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Effect of Different Levels of Postbiotic and Phytobiotic Combination as on Growth Performance, Economic Production, and Immune Response of Broiler Chickens

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Abstract

A total of two hundred and eighty eight day old unsexed chicks from a Ross 308 cross were randomly assigned to eight treatment and four replicates and each replicator had twelve birds. The experimental groups includes, N-control= negative control (basal diet), P-control = basal diet + 0.01% Doxin200 (positive control) (w/w), 0.1% Pos+Phy = basal diet + 0.05% thyme oil (v/w) + 0.05% postbiotic, 0.2% Pos+Phy = basal diet + 0.1% thyme oil (v/w) + 0.1% postbiotic, 0.3% Pos+Phy = basal diet + basal diet + 0.15% thyme oil (v/w) + 0.15% postbiotic, and 0.4% Pos+Phy = basal diet + 0.2% thyme oil (v/w) + 0.2% postbiotic. The broiler European index was greatly improved, particularly at levels of 0.3% Pos+Phy and 0.4% Pos+Phy, which corresponded to (385.38) and (351.57), respectively. Growth performance ageneraly improved, significantly heigh of BWG and Low FCR showed in birds fed 0.3% and 0.4% Pos+Phy, and the BW of birds fed compaination significantly (p

Keywords

Probiotics Byproduct; Thyme Oil; Combination of Pos+Phy; Broiler Performance; New Feed Additives

ORIGINAL ARTICLE

Effect of Different Levels of Postbiotic and Phytobiotic Combination on Growth Performance, Economic Production, and Immune Response of Broiler Chickens

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Abstract

Postbiotics are byproducts originating from probiotic-driven fermentation in the intestines. As probiotics digest prebiotics, they produce postbiotics, which are essentially the remnants of probiotic function. Postbiotics, as non-living cells, enhance broiler performance by providing organic and fatty acids, minerals, and vitamins. Phytobiotic additives advance digestion, immunity, appetite, bactericidal and antiviral traits, and antioxidant activity. These benefits collectively enhance animal product quality and growth. The goal of the study is to determine the optimal level of postbiotic and phytobiotic combinations to use as antibiotic alternatives to improve the immune system and economic production in broiler chickens. A total of 288 one-day-old unsexed chicks from a Ross 308 cross were randomly assigned to six treatments and four replicates, and each replicator had twelve birds. The experimental groups include N-control = negative control (basal diet), P-control = basal diet + 0.01% Doxin200 (positive control) (w/w), 0.1% Pos + Phy = basal diet + 0.05% thyme oil (v/w) + 0.05% postbiotic, 0.2% Pos + Phy = basal diet + 0.1% thyme oil (v/w) + 0.1% postbiotic, 0.3% Pos + Phy = basal diet + basal diet + 0.15% thyme oil (v/w) + 0.15% postbiotic, and 0.4% Pos + Phy = basal diet + 0.2% thyme oil (v/w) + 0.2% postbiotic. The broiler European index was greatly improved, particularly at levels of 0.3% Pos + Phy and 0.4% Pos + Phy, which corresponded to 385.38 and 351.57, respectively. Growth performance generally improved, with significant body weight gain and a low feed conversion ratio shown in birds fed 0.3% and 0.4% Pos + Phy, and the live body weight of birds fed combination was significantly ($p < 0.05$) higher compared to N-Control and p-Control. The titer of antibodies against Newcastle and infectious bursal disease was significantly increased in the bird's group given postbiotic and phytobiotics, especially at levels of 0.3 and 0.4% Pos + Phy in comparison to birds in the P-control and N-control. In summary, the ongoing incorporation of postbiotics and phytobiotics into broiler diets can lead to a synergistic effect, promoting enhanced growth, improved economic production, and bolstered immune responses in broiler chickens.

