IJAS Iranian Journal of Applied Animal Science

Effects of Combinations of Postbiotics and Phytobiotics on Growth Performance, Meat Quality, Gut Morphology, and Tibia Bone Characteristics in Broiler Chickens

Research Article

J.M.M. Doski[1] and K.Y. Kareem[1*]

[1] Department Animal Resource, College of Agricultural Engineering Science, Salahaddin University, Erbil, Iraq

Received on: 2 Jun 2023
Revised on: 22 Jul 2023
Accepted on: 11 Aug 2023
Online Published on: Dec 2023

*Correspondence E-mail: karwan.kareem@su.edu.krd

Online version is available on: www.ijas.ir

ABSTRACT

In the current study, the impact of the dietary combination of postbiotics and phytobiotics was examined on the growth performance, carcass characteristics, meat quality, gut morphology, and tibia bone characteristics of broiler chickens. This experiment was conducted in the Duhok government's private-sector poultry house for 35 days. The broiler chickens were allocated into eight treatment groups. Each treatment had four replicates, while each replicate had twelve birds. The treatment groups included T1= basal diet (negative control), T2= basal diet + 0.01% (w/w) Doxin 200 (positive control), T3= basal diet + 0.3 % (v/w) postbiotic, T4= basal diet + 0.3% black cumin oil (v/w), T5= basal diet + 0. 3% thyme oil (v/w), T6= basal diet + 0.15% postbiotic + 0.15% black cumin oil (v/w), T7= basal diet + 0.15% postbiotic + 0.15% thyme oil (v/w), T8= basal diet + 0.15% postbiotic + 0.075% black cumin oil + 0.075% thyme oil (v/w). According to the results, adding postbiotics and phytobiotics to broiler feed significantly increased weight growth, feed conversion ratio, and economic index, particularly in birds in the T7 group. The meat traits had improved nevertheless and were now in the normal range. Additionally, gut morphological characteristics had improved and, particularly in bird groups given T7, had demonstrated the greatest rate of villa height/crypt depth (Vh/Cd) ratio and absorptive surface area. In contrast to broiler-fed antibiotics, the characteristics of the tibia bone were enhanced by a combination of postbiotics and phytobiotics. The greatest prospective substitute for antibiotic growth promoters in broiler chicken is the combination of 0.3% postbiotics and phytobiotics (thyme oil) as new feed additives.

KEY WORDS broiler productivity, guts health, probiotics byproduct, thyme oil.

INTRODUCTION

The poultry industry is growing due to increasing demand for broiler production, population growth, urbanization, and the low price of chicken meat, which makes poultry products popular as dietary protein resources (Zuidhof *et al.* 2014). However, increased production capacity has led to an increase in diseases affecting poultry, causing economic losses for owners. This led to the extensive use of antibiotics to prevent diseases and increase production (Durso and Cook, 2014).

Unfortunately, the forced and indiscriminate use of antibiotics in poultry has raised concerns regarding the consumption of meat and eggs and their negative impact on human health (Shazali *et al.* 2014). Therefore, many countries in the world have banned the use of antibiotics in poultry, as well as the US Food and Drug Administration, to prevent antibiotic-resistant poultry-borne pathogens

(Tabashsum *et al*. 2023). In this context, specialists have recently found many natural alternatives to antibiotics for the purpose of obtaining safe, healthy, and disease-free poultry products (Abd El-Ghany, 2020). Natural alternatives as growth promoters and health enhancers are prebiotics, probiotics, symbiotics, postbiotics, and prebiotics-postbiotics mix (Rine *et al*. 2021). The action of these additives differs among themselves, as probiotics stabilize beneficial microbe colonies in the digestive tract of birds, thus preventing the colonization of harmful bacteria in them (Halliwell, 2001). Recently, postbiotics, or fermented products from lactic acid bacteria group fermentation, which have a positive effect on poultry gut microflora, were used as new feed additives and a promising alternative to antibiotics (Kareem *et al*. 2016). It is the probiotic products from Lactobacillus plantarum that have been the subject of many recent types of research (Humam *et al*. 2020). The energetic substances of postbiotics have a useful effect on broiler performance (Loh *et al*. 2014; Kareem *et al*. 2015). Although probiotics have advantageous impacts, a considerable number of them, particularly probiotic plasmids, possess antibiotic-resistance genes that can be transferred among various organisms (Ramiah *et al*. 2014). Another alternative to feed additives was probiotics, which contain four main classes of promising subgroups: botanicals, oleoresins, herbs, and essential oils (Yitbarek, 2015). These herbal products (thyme, ginger, pepper, black cumin, etc.) have a positive effect on chicken growth performance and health due to their stimulating appetite, increasing internal secretions, antioxidant, antimicrobial, gut manipulation, and immune-enhancing properties (Abd El-Hack *et al*. 2022). The essential oils extracted from some of these plants, such as black cumin and thyme, can be used safely in humans, livestock, and poultry (Grashorn, 2010). Based on previous studies, we can make the hypothesis that a combination of postbiotic and phytobiotics may alter gut morphology, promote growth performance, and improve meat quality and health status in broiler chickens. However, little has been known about the effect of mixing postbiotics and phytoncide in broiler chicken diets. Therefore, this study was conducted to examine the effects of a combination of postbiotics and phytobiotics on growth performance, carcass characteristics, meat quality, gut morphology, and the characteristics of the tibia bone in broiler chickens.

MATERIALS AND METHODS

Postbiotic and phytobiotic preparations

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. de-Mann Rogosa Sharpe (MRS) broth was used to twice revive the stock cultures. After that, spread plates were added and the incubation process was kept static for an additional 48 hours at 30 ˚C. One colony was then selected and put into 10 ml of MRS broth, where it was allowed to grow for 24 hours. To begin, a total of 3 liters of de Man, Rogosa, Sharpe (MRS) broth culture was meticulously prepared following the guidelines provided by the manufacturer. This involved dissolving 52.2 grams of the MRS broth powder in one liter of distilled water. The resulting solution was then subjected to autoclaving at a temperature of 121˚C for duration of 15 minutes. Utilizing a 2 ml portion of the prepared inoculum, the bacteria were introduced into a larger volume of 1000 mL of the respective MRS broth (growth medium). Then it was incubated there for the following 48 hours at 30 ˚C. The bacterial cells were separated using centrifugation at 10,000 rotation per minute (rpm) for 15 minutes. After that, the culture was ready to be used as an inoculum (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.

Bird's management and experimental design

This study was conducted at a local commercial poultry project house in Duhok governorate, Iraq. A total of 384 birds' one-day-old, unsexed Ross 308 broiler chicks were purchased from a local commercial hatchery in Erbil Gov. and transportation was by controlled-environment chick vehicles. The fields' house was environmentally controlled; the lighting and temperature program was according to Ross 308 guide (Aviagen, 2019). The birds were reared in a flooring system in wire pens 110 × 110 × 60 cm (length×width×height). The 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 allocated into 8 treatment groups. Each group had 4 replicates while each replicate had 12 birds. The treatment groups included: T1) basal diet (negative control), T2) basal diet + 0.01% (w/w) Doxin 200 (positive control), T3) basal diet + 0.3% postbiotic, T4) basal diet + 0.3% black cumin oil, T5) basal diet + 0.3% thyme oil (v/w), T6) basal diet + 0.15% postbiotic + 0.15% black cumin oil, T7) basal diet + 0.15% postbiotic + 0.15% thyme oil, T8) basal diet + 0.15% postbiotic + 0.075% black cumin oil + 0.075% thyme oil. Birds received water and feed *ad libitum* to the birds until 35 days of age. The birds were vaccinated against Newcastle Disease (NDV), Infectious Bursal

Disease (IBD), and Infectious Bronchitis (IB) Virus by-drinking water at 7, 14, and 21 days respectively. By the recommendations of the National Research Council (NRC, 1994) and Aviagen, Ross308 (Nutrition Specifications, 2022), broiler chickens were fed commercially balanced diets made with local feed ingredients over periods of 1 to 10, 11 to 24, and 25 to 35 days, respectively (Table1).

