler chickens. However, birds fed 0.4 Pos+Phy (basal diet + 0.2% postbiotic + 0.2% thyme oil) had a higher total weight gain and lower feed conversion ratio than those fed antibiotics. The faecal bacteria and the villus absorptive surface area were improved in birds fed 0.4% Pos+Phy compared to birds fed antibiotics. Thus, the high levels of 0.4% of a combination of postbiotics and phytobiotic could be used as a substitute for antibiotics in broiler diets to improve growth performance, guts health, and faecal bacteria in broiler chickens.

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