Keywords: Probiotics byproduct, Thyme oil, Combination of Pos + Phy, Broiler performance, New feed additives

1. Introduction

The widespread use of antibiotics in poultry for economic improvement led to the emergence of antibiotic-resistant pathogens, leading to numerous countries and the United States Food and Drug Administration (FDA) banning their use [1]. Recently, specialists have discovered natural alternatives to antibiotics for safe, healthy, and disease-free poultry products [2–5]. Prebiotics, probiotics, symbiotics, and postbiotics are natural growth promoters and health enhancers [6]. Probiotics have positive effects, but many of them, particularly probiotic plasmids, possess antibiotic-resistance genes that may be passed from one organism to another [7]. Postbiotics, fermented

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products made from lactic acid bacteria, are currently being utilized more safely as feed additions as a possible antibiotic substitute for enhancing the gut flora and immune system of chickens [8]. Postbiotics enhance broiler performance by providing organic and fatty acids, minerals, and vitamins [9,10]. Thyme oil enhances chicken growth, health, and immune function by stimulating appetite, improving gut health, and promoting antioxidants [11]. Essential oils from plants like black cumin and thyme are safe for humans, livestock, and poultry [12]. According to earlier research, adding postbiotics and phytobiotics to the diet can help broiler chickens grow more quickly and have stronger immune systems. It's unknown how these additions will affect the diets of broiler chickens, though. The objective of the research is to determine the optimum degree of postbiotic and phytobiotic effects on the immune system and economic production in broiler chickens.

2. Methodology

2.1. Postbiotic and phytobiotic preparations

The primary cultivation of *Lactobacillus plantarum* was created at the Laboratory of Animal Resources in the College of Agricultural Engineering Science at Salahaddin University-Erbil, Iraq. In the first stage, *Lactobacillus plantarum* was isolated from a fermented dairy product and stored in de-Mann Rogosa Sharpe broth. Stock cultures were revived and incubated at 30 °C for 48 h. A single colony was chosen and injected into 10 ml of MRS broth then (1% (v/v) of inoculum was inoculated into the media) for 24 h, categorizing it as ready for fermentation inoculum. The second stage is after sterilizing work tools with ethanol and distilled water. The cultures for growing lactic acid bacteria are prepared by adding 52 g of MRS broth to 1 L of distilled water in a sealed glass vial. The solution is sterilized at 121 °C for 15 min. After cooling, 2 ml of *lactobacillus plantarum* are cultivated per liter of MRS broth and incubated at 30 °C for 48 h. The live bacteria are separated from the fermented materials by centrifugation at 10,000 rpm (rotation per minute) for 15 min. After being separated, the postbiotic was kept at 4 °C until use [8]. The thyme oil extract used in the phytobiotic was purchased from the Aram Factory in Duhok Government. The prescribed dosages of doxycycline and tylosin from the Dutch antibiotic manufacturer Interchemie Doxin-200 WS were utilized at a ratio of 1:1–2 as recommended.

2.2. Birds management and experimental design

The research was done at the Duhok government's directorate of agricultural research between November 1 and December 25, 2022. However, 288 one-day-old unsexed Ross308 broiler chicks were purchased from Evan poultry hatchery in Erbil by controlled-environment chick vehicles. The field houses were environmentally controlled, and the lighting and temperature program was according to Ross 308 Guide—Aviagen 2022. Birds were reared in a flooring system in wire pens 110 × 110 × 60 cm (length × width × height). Postbiotics, thyme oil, and black cumin oil were added to basal diets. Feed was sprayed in prescribed amounts, mixed, and packaged in polyethylene bags for each treatment group. The birds were divided into six treatments and four replicates, and each replicator had 12 birds. The treatment groups included: N.control = negative control (basal diet), P.control = positive control (basal diet + 0.01% (w/w) doxin 200), 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 + basal diet + 0.15% thyme oil + 0.15% postbiotic, and 0.4% Pos + Phy = basal diet + 0.2% thyme oil (v/w) + 0.2% postbiotic. Birds were fed over age-stage periods of 1–10, 11–24, and 25–35 days, respectively, which were balanced and formulated according to NRC, 1994, and Aviagen, Ross 308, Nutrition Specifications, 2022 Table 1. At 7, 14, and 21 days, respectively, the birds received vaccinations against the infectious bronchitis virus, infectious bursal disease, and Newcastle disease.