Table 1 Ingredients and composition of experimental basal diet

Ingredient (%)	Growth periods		
	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-35 d)
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
Vegetable oil	2.3	3	3.2
Limestone	1.3	1.3	1.3
Dicalcium phosphate	0.6	0.6	0.7
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
Anti-toxine	0.05	0.05	0.0
Anti-coccidiostat	0.05	0.05	0.0
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 (%)	0.63	0.88	1.07
P (%)	0.58	0.6	0.6

[1] (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: vtamin A: 12000 IU; vtamin D3: 2200 IU; vitamin E: 10 mg; vitamin K3: 2 mg; vitamin B1: 1 mg; vitamin B2: 4 mg; vitamin B6: 1.5 mg; vitamin B12: 10 mg; Niacin: 20 mg; Pantothenic acid: 10 mg; Folic acid: 1 mg; Biotin: 50 mg; Copper: 10 mg; Iodine: 1 mg; Iron: 30 mg; Manganese: 55 mg; Zinc: 50 mg and Selenium: 0.1 mg.
[2] (48%) ratio of crude protein.
ME: metabolizable energy.

Sampling and data collection

Live body weight (LBW), body weight growth (BWG), feed intake (FI), and feed conversion ratio (FCR) were all monitored weekly during the treatment period, which lasted from one day to 35 days of age. The Europe Broiler Index (EBI), also known as the production index, was developed to compare the broiler outcomes from various treatments. The EBI standardizes technical outcomes by taking feed conversion, mortality, and daily weight gain into account. The formula (Average weight gain (g)/day % survival rate) / Feed conversion × 10 was used to determine EBI. At the end of the experiment, eight birds from each treatment group (4 males and 4 females) close to the group's average body weight were selected to estimate growth performance, biochemistry, meat quality, gut morphology, and tibia bone characteristics.

Gut morphology

The histomorphology of the jejunum was assessed using the methodology outlined by Humam *et al.* (2019). After the chickens were slaughtered and underwent cleaning following a Halal slaughter procedure, the jejunum was extracted for examination. The measurements took place at the Vin Medical Laboratory in Duhok City (Figure 1).

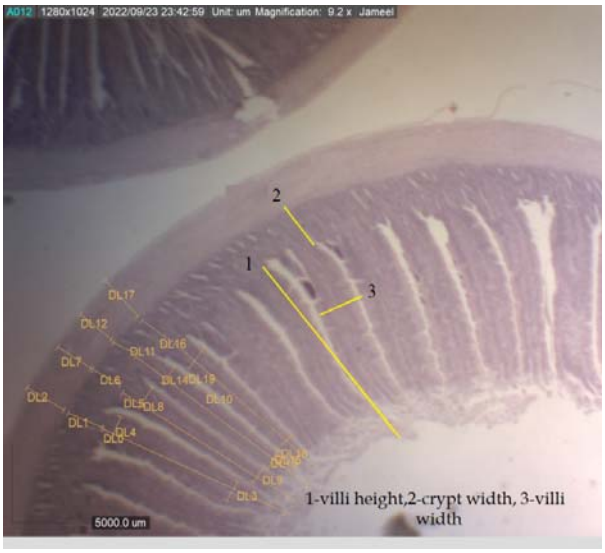

Figure 1 Villus morphology measurements

Subsequently, the slides were stained with hematoxylin and eosin and then mounted for examination under light microscopes. Several parameters were measured, including the depth of invagination between adjacent villi (known as crypt depth), the height from the tip of the villi to the junction between villi and crypts (referred to as villi height), muscle depth, villi tip width, villi base width, and villous area (millimeters). To facilitate these measurements, software called Dinocapture 2.0 version 1.4.0 was employed. In addition, histological sections were initially examined under a low-power microscope (10x). Furthermore, the mean absorptive surface area (ASA) was calculated using the following formula: ASA= (villus width×villus length) + (villus width/2+crypt width/2)^2 - (villus width/2)^2 / (villus width/2+crypt width/2)^2, following the methodology by Kisielinski *et al.* (2002).

Meat quality analysis

Drip loss

The drip loss measurement was carried out on individual breast samples. After the slaughtering process, approximately 30-35 grams of fresh meat samples were collected

from the pectoralis major muscle. These samples were individually weighed, and these initial weights were recorded as (W1).

To preserve the samples, they were placed in polyethylene plastic bags, which were then hermetically sealed. These vacuum-sealed packages were subsequently stored in a refrigerator at a temperature of -20 ˚C. On the seventh day following storage, the ultimate weight (W2) was determined, following the procedure outlined by Abdulla *et al.* (2017).

This measurement was promptly taken after the samples were removed from the bags and dried using a soft bathroom tissue. To calculate the percentage of drip loss, the formula proposed by Hayat *et al*. (2021) was utilized. The formula is expressed as follows:

Drip loss (%)= [(W1−W2) / W1] × 100

Where:
W1: weight (in grams) of the muscle sample before storage.
W2: weight (in grams) of the muscle sample after storage.

Cooking loss

The day-7 frozen breast muscle subsamples were placed in a 4 ˚C chiller overnight to thaw after being removed from a -20 ˚C freezer (Abdulla *et al.* 2017). Polyethylene bags containing meat samples weighing approximately 30 g each were subjected to submersion in a water bath that had been pre-set to 80 ˚C. The samples were allowed to remain in the water bath until their internal temperature reached 78 ˚C. After attaining the desired internal temperature, the specimens were subjected to an additional 10-minute cooking period (Kareem *et al*. 2015). The samples were subjected to a cooling process by placing them in bags and running tap water for 30 minutes. Afterward, the samples were gently removed from the cooking water using paper towels without exerting any pressure and subsequently weighed again. The cooking loss percentage was calculated using the equation of Hayat *et al.* (2021).

The formula for calculating cooking loss as a percentage is expressed as follows:

[(W1−W2) / W1] × 100

Where:
W1: weight of the muscle sample before being cooked in the water bath (in grams).
W2: weight of the muscle sample after being cooked in the water bath (in grams).

Color value

Before use, the device was initially calibrated using reference tiles that were black and white. At 7 days postmortem, each sample was measured with its L* (lightness), a* (redness), and b* (yellowness) measurements taken in triplicate, utilizing the ColorFlex® system (Hayat *et al.* 2021).