2.3. Sampling and data collection

The chicks were monitored daily for their health and management status, and their weight and feed intake were recorded weekly. The Europe Broiler Index (EBI), also known as the production index, was developed to compare broiler economic production from various treatments. At the end of the experiment, eight birds from each treatment group (4 males and 4 females) were chosen near the mean body weight of the group to estimate growth performance and biochemistry (antibody titers against IBD and NDv). According to [34] The following formula was used to calculate growth performance:

1- Feed conversion ratio (g feed: g weight gain) = (the average amount of feed consumed (g) at a week)/(average weight gain (g) in a week)

Table 1. The experimental basal diet for age stage, Starter (1–10), Grower (11–24), and Finisher (25–35) days.

Ingredient (%)	Starter diet	Grower diet	Finisher diet
Broilers concentrate (5%)[1]	5	5	3
Corn	45	50	49
Soybean meal (48%)[2]	31	27.4	23
Wheat	14.1	12	19.2
Veget. 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
Threo.	0.1	0.1	0.1
Anti-toxine	0.05	0.05	0.00
Anti-coccidiostat	0.05	0.05	0.00
NIR analyses of diets (%):			
M.E. kcal/kg)	2922	2986	3050
CP (%)	22.33	21.44	20.14
CF (%)	3.3	4.36	5.17
C. fiber (%)	2.58	2.69	2.84
Ash (%)	8.63	7.15	5.66
Ca (%)	0.63	0.88	1.07
P (%)	0.58	0.6	0.6

5%, means the ratio mixing to feed,Cp:40%,CF:2.3%,CF:4.5% MLysine digestible:3.5, Methionine dig.:3.4,Meth.+Cystine dig.:4.1, Tryptophan dig.0.53, Vitamin and mineral mix supplied/kg of diet, Vit A: 12000 IU, Vit D3: 2200 IU, Vit E: 10 mg, Vit K3: 2 mg.
2) 48% ratio of crude protein.

2- Mortality percentage = the number of dead birds/the total number of birds at the end of the week.
3- Live body weight (g/bird/group) = (birds LBW)/(number of birds at the end of the week).
4- Weight gain (g/bird) = (the average live body weight at the end of the week (g)) – (the average live body weight at the beginning of the week (g)).
5- Europe Broiler Index: {(Viability (100 - Mortality %)} x {(TWG ÷ days)}/{(FCR (kg feed/kg gain) x 10} X 100, accurding to [13].

2.4. Test of antibody titer against virus

At 35 days old, eight broiler chickens in each treatment group had their serum blood samples taken up to the moment of slaughter. ELISA systems effectively quantify antibody levels for infectious bursal disease and Newcastle disease, enabling the monitoring of immune status in flocks and determining vaccination timing. An ELISA test (IBD ELISA CK 113, ND ELISA CK 116, BioChek Limited, United Kingdom) was used to test serum to measure the antibody titers of all bird groups in the 5th week according to the recommended technique by the producer [14].

2.5. Statistical analysis

SAS 9.1 software was used to do statistical analysis utilizing a completely randomized design method (CRD) model [15]. Data obtained for growth performance and antibody titer were subjected to the generalized linear model of SAS. The significant differences between the treatment means were compared using Duncan's multiple range test at the probability level ($p < 0.05$).