The light source used was illuminant D65 and a 10° standard observer with an aperture size of 5 cm was employed. The frozen meat specimens were defrosted throughout the night in a refrigeration unit set at 4 ˚C. The meat samples were prepared, bloomed for 30 minutes, and then put into the ColorFlex® device (NR20XE Precision Colorimeter), which works by scanning. To calculate the average color values of the breast meat, three distinct site measurements were made; for the second and third measurements, the cup was spun 90 degrees clockwise (Kareem *et al.* 2015; Humam *et al*. 2020).

pH value determination

The method outlined by Kareem *et al.* (2015) was utilized to determine the pH value measurement of each breast muscle. The portable pH meter (Eutech pH 700 pH/mV/˚C/˚F Bench Meter) was utilized for the indirect determination of the pH of the breast muscles. Before its utilization, the pH meter underwent calibration using a standard buffer solution with pH values of 4.0 and 7.0. Each breast meat sample, weighing approximately 0.5 g, underwent homogenization (WTW Multi 3420, Germany) for 20 seconds in 10 mL of distilled water.

Tibia bone parameter determination

The tibia bones located on the right side of the birds were obtained subsequent to slaughter. These bones were subjected to boiling at a temperature of 100 ˚C for 8 minutes. The excision of the muscle tissue was conducted with precision using a scalpel blade.

The digital balance was used to measure the weight of the bones. All bones were weighed in grams (after being dried at 38 ˚C for a period of 48 hours), measured in length (mm), medullary canal diameter (mm), and maximum and minimum diameter (mm) (Figures 2, 3, 4, and 5) with a digital caliper. Then, after the bones were oven-dried and the ashes were determined, the calcium and phosphor values and percentage of ash were determined by using the third-generation diode array NIR analyzer from PerkinElmer called the DA 7250 NIR analyzer USA, which is intended primarily for use in the food and agricultural industries, in only 6 seconds.

The tibiotarsal and robusticity indexes are determined using the following formula: tibiotarasl index= (diaphysis diameter–medullary canal diameter) / (diaphysis diameter) × 100; robusticity index= bone length/cube root of bone weight (Hafeez *et al.* 2020).

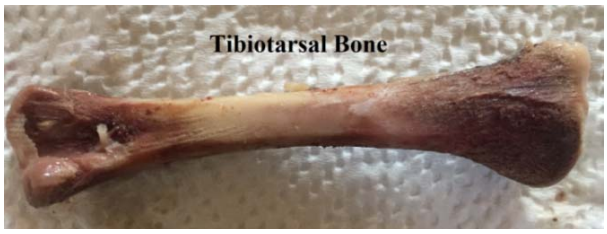

Figure 2 Tibiotarsal bone of broiler chickens

Figure 3 Tibiotarsal length

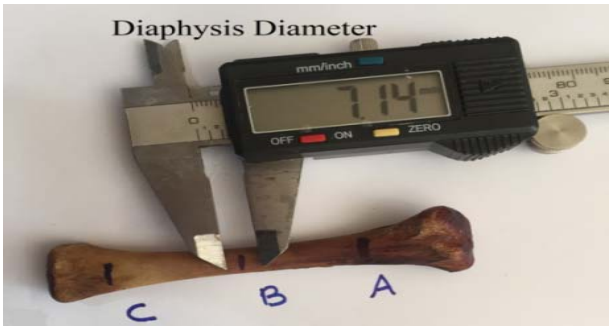

Figure 4 Tibiotarsal diameter (B+A/2)

Figure 5 Medullary canal

Statistical analysis

Statistical analysis was conducted by SAS version 9.1 software using a completely randomized design procedure (CRD) model (SAS, 2012). Data obtained for the growth performance, carcass yield, meat quality, gut parameter, and tibia bone were subjected to the generalized linear model of SAS. Duncan's multiple range tests was used to compare the significant differences of the treatment means at the probability level of ($P<0.05$).

RESULTS AND DISCUSSION

The effects of postbiotics and phytobiotics supplements and their mixture on FBW, TWG, FI, and FCR are shown in Table 2. Birds fed with T3, T6, and T7 had higher final BW and total WG than other treatments ($P<0.05$). The final BW and WG of birds fed the T6 and T7 were similar to the positive control diet ($P>0.05$) and higher than the negative control diet. There was no significant difference among the treatments for FCR ($P>0.05$). Birds fed with T3, T4, T6, T7, and T8 had higher statistical FI compared with birds fed the negative and positive control diet. While the highest FI was recorded in birds group T6 but doesn't significant among other experiment treatment groups.

The effects of postbiotics and phytobiotic and their combination supplementations showed significant differences in the mortality percentage of broiler chickens, as shown in Figure 6. The percentage of mortality decreased significantly showed between all treatment groups of birds that received postbiotic and phytobiotic combination (T3, T4, T5, T6, T7 and, T8) compared to the positive and negative control. Furthermore, no statistically significant difference between birds in treatment groups (T4, T5, T6, T7, and T8) compared to other treatments group (T1, T2, and T3). However, showed positive impacts of the postbiotics on the mortality rate in the bird's treatment group (T3) and recorded (4.2%) which that the lower percentage of mortality among all treatment groups. While a higher rate of mortality was recorded in positive control groups (16.7%).

The results of the economic production index in Figure 7 showed that the European broiler index (EBI) was increased significantly in all treatment groups fed postbiotic and phytobiotic as feed additives with compare to the negative control. No significant differences in EBI were shown across T3, T5, T6, T7, and T8 compared to the positive control. No significant differences ($P<0.05$) in EBI were found in birds in the T7, T3, and T6 (381.2, 363, and 355.9), respectively, compared to the positive control (360.1). Additionally, no significant differences were observed between (T4, T5, and T8) and all other treatment groups.

Table 2 Effect of a combination of postbiotics and phytobiotics on growth performance (at 35th day)

Treatments[1]	Initial weight (g)	Live body weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion ratio
T1	38.25	2037.69^{b}	1999.44^{b}	2993.76^{b}	1.50
T2	37.82	2097.12^{ab}	2059.30^{ab}	3038.76^{b}	1.48
T3	37.92	2142.66^{ab}	2104.60^{ab}	3107.12^{ab}	1.47
T4	37.95	2084.83^{ab}	2046.88^{ab}	3116.19^{ab}	1.52
T5	37.93	2068.13^{ab}	2030.21^{ab}	2979.40^{b}	1.47
T6	38.07	2175.18^{a}	2137.25^{a}	3273.89^{a}	1.53
T7	37.75	2173.96^{a}	2136.21^{a}	3152.25^{ab}	1.47
T8	38.12	2066.52^{ab}	2028.40^{ab}	3112.22^{ab}	1.53
SEM	0.06	21.52	21.51	23.11	0.01
P-value	0.621	0.0001	0.0001	0.020	0.43

T1: basal diet (negative control (NC)); T2: NC + 0.01% (v/w) Doxin 200 (positive control); T3: NC + 0.3% (v/w) postbiotic; T4: NC + 0.3% black cumin oil; T5: NC + 0.3% thyme oil; T6: NC + 0.15% postbiotic + 0.15% black cumin oil; T7: NC + 0.15% postbiotic + 0.15% thyme oil and T8: NC + 0.15% postbiotic + 0.075% black cumin oil + 0.075% thyme oil.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