3. Results

3.1. Productive performance

The chick's live body weight (LBW) fed at the 0.4% pos + phy level for the age stages (1–10) (11–24) and (25–35) days was significantly ($P < 0.05$) higher compared to the group fed at the levels 0.1, 0.2, or 0.3% pos + phy levels as well as compared to the birds fed on antibiotic Table 2. No significant ($P > 0.05$) variations were seen between birds fed 0.3% pos + phy and birds in positive control at the mentioned periods. The results also indicate that there was a significant increase ($P < 0.05$) in the average weight gain (WG) for the periods 1–10 and 11–25 days of age for the two groups of birds fed with the container diets of 0.3 and 0.4% Pos + Phy. No significant ($P > 0.05$) differences were observed in the average WG for the period of 24–35 days between all treatments. There are no significant differences in the WG of the birds fed 0.2, 0.3% Pos + Phy, and 0.4% Pos + Phy compared to the average WG of birds receiving antibiotics. But throughout the duration of the whole 1-35-day period, the treatment birds got at 0.4% Pos + Phy showed a substantial advantage in weight gain increase compared to all other experimental treatments. The study found a significant increase in daily feed intake (FI) for birds fed different levels of post-phy compared to the positive control group. No significant differences were observed in feed intake between birds in the 1–10 days. No significant differences were observed in FI between groups receiving different levels of postbiotics and phytobiotics, and the negative control was compared to the P. control. However, there was a significant improvement in feed conversion ratio (FCR) in all age period groups fed different levels of Pos + Phy and P.control compared to the N-control ($P < 0.05$). In the whole stage age period (1–35) days, a significant reduction in bird FCR was shown in two treatments that fed 0.3 and 0.4% Pos + Phy compared to 0.1 and 0.2% Pos + Phy and the N-

Table 2. Effects of different levels of postbiotics and phytobiotics combination on broiler chicken's growth performance (at 35th day).

Treatments[1]								
Age stages/day	N-control	P-control	0.1% Pos + Phy	0.2% Pos + Phy	0.3% Pos + Phy	0.4% Pos + Phy	p-value	SEM[2]
Live body weight (LBW)-(g/bird)								
Initial weight	39.38	39.05	38.93	39.08	38.88	39.10	0.767	0.1
1–10	594.5^{c}	688.8^{a}	601.7^{c}	646.9^{b}	681.3^{ab}	686.6^{a}	0.0001	9.2
11–24	1147.3^{d}	1274.4^{b}	1153.7^{d}	1203.1^{c}	1268.9^{b}	1314.8^{a}	0.0001	13.7
25–35	1982.5^{d}	2163.5^{b}	2047.8^{cd}	2083.7^{c}	2172.7^{b}	2248.3^{a}	0.0001	20.3
Weight gain (WG)- (g/bird)								
1–10	552.9^{b}	585.5^{b}	551.9^{b}	556.2^{b}	587.7^{b}	628.2^{a}	0.005	7.5
11–24	673.9^{c}	818.0^{a}	712.9^{bc}	771.2^{ab}	813.2^{a}	818.2^{a}	0.002	14.7
25–35	716.3	720.9	744.1	717.3	733.1	762.9	0.278	6.6
1–35	1943.1^{d}	2124.5^{b}	2008.9^{cd}	2044.6^{c}	2133.8^{b}	2209.2^{a}	0.0001	20.4
Feed intake (FI) -(g/bird)								
1–10	531.6	515.5	533.6	499.9	515.6	519.9	0.563	5.5
11–24	1277.0^{ab}	1181.9b	1268.6^{ab}	1301.8^{a}	1286.65^{ab}	1349.0^{a}	0.0789	16.5
25–35	1629.2^{a}	1519.6b	1616.4^{a}	1566.5^{ab}	1565.4^{ab}	1597.2^{a}	0.021	108
1–35	3437.73^{a}	3216.9b	3418.6^{a}	3368.2^{a}	3367.6^{a}	3466.1^{a}	0.006	22.3
Feed conversion ratio (FCR) -(g feed/g weight)								
1–10	0.963^{a}	0.883^{ab}	0.969^{a}	0.899^{ab}	0.879^{ab}	0.828^{b}	0.047	0.015
11–24	1.900^{a}	1.455^{c}	1.796^{ab}	1.694^{abc}	1.588^{bc}	1.649^{abc}	0.026	0.043
25–35	2.277^{a}	2.112b	2.173^{ab}	2.187^{ab}	2.138^{ab}	2.095^{b}	0.155	0.021
1–35	1.770^{a}	1.514^{d}	1.702^{ab}	1.648^{bc}	1.578^{cd}	1.569^{cd}	0.0002	0.021

a-d meanings with different superscripts within the rows are significantly different (P < 0.05).
N.control = negative control (basal diet), P.control = positive control (basal diet + 0.01% (w/w) doxin200), 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 + basal diet + 0.15% thyme oil + 0.15% postbiotic, and 0.4% Pos + Phy = basal diet + 0.2% thyme oil (v/w) + 0.2% postbiotic.
SEM, standard error of the mean (pooled).

control, but there were no significant differences compared to the P-control.

3.2. Broiler chickens' immune response

Effects of the combination of postbiotics and phytobiotics on antibody titers against viruses of infectious bronchitis bursal disease (IBD), and Newcastle disease (NDv) in broiler chickens are presented in Table 3. The results showed that antibody titers against ND were significantly ($P < 0.05$) increased in broiler chickens provided with 0.4% Pos + Phy combinations, which recorded the highest levels of titers compared to other birds fed 0.1, 0.2, and 0.3% Pos + Phy and the N-control and P-control. No significant ($P > 0.05$) differences occurred on the titers against ND among treatment groups provided with a diet level of 0.1, 0.2, and 0.3% Pos + Phy, respectively, compared to the negative and positive controls. In addition, results showed that antibody titers against IBD were significantly increased in all experimental treatments provided with different levels of postbiotic (0.1, 0.2, 0.3, and 0.4% Pos + Phy) compared to the positive control provided with antibiotics. Also, no significant differences occurred in the titers of IBD among all experimental treatments that fed different levels of experimental feed (0.1, 0.2, 0.3, and 0.4% Pos + Phy) compared to N-control.

Table 3. Effects of different levels of Pos + Phy on antibody titer against (IBD) and (ND).

Treatments	Antibody against ND	Antibody against IBD
N-control	2038.0^{c}	705.3^{bc}
P-control	1961.5^{c}	575.3^{c}
0.1% Pos + Phy	2140.8^{bc}	998.8^{ab}
0.2%pos + phy	2463.0^{b}	1254.3^{a}
0.3%Pos + Phy	2526.8^{b}	1276.3^{a}
0.4%Pos + Phy	2922.5^{a}	1270.3^{a}
P-value	0.0004	0.0003
SEM[1]	17.069	14.378

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

3.3. Broiler chickens' mortality percentage

"Figure 1," shows the effects of various levels of combination postbiotics and phytobiotics on the final mortality rate of broiler chickens. There were no significant ($P > 0.05$) variations in the mortality rate of broiler chickens fed an experimental diet across all treatment groups. However, there are statistical differences in the percentage of mortality of broiler chickens between experimental treatments, with birds in 0.4% Pos + Phy having the