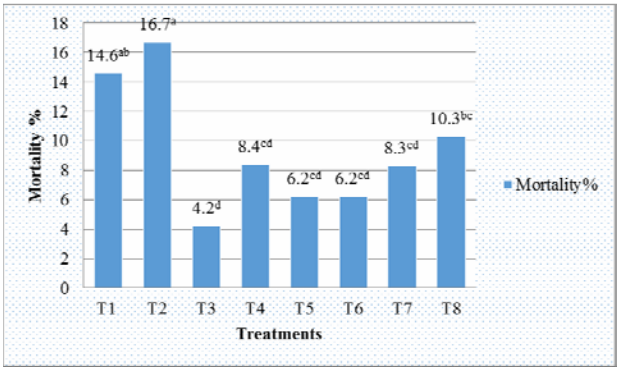

Figure 6 Effects of a combination of postbiotics and phytobiotics on broiler chickens' mortality percentage
The means within the same column with at least one common letter, do not have significant difference (P>0.05)
SME= 0.895

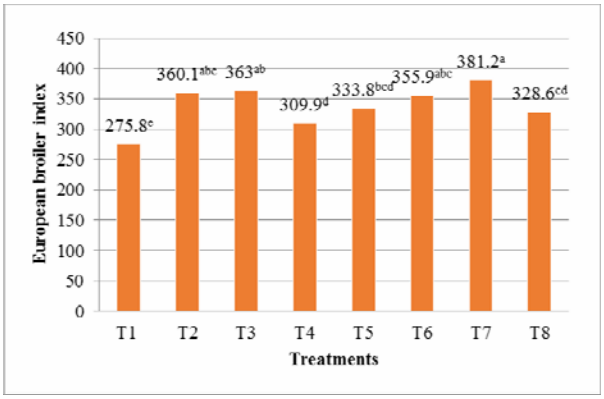

Figure 7 Effects of a combination of postbiotics and phytobiotics on broiler chickens' European broiler index
The means within the same column with at least one common letter, do not have significant difference (P>0.05)
SME= 6.635

The highest EBI was recorded in birds fed a combination of postbiotics and phytobiotics (thyme oil) in treatment group T7 (381.2).

Table 3 shows the results of meat quality (L*), (a*), (b*), drip loss, cooking loss, and pH value of broiler chicken fed a combination of postbiotics and phytobiotics. No significant differences were shown in redness, yellowness, and pH values of the pectoralis major muscles of broiler chicken fed a combination of postbiotics and phytobiotics across the overall treatment group. While there was a significant difference (P<0.05) effect in lightness (L*) in all bird groups fed postbiotics and phytobiotics compared to the positive control. However, broiler pectoralis major muscles lightness in (T3, T4, T6, T7, and T8) were significantly similar to bird groups in positive control T2, fed antibiotics, but had a higher lightness, when compared to the negative control.

Furthermore, the breast fillets color of the overall bird's group measured lightness, redness, and yellowness values had a range in a normal distribution, according to the standard range of breast fillets color: dark (lightness<56), normal (56≤lightness≤62), and pale (lightness>62), redness (b*) its range: 3 to 12 and yellowness (a*) range: 0 to 13.

Regarding, drip loss and cooking loss of broiler chicken pectoralis major muscles were observed no significant differences were affected in all treatments group by supplement combination diets (postbiotics and phytobiotics) compared to the bird's group fed negative control, and positive control (P>0.05).

The effects of feed combinations of postbiotics and phytobioticss on the intestine morphology measurements (villus length, crypt depth, villus width, crypt width, and villus area) of broiler chickens fed are presented in (Table 4).

Table 3 Effect of of a combination of postbiotics and phytobiotics on meat quality of broiler chicken (at 35th day)

Treatmeants[1]	Lightness	Redness	Yellowness	Drip loss %	Cooking loss %	pH
T1	52.37^{c}	8.64	7.27	5.64	29.34	5.74
T2	57.73^{a}	8.28	6.66	4.57	26.33	5.77
T3	55.64^{ab}	7.75	8.05	4.84	30.00	5.83
T4	56.12^{ab}	7.78	8.63	4.73	30.99	5.82
T5	54.41^{bc}	8.36	8.85	4.91	28.99	5.73
T6	55.92^{ab}	8.06	8.53	5.22	28.12	5.73
T7	56.30^{ab}	8.22	7.28	4.72	28.71	5.87
T8	57.41^{ab}	8.31	8.43	4.83	28.24	5.79
SEM	0.52	0.29	0.24	0.12	0.36	0.02
P-value	0.009	0.787	0.337	0.024	0.446	0.234

T1: basal diet (negative control (NC)); T2: NC + 0.01% (v/w) Doxin 200 (positive control); T3: NC + 0.3% (v/w) postbiotic; T4: NC + 0.3% black cumin oil; T5: NC + 0.3% thyme oil; T6: NC + 0.15% postbiotic + 0.15% black cumin oil; T7: NC + 0.15% postbiotic + 0.15% thyme oil and T8: NC + 0.15% postbiotic + 0.075% black cumin oil + 0.075% thyme oil.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effect of a combination of postbiotics and phytobiotics on tibia bone characteristics of broiler chicken (at 35th day)

Bone characteristics	Treatments								SEM	P-value
	T1	T2	T3	T4	T5	T6	T7	T8		
Tibiotarsal weight (g)	4.46	4.88	5.02	5.00	5.00	4.89	5.00	4.64	0.069	0.697
Tibiotarsal length (mm)	88.39	90.99	89.58	90.81	91.28	89.48	90.84	87.97	0.368	0.169
Diaphysis diameter (mm)	9.05^{c}	10.24^{ab}	9.87^{b}	10.63^{a}	10.19^{ab}	10.31^{ab}	10.36^{ab}	10.11^{ab}	0.083	0.0001
Medullary canal (mm)	5.14	5.57	5.06	5.42	5.37	5.25	5.41	5.11	0.057	0.278
Tibiotarsal index	43.19^{b}	45.64^{ab}	48.58^{a}	49.06^{a}	47.24^{a}	49.09^{a}	47.60^{a}	49.53^{a}	0.532	0.035
Robusticity index	5.32	5.38	5.28	5.31	5.35	5.29	5.32	5.28	0.021	0.958
Calcium (Ca) mg/100 gm	8.11^{b}	8.33^{b}	9.19^{b}	10.77^{a}	10.94^{a}	8.30^{b}	9.31^{b}	8.67^{b}	0.460	0.001
Phosphor (P) mg/100 gm	184.7^{a}	125.0^{c}	188.5^{a}	129.5^{c}	161.3^{b}	168.5^{ab}	169.8^{ab}	155.8^{b}	0.242	0.0001
Ash %	0.902	0.848	0.838	1.020	1.027	0.950	0.890	0.803	0.033	0.61

T1: basal diet (negative control (NC)); T2: NC + 0.01% (v/w) Doxin 200 (positive control); T3: NC + 0.3% (v/w) postbiotic; T4: NC + 0.3% black cumin oil; T5: NC + 0.3% thyme oil; T6: NC + 0.15% postbiotic + 0.15% black cumin oil; T7: NC + 0.15% postbiotic + 0.15% thyme oil and T8: NC + 0.15% postbiotic + 0.075% black cumin oil + 0.075% thyme oil.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effect of a combination of postbiotics and phytobiotics on gut morphology of broiler chicken (at 35th day)