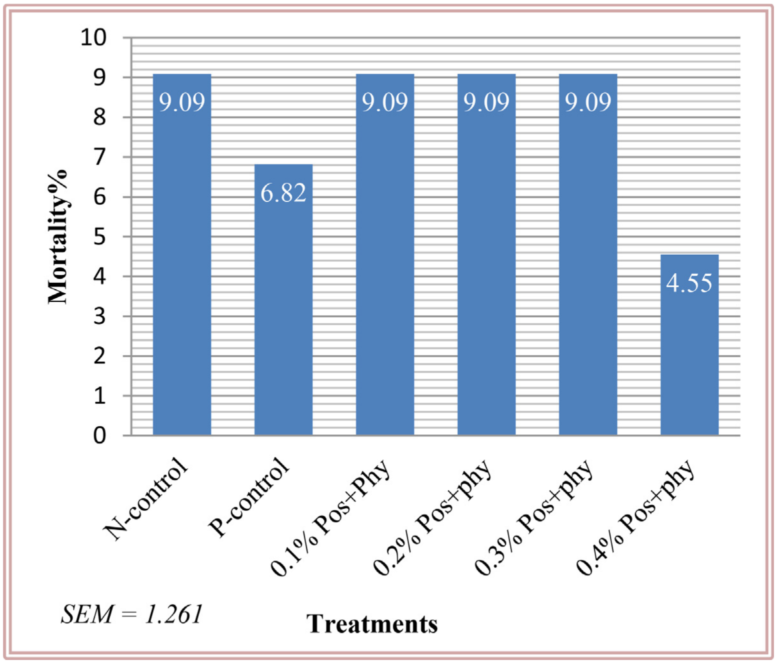

Figure 1. Effects of a combination of different levels of postbiotics and phytobiotics on broiler chicken's mortality. SEM, standard error of the mean (pooled).

lowest percentage of mortality (4.55%) compared to all experimental treatment groups.

3.4. Broiler chickens' European Broiler Index (EBI)

Table 4 demonstrated the effects of various levels of combination postbiotics and phytobiotics on the economic production index, or broiler Europe index. The BEI increased significantly ($P < 0.05$) in the treatment group fed at levels of 0.3 and 0.4% Pos + Phy compared to the bird groups fed at levels of 0.1% and 0.2% Pos + Phy. No significant ($P > 0.05$) differences were shown among birds fed levels of 0.3 and 0.4% Pos + Phy experimental diets and birds fed antibiotics in the positive control. The BEI was also significantly increased when the levels of diet supplements in the experimental treatment groups were increased from 0.1% to 0.4% Pos + Phy. The highest value of BEI was recorded in 0.4Pos + Phy (385.38), which was fed a combination of postbiotics and phytobiotics (thyme oil), which was also significantly similar to the positive control that was recorded (374.04), which was fed antibiotics.

Table 4. Effects of different levels of postbiotics and phytobiotics combination on Broiler Europe index (BEI).

Treatments	Broiler Europe index
N-control	284.06^{c}
P-control	374.04^{a}
0.1% Pos + Phy	300.15^{c}
0.2% pos + phy	322.73^{bc}
0.3% Pos + Phy	351.57^{ab}
0.4% Pos + Phy	385.38^{a}
P-value	0.002
SEM[1]	1.261

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

4. Discussion

4.1. Growth performance

The findings showed that varied levels of postbiotic and phytobiotic in broiler chickens at various stages of age considerably enhanced growth performance, particularly the high levels of feed additives 0.3% Pos + Phy and 0.4% Pos + Phy. It will be known that postbiotics from *L. plantarum* have bacteriostatic and bactericidal effects, which may reduce or eliminate the growth of pathogenic bacteria in the gut. The present findings are similar to those of [16], who found that the average of LBW, WG, and FI increased significantly in bird groups when they received dietary supplements containing 0.1% phytobiotic and 0.15% probiotic combinations, and the feed conversion ratio decreased significantly compared to the control. The results were in agreement with [17], who observed that LBW, WG, FCR, and FI were significantly improved in all experimental feed additives (12% phytobiotics+0.5 probiotic) in the final stage of age. While results by

[18], disagree with our study that LBW, FI, and FCR were not affected by dietary treatments containing 0.1 g/kg commercial probiotic and 5−7.5 g/kg ground thyme compared to the control, Also, the results were in agreement with [19,20], who noticed that birds fed 0.3% postbiotic of different strain diets had significantly higher average LBW, WG, and cumulative FI, and the FCR was significantly lower than the treatment group of birds that received the antibiotic and basal diet. In addition, the results agree with [8], who found that birds fed a combination of various strains of 0.3% postbiotics and 0.8%−1.0% prebiotics (inulin) had higher significant averages of final LBW and total WG than other treatments and a lower FCR compared to those birds fed a basal diet in the control, but were similar to those birds in other treatments fed antibiotics and another strain of postbiotics. However [21], results agree with our current study results that LBW and WG were significantly increased in quail birds when presented with dietary supplements at level 0.4% postbiotic, but disagree with the current study that no significant differences have occurred on FCR and FI when presented different levels of postbiotic (0.2, 0.4, and 0.6%) compared to control. Moreover [22], agrees with our results and shows that LBW, cumulative WG, total FI, and FCR were improved significantly in broiler chicken fed at levels 0.3 and 0.45% (postbiotic + prebiotic inulin) compared to other treatments fed at levels (0.15 and 0.6%) and with the negative and positive control. In addition [23], agrees with our finding that the LBW and WG were significantly increased, and the FCR was decreased in broiler chickens fed dietary supplements with 0.2% phytobitics compared to negative and positive controls [24]. Also, agree with our finding that feeding birds 100 mg/kg of thymol oil showed significantly improved final LBW, FCR, and feed consumption compared to the control.

The bioactive substances may be responsible for the increase in the bird's growth performance due to the composition of the phytobiotics, as it is known that thymol and carvacrol are the most important biologically active substances in thyme oil that can stimulate appetite and the secretion of digestive enzymes. It may also be due to the nutrients found in postbiotics, such as protein compounds, organic acids, peptides, vitamins, and short-chain fatty acids. The enhanced growth performance could potentially result from the synergistic interplay between postbiotics and phytobiotics.

The biological mechanisms of action result in the intended effects on feed consumption, nutritional bioavailability, promoting the production of digestive enzymes, and enhancing gastrointestinal motility; they also have anti-inflammatory, antispasmodic, and antioxidant actions [25] Although the presence of many typical nutrients in postbiotic compositions, such as vitamin B12, vitamin K, folic acid, and several amino acids, as well as lipopolysaccharides, enzymes, short-chain fatty acids, bacterial lysates, and cell-free supernatants, which may have a role in preventing respiratory tract infections and have antibacterial, anti-inflammatory, antiproliferative, and antioxidant activities, maybe the reason to improve the bird's health and then to improve the growth performance [26] In addition, the improved growth performance in birds fed a diet combination of postbiotics and inulin may be due to the bacteriocins, organic acids, and vitamins present in the postbiotics [22]. Phytobiotics can significantly affect the bird's growth performance, especially when exposed to stressful environmental conditions. They can do this by enhancing the health of the intestinal tract, increasing digestive enzymes, and increasing absorption [18]. Another reason for the enhancement of growth performance may be due to modifying the microbial population and nutrient absorption due to the alteration of the gut pH [17]. Moreover, environmental conditions, farm management, feeding plans, and pen hygiene could also impact broiler chickens' growth performance.

4.2. Broiler chicken's immune response

The ELISA antibody titer against Newcastle disease virus and infectious bursal disease was significantly improved after feeding different levels of the combination of postbiotics and phytobiotic in the present study in broiler chicken serum as compared with birds in the positive and negative control groups. The same results found by [27] showed that birds receiving 0.5−1 kg of postbiotic/ton feed or 4 ml of postbiotic/litter drinking water significantly enhanced performance, productivity, immune response, and antibody titers against IBDv and Newcastle disease virus when compared with challenged non-treated chickens. Similar results were observed by other researchers when 1.5 g/kg of probiotics and prebiotics (MOs) were added to broiler diets and showed higher antibody titers against IBD compared to control and level 1 g/kg feed [28]. Also, our findings agreed with [29], who observed higher titers of antibodies against Newcastle disease, Gumboro disease, and infectious bronchial viral disease in broiler chickens fed 0.3% each of postmitotic, probiotic, and prebiotic compared to negative and positive controls. The same outcomes of our study were also noted by [30], The immune response indicated higher serum ELISA antibody titers against infectious bursal disease (IBD) and higher weights of immunological organs such as the thymus, spleen, and bursa of Fabricius in the bird groups fed thyme oil compared with the control group ($P < 0.05$). Contrary to current studies [31], found that supplementation with thyme or oregano essential oils for 35 days had no impact on the NDV antibody titer levels of broiler chickens. An immunoglobulin level in the blood is an important indicator for estimating the presence of antibodies. That was conducted to investigate if there is an immune reaction resulting from antigens in the blood or if the immune system produces the specialized proteins (antibodies) to identify and destroy foreign invaders (antigens), such as viruses and bacteria [32]. These findings, including higher antibody titers against ND and IBD, may be due to active compounds in thyme oil such as flavonoids, which enhance vitamin C activity, antioxidant properties, and immune function [30]. However, it might be the result of the stimulation of immune cells by cytokines, which are secreted by lactic acid bacteria and microbes [33]. In addition, it could be related to postbiotic structures such as peptidoglycan, which makes up 90% of the dry weight of *Lactobacilli*, and stimulates the immune response in birds, lipopolysaccharides, and the polymeric compounds teichoic and lipoteichoic acids, which are found in the cell walls of gram-positive bacteria [27]. These observations, which involve increased antibody titers against ND and IBD, could be attributed to the synergistic impact of active components present in both thyme oil and postbiotics. These components enhance beneficial bacteria, anti-inflammatory cytokines, antioxidant properties, and immune function.