Treatments[1]	Villi height (mm)	Crypt depth (mm)	Muscle width (mm)	Villi width (mm)	Crypt width (mm)	Vh/Cd ratio	Absorptive surface area (mm)[2]
T1	0.942^{f}	0.159^{e}	0.233^{b}	0.079^{c}	0.087^{c}	6.1^{dc}	5.9^{bc}
T2	1.155^{e}	0.209^{d}	0.257^{b}	0.073^{c}	0.090^{c}	5.7^{d}	5.1^{c}
T3	1.795^{a}	0.292^{a}	0.303^{a}	0.171^{a}	0.195^{a}	6.2^{dc}	6.2^{b}
T4	1.713^{b}	0.262^{b}	0.262^{b}	0.153^{a}	0.193^{a}	6.6^{dc}	6.0^{bc}
T5	1.620^{c}	0.209^{d}	0.229^{b}	0.157^{b}	0.173^{a}	7.9^{ab}	6.9^{ab}
T6	1.507^{d}	0.204^{d}	0.234^{b}	0.128^{b}	0.143^{b}	7.6^{ab}	7.4^{a}
T7	1.795^{a}	0.224^{cd}	0.241^{b}	0.113^{b}	0.138^{b}	8.4^{a}	7.4^{a}
T8	1.693^{b}	0.238^{bc}	0.241^{b}	0.117^{b}	0.127^{b}	7.0^{bc}	6.8^{ab}
SEM	0.02	0.01	0.01	0.01	0.01	0.62	0.59
P-value	0.0001	0.0001	0.0001	0.001	0.0001	0.0001	0.0001

T1: basal diet (negative control (NC)); T2: NC + 0.01% (v/w) Doxin 200 (positive control); T3: NC + 0.3% (v/w) postbiotic; T4: NC + 0.3% black cumin oil; T5: NC + 0.3% thyme oil; T6: NC + 0.15% postbiotic + 0.15% black cumin oil; T7: NC + 0.15% postbiotic + 0.15% thyme oil and T8: NC + 0.15% postbiotic + 0.075% black cumin oil + 0.075% thyme oil.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Figure (1) displays photos of the small intestine's crypt depths and villi heights taken with a light microscope. The postbiotics and phytobiotics had a positive effect on gut morphology; overall, treatments fed postbiotics and phytobiotics had significantly (P<0.05) higher villi measurements compared to the control.

Villi height, crypt width, and villous area in all bird groups (T3, T4, T5, T6, T7, and T8) fed postbiotics and phytobiotics increased compared to the negative control (T1) and positive control fed antibiotics (T2). The bird's group in T3 and T7 had detected the highest villi height across all treatment groups.

No significant differences were observed in the crypt depth of the bird's group (T5, T6, and T7) fed phytobiotics and postbiotics compared to the group fed antibiotics (T2). Furthermore, chicken groups fed postbiotics and phytobiotics and antibiotics had higher villi crypt depth compared to the negative control. The highest villi crypt depth was detected in chicken groups T3, T4, and T8. The villi crypt width of broilers fed a combination of postbiotics and phytobiotics (T3, T4, T5, T6, T7, and T8) significantly increased compared to the negative and positive controls. No significant differences (P>0.05) in muscle depth were shown in all treatments fed a combination of phytobiotics and postbiotics compared to negative and positive controls. However, muscle depth in T3 is greater compared to all treatment groups. Also, Vh/Cd ratios were significantly increased in birds in T5, T6, T7, and T8 compared to negative control, positive control, T3, and T4. A higher ratio of Vh/Cd was detected in T7. However, it showed that the absorptive surface area of villi increased significantly (P<0.05) in all experimental treatment groups fed postbiotics and phytobiotics compared to birds in T2 fed antibiotics. No significant differences were observed across T5, T6, T7, and T8 in absorptive surface area. However, a higher absorptive surface area was recorded in T7 and T8 compared to all other treatment groups and negative and positive controls.

The results of tibia bone quality are shown in Table 5. The right tibia bones of the birds group fed dietary contain postbiotics and phytobiotics has not significantly (P>0.05) affect any of the examined bone parameters, such as tibiotarsal weight, tibiotarsal length, medullary canal, robusticity index, and percentage of ash, compared to the positive or negative control. Nevertheless, observed only quantitatively higher tibiotarsal weight, length, and ash% in all birds' groups compared to the control. However, there were significant differences (P<0.05) in the diaphysis diameter, tibiotrasal index, calcium, and phosphor. The highest levels of diaphysis diameter were recorded in overall treatments of the bird's group fed postbiotics and phytobiotics compared to the negative control but did no significant differences with positive control. The tibia bone calcium level in the bird group fed phytobiotic (thyme and black cumin oil) in T4 and T5 was found to be higher than in the group fed postbiotic and control. No significant differences in tibia bone calcium levels were found between the birds' group fed postbiotics and phytobiotics in T3, T6, T7 and, T8 compared to the positive control. The findings showed that the tibia bone phosphor levels in the bird's group fed postbiotics and phytobiotics (T3, T6, and T7) was highest significantly compared to groups fed antibiotic control. No significant differences were observed within the bird's group at (T5, T6, T7, and T7), and a lower level of phosphor was detected in T4.

Results from the current study showed that broiler chickens fed combinations of postbiotics and phytobiotics had a positive influence on growth performance and got similar results to the bird's group that fed antibiotics and negative control. The current results are in agreement with those of Kareem *et al.* (2016) who found that birds fed postbiotics and inulin had higher FBW, WG, and lower FCR compared to the negative and positive controls. Similar to the findings by Abd El-Ghany *et al.* (2022) reported that the application of postbiotic compounds in feed and water significantly improved the average body weight and feed conversion ratio of broilers compared to the control. However, final body weight, body weight gain, feed conversion ratio, and Europe broiler index were found to improve significantly in groups fed phytobiotics and probiotics compared to the positive control (Hussein *et al.* 2020b).

Also, Kareem, (2020) reported the same outcome: the bird groups that were fed 0.4% postbiotic improved the growth performance of quail. However, Mohammed and Kareem *et al.* (2022) reported no significant differences in broiler chicks fed 0.03% postbiotics in FBW, WG, total FI, and, FCR compared to the control. All feed additives were found to enhance growth performance and feed efficiency (Hussein *et al.* 2020a). Another result by Ferdous *et al.* (2019) showed the highest FBW, and WG with the lowest FCR in birds fed phytobiotic and probiotic. Similarly, Kareem *et al.* (2021) reported that a broiler-fed diet with combinations of various levels of postbiotic and inulin (0.015 and 0.03%) had significantly improved weight gain and feed conversion ratios. This could be due to the fact that phytobiotics (thyme and black cumin oil) containing high levels of bioactive phytochemicals such as carvacrol, thymol, and thymoquinone responsible for the antibacterial properties, exert stimulatory effects on pancreatic digestive enzymes and their positive effect on nutrient digestibility or even their appetite stimulation (Martel *et al.* 2020). Increased intestinal absorption has been hypothesized to enhance performance (Al-khalaifa *et al.* 2019). The organic acids and bactericins as antimicrobial metabolites in postbiotics can reduce the gastrointestinal pH and increase the beneficial bacteria population (Aguilar-Toala *et al.* 2018).

Postbiotics possess bacteriostatic and bactericidal properties, which result in a reduction of pathogenic bacterial load within the gastrointestinal microbiota. Additionally, postbiotics exhibit an inhibitory effect against various pathogens (Kareem *et al.* 2014; Kareem *et al.* 2016; Kareem *et al.* 2021).