4.3. Mortality percentage and broiler Europe index

Statistically, there was no significant impact on total mortality percentage between groups fed variant levels of Pst + Phy or antibiotic, but calculatedly, it decreased in birds fed 0.4% Pos + Phy. While the broiler Europe index was significantly affected by different levels of postbiotic and phytobiotic combinations, especially high levels of 0.3 and 0.4% Pos + Phy. The same results for our present finding were found by [34], who reported that the mortality percentage of broilers was not significantly different ($P > 0.05$) among all experimental treatments fed dietary with 0.2% postbiotic strains TL1 and RS5 compared to birds in the negative control (basal diet) and positive control fed 0.01% oxytetracycline. On the other hand, the finding agrees with our current study results that the birds fed 0.2% (v/w) postbiotic strain RI11 had the significantly highest value of the Europe broiler index compared to the control.

Furthermore [17], also agree, with our finding results, they observed that EBI and survival percentage of broiler chicken were increased in treated groups fed phytobiotic and probiotics with comparison to a positive control (0.01% antibiotic), when they feeding 0.12 g/kg phytobiotics or a combination of 0.12 g/kg phytobiotics and 0.5 g/kg probiotics as dietary supplements. In addition [19], agree with our findings, found no significant differences in the percentage of mortality between all treatment groups fed 0.3% postbiotics of various strains (RI11, RS5, and UL4) compared to the negative control and positive control (0.02% oxytetracycline). Also, agree with our findings and reported no significant difference in mortality rate in bird groups that were fed postbiotics compared to challenge groups without the postbiotic in the most severely challenged birds with *Clostridium perfringens*.

The reason for the improvement in both the mortality rate and the economic production index in our current study may be due to the result of the effects of optimum levels of the combination of the two feed additive compounds included in the experimental supplements, postbiotics, and phytobiotics, as the main active substances in them are thymol and carvacrol in the phytobiotics, as well as bacteriocins, fatty acid chains, and peptides in the postbiotics. In addition to being rich in nutrients such as vitamins and mineral elements, which are important for improving production and health in birds. Our current study demonstrates that a combination of different levels of a postbiotic plus phytobiotics (thyme oil extracts) may exert additive effects on the efficiency of economic production in broiler chickens. In this regard [13], also evaluated the performance of broiler chickens in terms of the European Broiler Index and the European Production Efficiency Factor, which include the daily average of WG, FCR, and survival ratio, that higher values of these indicators show that the flock is healthy and the birds' BWG had high uniformity rate. The addition of the feed additive component used in this study led to an improvement in the average live body weight and the feed conversion ratio, which led to an increase in the economic production index values to reach 385.38 in the groups of birds fed 0.4% Pos + Phy of the experimental supplements. These improvements may accrue after postbiotics were added to the diet of the chickens through improved immunity, gut health, tissue integrity, and gut microbiota regulation.

5. Conclusion

We reached the conclusion that levels of 0.4% Pos + Phy (base diet with 0.2% thyme oil + 0.2% postbiotic) should be utilized in broiler chicken feed to provide the highest growth performance, immunological responses, and economic production.

Disclosure statement

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