Therefore, postbiotics and phytobiotics can act similarly to antibiotics in terms of promoting growth. During this

trial, the cumulative percentage of mortality in this study was a significant (P<0.05) low, and high EPI compared with the bird group fed the basal diet, representing the positive effect of feed additives (postbiotics and phytobiotics) on the mortality rate, and economic index. The lower mortality rate may be attributed to the inhibitory effects of these additives on intestinal microorganisms through the modification of the intestinal pH value (Abdel-Hafeez *et al*. 2017; Hussein *et al.* 2020b). These findings were similar to those found by Hussein *et al*. (2020a) indicating that the addition of probiotics and phytobiotics as feed additives decreased the mortality rate and increased the EPI. Additionally, this result is consistent with that reported by Danladi *et al.* (2022), who found that broiler chickens fed postbiotics (0.2%) had the highest level of the European broiler index and the lowest mortality rate when compared to the group of birds given antibiotics.

The results of the current study show that, when compared to bird groups that were fed antibiotics or a basal diet, the addition of postbiotics and phytobiotics did not have a statistically significant impact on the percentage of drip loss, cooking loss, or pH value of breast meat samples, while the lightness was improved. A similar trend was observed by Kareem *et al.* (2015) for cooking loss and pH value when they fed broiler postbiotic and inulin, in contrast, the percentage of drip loss was reduced. At the same time, Orlowski *et al.* (2018) findings in the cornet study indicated that drip loss and pH did not significantly differ in broiler chickens that drank phytogenic compared to the control. Conversely, another study done by Mohammed and Kareem (2022) observed that breast meat of broiler chicken fed postbiotic had a positive effect on cooking loss, drip loss, and pH value compared to the bird's group fed the antibiotic or basal diet. Also, Kareem *et al.* (2015) found an increase in drip loss of broiler chicken breast meat fed postbiotic and inulin. Color is one of the main indicators of the quality of most foods. The pH is frequently used as an important indicator for evaluating meat quality since it is related to the meat's color, and water-holding ability, which is largely governed by the postmortem conversion of muscle glycogen to lactic acid (Tang *et al*. 2021). If comparing the average pH values of breast meat obtained in this study with the proposed criteria, it could be noticed that the breast meat of broiler chicken fed with a combination of postbiotics and phytobiotics could be classified as normal quality meat; the range was between 5.73 and 5.87. Similar findings were reported by Kareem *et al*. (2015), birds fed postbiotic and inulin had no significant difference in lightness, redness, and, yellowness compared to birds fed antibiotics.

Also, Mohammed and Kareem (2022) obtained the same results and detected that birds fed postbiotic had no significant differences in lightness and redness compared to birds fed antibiotics. On the other hand, Popovic *et al.* (2019), found in their study that phytobiotics (essential oil of thyme and oregano) added to the diet of broiler chickens did not affect the value of this indicator pH value, redness (a*), and yellowness (b*) color, but lightness (L*) color and drip loss were significantly improved (P<0.05) compared to control treatment which is consistent with the findings obtained in this study. The lightness L* value is the main parameter that determines the poultry meat color. Garcia *et al.* (2010) mentioned that pale soft and exudative (PSE) meat lost more than 14.61% water during heat treatment, which the authors explain as a reduction of WHC in meat due to protein denaturation at a lower pH value. Fillets with higher values are indicative of lighter color, which is associated with low pH (pH< 5.6), while values falling below this range are indicative of darker fillets with high pH (pH>5.9) according to Ristic and Damme (2013). The property in question exhibits a strong correlation with other attributes, namely pH, water-holding capacity, emulsifying capacity, and texture, as stated by Garcia *et al.* (2010). The correlation between pH and meat quality attributes such, as color, and water-holding capacity is well-established (Tang *et al.* 2021).

The villi are the main components responsible for the absorption of nutrients in the small intestine, and improving gut morphology has a positive role in growth performance. Villus height and crypt depth are important indicators of gut function and animal health (Humam *et al.* 2019). The results of the present study showed that the chick's group fed postbiotics and phytobiotics generally had detected a positive enhancement and improved gut morphology. Contrary to the above findings, Peng *et al.* (2016) reported that supplementation of *Lactobacillus plantarum* in broiler diets did not affect gut morphology and no significant differences in villus height, crypt depth, and villus height to crypt depth ratio compared to control. However, Hafeez *et al.* (2020) found villus length and width decreased (P<0.05) in phytobiotics (black cumin) fed broilers, whereas the crypt depth, and the villus length to crypt depth ratio were not changed (P<0.05). However, in agreement with our finding, Humam *et al.* (2019) also found that dietary supplementation with postbiotics produced by *Lactobacillus plantarum* significantly increased the villi height, crypt depth, and villi height: crypt depth compared with the control. Also, Zangana and Mohamad (2016) observed an increase in the villi height and crypt depth of broiler-fed probiotics. Furthermore, Basit *et al.* (2020) mentioned dietary supplementation of phytobiotics improved gut morphology, and positively modulated and maintained the dynamics of cecal microbiota with enhanced nutrient digestibility, thus, increasing the growth performance, through increasing the villus height, crypt depth, and, villus height/crypt depth.

The improvements in both the height of the villi and the depth of the crypts may be attributed to the positive effect of the postbiotics composition, which contains a group of amino acids, a group of vitamins, mineral elements, and volatile fatty acids that is an important source of food for the intestinal cells to sustain and renew them continuously, and then increasing the length of the villi and the depth of the crypts to contribute better to digestion and absorption (Zangana and Mohamad, 2016).

In the current study, tibiotarsal weight, tibiotarsal length, medullary canal, robusticity index, and percentage of ash were not affected by the postbiotics and phytobotics of broiler chicken. While diaphysis diameter, calcium, and phosphor were improved. This observation corroborates the findings of Kareem *et al.* (2015), who reported that the tibiotarsal index and robusticity were not affected by postbiotics and inulin. In a similar study by Hafeez *et al.* (2020), bone length, bone weight, and tibiotarsal index in broilers were not affected by being supplemented with phytobiotics (black cumin). Unlike the present results, Hafeez *et al.* (2020) showed that the robusticity index decreased significantly in the bird group fed phytobiotics and mentioned that a lower robusticity index indicates stronger bones. At the same time, Hafeez *et al.* (2020) reported that all of the bone parameters under consideration, including tibia bone weight, length, ash %, robusticity index, and tibiotarsal index were not affected ($P<0.05$) due to the inclusion of a phytobiotic (black cumin) at different levels in the broiler. In contrast, other studies have reported positive effects of broiler tibia bone-fed postbiotics and phytobiotics by Al-Qahtani *et al.* (2021) found that birds' tibia bone length and weight improved when fed probiotics, but on the other hand, diaphysis diameter, tibiotarsal index, and robusticity were not affected. Also, Behlul and Yusuf (2021) agree with our results: Bone Ca, Mg, and P contents were higher in the group with phytobiotic (thyme essential oil) added to the diet than in the control group. Also, Fuentes *et al.* (2013) observed the tibia bone parameter, diaphysis diameter, calcium, and phosphorus in birds-fed probiotics (LAB). The importance of the leg bone comes from the intensive production and raising of broiler chickens that are particularly susceptible to leg disorders due to selective breeding for rapid growth and rapid weight gain with large breast muscles, which leads to an imbalance between body size and the weight-supporting skeletal system (Meyer *et al.* 2019). Bone minerals are an essential source of minerals for metabolic needs that provide strength and hardness to bone tissue in birds, and their development can be enhanced with dietary supplementation (Javid *et al.* 2022).

CONCLUSION

The study demonstrated that the addition of postbiotics and phytobiotics had beneficial effects on total body weight, feed conversion ratio, meat quality, gut morphology, and the tibia bone of broiler chickens. However, birds in T7 fed (basal diet+0.15% postbiotic+0.15% thyme oil) had higher total body weight and broiler Europe Index, and also had a lower feed conversion ratio and percentage of mortality than the other treatments. The combination of postbiotic and phytobiotic had positive effects on meat and tibia bone quality and improved the percentage of tibia bone minerals, such as calcium and phosphorus. However, the tibiotarsal index and the villus absorptive surface area were increased as compared to the birds fed antibiotics. Thus, a combination of postbiotics and phytobiotics, especially T7, could be used as a substitute for antibiotics in diets to improve the growth, meat quality, tibia bone, and gut health of broiler chickens.

ACKNOWLEDGEMENT

We would like to give thanks to all those who supported us in conducting this study in the Department of Animal Production, College of Agricultural Engineering Science, University of Salahaddin-Erbil, Kurdistan Region, Iraq.

REFERENCES

Abd El-Ghany W.A., Fouad H., Quesnell R. and Sakai L. (2022). The effect of a postbiotic produced by stabilized non-viable *Lactobacilli* on the health, growth performance, immunity, and gut status of colisepticaemic broiler chickens. *Trop. Anim. Health Prod.* **54,** 286-295.

Abd El-Ghany W.A. (2020). Paraprobiotics and postbiotics: contemporary and promising natural antibiotics alternatives and their applications in the poultry field. *Open Vet. J.* **10(3),** 323-330.

Abd El-Hack M.E., El-Saadony M.T., Salem H.M., El-Tahan A.M., Soliman M.M., Youssef G.B., Taha A.E., Soliman S.M., Ahmed A.E., El-kott A.F., Al Syaad K.M. and Swelum A.A. (2022). Alternatives to antibiotics for organic poultry production: Types, modes of action and impacts on bird's health and production. *J. Poult. Sci.,* **101(4),** 1-11.

Abdel-Hafeez H.M., Saleh E.S., Tawfeek S.S., Youssef I.M.A. and Abdel-Daim A.S. (2017). Effects of probiotic, prebiotic, and synbiotic with and without feed restriction on performance, hematological indices and carcass characteristics of broiler chickens. *Asian-Australasian J. Anim. Sci.* **30(5),** 672-682.

Abdulla N.R., Mohd Zamri A.N., Sabow A.B., Kareem K.Y., Nurhazirah S., Ling F.H., Sazili A.Q. and Loh T.C. (2017).

Physico-chemical properties of breast muscle in broiler chickens fed probiotics, antibiotics or antibiotic–probiotic mix. *J. Appl. Anim. Res.* **45,** 64-70.

Aguilar-Toalá J., Garcia-Varela R., Garcia H., Mata-Haro V., González-Córdova A., Vallejo-Cordoba B. and Hernández-Mendoza A. (2018). Postbiotics: An evolving term within the functional foods field. *Trends Food Sci. Technol.* **75,** 105-114.

Al-Khalaifa H., Al-Nasser A., Al-Surayee T., Al-Kandari S., Al-Enzi N., Al-Sharrah T., Ragheb G., Al-Qalaf S. and Mohammed A. (2019). Effect of dietary probiotics and prebiotics on the performance of broiler chickens. *J. Poult. Sci.* **98(10),** 4465-4479.

Al-Qahtani M., Ahiwe E.U., Abdallh M.E., Chang'a E.P., Gausi H., Bedford M.R. and Iji P.A. (2021). Endogenous enzyme activities and tibia bone development of broiler chickens fed wheat-based diets supplemented with xylanase, β-glucanase and phytase. *J. Anim. Biosci.* **34(6),** 1049-1060.

Aviagen. (2019). Ross 308: Broiler Performance Objectives and Nutrition Specifications. Aviagen Ltd., Newbridge, UK

Basit M.A., Kadir A.A., Loh T.C., Abdul Aziz S., Salleh A., Zakaria Z.A. and Banke Idris S. (2020). Comparative efficacy of selected phytobiotics with halquinol and tetracycline on gut morphology, ileal digestibility, cecal microbiota composition and growth performance in broiler chickens. *Animals.* **10(11),** 2150-2161.

Behlül S. and Yusuf C. (2021). Effects of essential oils and their combinations added to broiler diets on the mineral contents of some tissues and bone breaking strength. *J. Rocz. Nauk. Pols. Towarz. Zootech*. **17(3),** 59-69.

Danladi Y., Loh T.C., Foo H.L., Akit H., Md Tamrin N.A. and Naeem A.M. (2022). Effects of postbiotics and paraprobiotics as replacements for antibiotics on growth performance, carcass characteristics, small intestine histomorphology, immune status and hepatic growth gene expression in broiler chickens. *Animls*. **12(7),** 917-925.

Durso L.M. and Cook K.L. (2014). Impacts of antibiotic use in agriculture: what are the benefits and risks? *Curr. Opin. Microbiol.* **19,** 37-44.

Ferdous M.F., Arefin M.S., Rahman M.M., Ripon M.M. R., Rashid M.H., Sultana M.R., Hossain M.T., Ahammad M.U. and Rafiq K. (2019). Beneficial effects of probiotic and phytobiotic as growth promoter alternative to antibiotic for safe broiler production. *J. Adv. Vet. Anim. Res.* **6(3),** 409-415.

Fuentes C.G.G., Orozco L.A.Z., Vicente J.L., Velasco X.H., Menconi A., Kuttappan V.A., Kallapura G., Latorre J., Layton S., Hargis B.M. and Téllez G. (2013). Effect of a lactic acid bacteria based probiotic, floramax-b11®, on performance, bone qualities, and morphometric analysis of broiler chickens: an economic analysis. *Int. J. Poul. Sci*., **12(6),** 322-327.

Garcia R.G., Freitas L.W., Schwingel A.W., Farias R.M., Caldara F.R., Gabriel A.M.A., Graciano J.D., Komiyama C.M. and Almeida P. (2010). Incidence and physical properties of pse chicken meat in a commercial processing plant. *Brazilian J. Poult. Sci*. **12(4),** 24-32.

Grashorn M.A. (2010). Use of phytobiotics in broiler nutrition – an alternative to infeed antibiotics? *J. Anim. Feed Sci.* **19(3),** 338-347.

Hafeez A., Sohail M., Ahmad A., Shah M., Din S., Khan I., Shuiab M., Nasrullah S., W., Iqbal M. and Khan R.U. (2020). Selected herbal plants showing enhanced growth performance, ileal digestibility, bone strength and blood metabolites in broilers. *J. Appl. Anim. Res.* **48(1),** 448-453.

Halliwell B. (2001). Role of free radicals in the neurodegenerative diseases therapeutic implications for antioxidant treatment. *Drugs Aging*. **18,** 685-716.

Hayat M.N., Kaka U. and Sazili A.Q. (2021). Assessment of physicochemical characteristics and microbiological quality in broiler chicken breast muscle (pectoralis major) subjected to different temperatures and lengths of cold transportation. *J. Foods.* **10(4),** 874-886.

Humam A.M., Loh T.C., Foo H.L., Samsudin A.A., Mustapha N.M., Zulkifli I. and Izuddin W.I. (2019). Effects of feeding different postbiotics produced by *Lactobacillus plantarum* on growth performance, carcass yield, intestinal morphology, gut microbiota composition, immune status, and growth gene expression in broilers under heat stress. *Animals.* **9(9),** 644-657.

Humam A.M., Loh T.C., Foo H.L., Izuddin W.I., Awad E.A., Idrus Z., Samsudin A.A. and Mustapha N.M. (2020). Dietary supplementation of postbiotics mitigates adverse impacts of heat stress on antioxidant enzyme activity, total antioxidant, lipid peroxidation, physiological stress indicators, lipid profile and meat quality in broilers. *Animals.* **10(6),** 982-991.

Hussein E.O.S., Ahmed S.H., Abudabos A.M., Aljumaah M.R., Alkhlulaifi M.M., Nassan M.A., Suliman G.M., Naiel M.A.E. and Swelum A.A. (2020a). Effect of antibiotic, phytobiotic and probiotic supplementation on growth, blood indices and intestine health in broiler chicks challenged with *clostridium perfringens*. *Animals.* **10(3),** 507-514.

Hussein E.O.S., Shamseldein H.A., Alaeldein M.A., Gamaleldin M.S., Mohamed E.A., Ayman A.S. and Abdullah N.A. (2020b). Ameliorative effects of antibiotic-, probiotic- and phytobiotic-supplemented diets on the performance, intestinal health, carcass traits, and meat quality of clostridium perfringens-infected broilers. *Animals.* **10,** 669-676.

Javid M.A., Masood S., Zaneb H., Rehman H.U., Nazir M.M., Waqas M.Y., Asif M., Basit M.A., Abbas G. and Sufyan A.B. (2022). Effect of dietary growth promoter supplementations on the serum biochemistry and morphometric characteristics of tibia bone in broiler chicken. *Brazilian J. Poult. Sci*. **24(4),** 201-212.

Kareem K.Y., Foo H.L., Loo T.C., Ooi M.F. and Asmara S.A. (2014). Inhibitory activity of postbiotic produced by strains of *Lactobacillus plantarum* using reconstituted media supplemented with inulin. *Gut Pathog*. **14(6),** 23-31.

Kareem K.Y., Loo T.C., Foo H.L., Asmara S.A., Akit H., Abdulla N.R. and Ooi M.F. (2015). Carcass, meat and bone quality of broiler chickens fed with postbiotic and prebiotic combinations. *Int. J. Prob. Preb.* **10(1),** 23-30.

Kareem K.Y., Loh T.C., Foo H.L. and Asmara S.A. (2016). Effects of dietary postbiotic and inulin on growth performance, IGF1 and GHR mRNA expression, faecal microbiota and volatile fatty acids in broilers. *BMC Vet. Res.* **12,** 163-

171.

Kareem K.Y. (2020). Effect of different levels of postbiotic on growth performance, intestinal microbiota count and volatile fatty acids on quail. *Plant Arch.* **2,** 2885-2887.

Kareem K.Y., Loh T.C. and Foo H.L. (2021). Effect of new feed additive on growth performance and immunoglobulin of broilers. *IOP Conf. Ser. Earth Environ. Sci*. **761,** 12110-12119.

Kisielinski K., Willis S., Prescher A., Klosterhalfen B. and Schumpelick V. (2002). A simple new method to calculate small intestine absorptive surface in the rat. *Clin. Exp. Med.* **2(3),** 131-135.

Loh T.C., Choe D.W., Foo H.L., Sazili A.Q. and Bejo M.H. (2014). Effects of feeding different postbiotic metabolite combinations produced by *Lactobacillus plantarum* strains on egg quality and production performance, faecal parameters and plasma cholesterol in laying hens. *BMC Vet. Res.* **5(10),** 149-158.

Martel J., Ojcius D.M., Ko Y.F. and Young J.D. (2020). Phytochemicals as prebiotics and biological stress inducers. *Trends Biochem. Sci.* **45(6),** 462-471.

Meyer M.M., Johnson A.K. and Bobeck E.A. (2019). A novel environmental enrichment device improved broiler performance without sacrificing bird physiological or environmental quality measures. *Poult. Sci.* **98(11),** 5247-5256.

Mohammed M.Y. and Kareem K.Y. (2022). A comparison study of probiotic, postbiotic and prebiotic on performance and meat quality of broilers. *Tikrit J. Agric. Sci.* **22(4),** 24-32.

NRC. (1994). Nutrient Requirements of Poultry, 9th Rev. Ed. National Academy Press, Washington, DC., USA.

Orlowski S., Flees J., Greene E.S., Ashley D., Lee S.O., Yang F.L., Owens C.M., Kidd M., Anthony N. and Dridi S. (2018). Effects of phytogenic additives on meat quality traits in broiler chickens. *J. Anim. Sci.* **96(9),** 3757-3767.

Peng Q., Zeng X., Zhu J., Wang S., Liu X., Hou C., Thacker P. and Qiao S. (2016). Effects of dietary *Lactobacillus plantarum* B1 on growth performance, intestinal microbiota, and short chain fatty acid profiles in broiler chickens. *J. Poult. Sci.* **95(4),** 893-900.

Popović S., Puvača N., Peulić I., Kostadinović P. and Đuragić O. (2020). The usefulness of dietary essential oils mixture supplementation on quality aspect of poultry meat. *J. Agron. Technol. Engin. Manag.* **2(6),** 335-343.

Ramiah S.K., Zulkifli I., Rahim N.A., Ebrahimi M. and Meng G.Y. (2014). Effects of two herbal extracts and virginiamycin supplementation on growth performance, intestinal microflora population and fatty acid composition in broiler chickens. *Asian-Australasian J. Anim. Sci.* **27(3),** 375-382.

Rine R.C., Shovon L.S., Pravas C.R., Azraf A.M., Anwar H. and Iqbal K.J. (2021). Prebiotics, probiotics and postbiotics for sustainable poultry production. *World's Poult. Sci. J.* **77,** 825-882.

Ristic M. and Damme K. (2013). Significance of pH-value for meat quality of broilers: influence of breed lines. *Vet. Glasnik*. **67(1),** 67-73.

SAS Institute. (2012). SAS®/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Shazali N., Foo H.L., Loh T.C., Choe D.W. and Abdul Rahim R. (2014). Prevalence of antibiotic resistance in lactic acid bacteria isolated from the faeces of broiler chicken in Malaysia. *J. Gut Pathog.* **6,** 1-9.

Tabashsum Z., Alvarado-Martinez Z., Wall M.J., Aditya A. and Biswas D. (2023). Combined effect of metabolites produced by a modified Lactobacillus casei and berry phenolic extract on Campylobacter and microbiome in chicken cecum contents. *J. Food Sci.* **88(6),** 2583-2594.

Tang X., Liu X. and Liu H. (2021). Effects of dietary probiotic (*Bacillus subtilis*) supplementation on carcass traits, meat quality, amino acid, and fatty acid profile of broiler chickens. *J. Front. Vet. Sci.* **8,** 767802-767811.

Yitbarek M.B. (2015). Phytogenics as feed additives in poultry production: a review. *Int. J. Extensive Res.* **3,** 49-60.

Zangana B.S.R. and Mohamad M.J. (2016). The effect of early feeding with three types of probiotic on histology characteristics and intestinal microflora of gut broiler. *Iraqi Poult. Sci. J.* **10(1),** 59-69.

Zuidhof M.J., Schneider B.L., Carney V.L., Korver D.R. and Robinson F.E. (2014). Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. *Poult. Sci.* **93(12),** 2970-2982.