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The effects of different levels of postbiotic and phytobiotic combination as feed additives on carcass, lipid profile, meat quality, and tibia bone in broiler chickens

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Abstract

Like probiotics, postbiotics function without the presence of living cells and are composed of bacteriocins and metabolites that are antibacterial. They improve chicken products and health by reducing acidity in the gut and preventing infectious diseases. A total of 288 one-day-old unsexed broiler birds (Ross308) were divided into sex-specific treatment groups, with each group having four replicates and each replicate containing twelve birds.The treatment groups were as follows: T1 = Standard diet (negative control), T2 = Standard diet + 0.01% doxin200 (positive control), T3 (0.1%) = Standard diet + 0.05% thyme oil + 0.05% postbiotic, T4(0.2%) = Standard diet + 0.1% thyme oil + 0.1% postbiotic, T5(0.3%) = Standard diet + 0.15% thyme oil + 0.15% postbiotic, T6 (0.4%) = Standard diet + 0.2% thyme oil + 0.2% postbiotic.

The results showed significantly ($P<0.05$) increased breast cuts and a decrease in back cuts, in birds given varied levels of combined postbiotic and phytobiotic (0.1%, 0.2%, 0.3%, and 0.4%) compared to the negative and positive controls. Serum levels of total cholesterol and triglycerides showed significant reductions, and there was an increase in HDL among all groups of birds that received different levels of dietary supplements, as compared to the positive and negative control. The meat cooking loss rate was significantly reduced in birds fed at a 0.4% level compared to all experimental groups. No significant differences ($P > 0.05$) in meat color were noticed by feed additives compared to the positive control and stayed within the normal range. Various levels of the combination diet significantly improved tibia bone health, especially at a level of 0.4% (T6). In conclusions, a level of 0.4% combined (0.2% postbiotics+ 0.2% phytobiotics(thyme oil) as viable alternatives to antibiotics in broiler diets.

Key words: Probiotics byproducts, thyme oil, antibiotic alternative, tibia health, meat products.
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Introduction

The expansion of the poultry industry is being propelled by consumer demand for more health-conscious meat options, particularly Main cuts of meat (breast and drumstick), which contains a higher percentage of polyunsaturated fatty acids, which are essential for human growth and provide energy, and vitamins, additionally had good on texture, flavor, palatability, and color [1]. Consequently, experts in the poultry field have recently taken an interest in utilizing natural feed additives like probiotics, prebiotics, phytobiotics, and postbiotics as alternatives to medications and safer growth promoters. The aim is to produce premium-quality meat that is not only healthier but also devoid of antibiotic residues and resistant to bacteria [2], [3]. Probiotics have been widely incorporated into poultry feed to foster a healthy intestinal environment and enhance growth performance. While they offer several benefits for animal health, the use of live probiotic cells can be challenging due to storage requirements, host-specific colonization, and persistence abilities. Timing of administration is crucial for temporary colonization, and identifying suitable probiotic strains can be complex. Moreover, there's a notable risk that probiotic bacteria within the host could acquire virulent genes from pathogenic microbes through horizontal transfer, leading to antibiotic resistance [4]. As an alternative, postbiotics or probiotic metabolites are added to animal feed [5]. Postbiotics share similar capabilities and mechanisms of action with probiotics but lack live cells. They encompass bacteriocins and other antimicrobial byproducts that can lower gut pH and inhibit the growth of opportunistic infections [6].

Postbiotics, an alternative to probiotics, have gained attention for their metabolic by-products, which operate similarly to living organisms [7]. New terms like postbiotics refer to soluble factors secreted by live bacteria or released after lysis, such as enzymes, peptides, and polysaccharides. They have a clear chemical structure, safety dose parameters, and a long shelf life. Postbiotics may improve host health by improving specific physiological functions, although the exact mechanisms remain unclear [8].

Furthermore, aromatic plants have gained traction as antibiotic alternatives in the poultry industry. "Phytobiotics" refers to a range of organic compounds including herbs, spices, and essential oils, renowned for their antibacterial and pharmacological effects. These substances are commonly introduced into broiler feed to promote growth, stimulate feed intake, combat microbes, provide antioxidants, act as anthelmintics, and offer alternative medicinal benefits [3]. The addition of phytobiotics can enhance the production of digestive enzymes, boost immunity, increase appetite, and exert bactericidal, antiviral, and antioxidant effects. Additionally, they contribute to improved animal product quality and growth performance [9]. Based on prior research, it's reasonable to hypothesize that different combinations of postbiotics and phytobiotics as feed additives in varying doses will enhance growth performance and positively influence meat quality characteristics and tibia bone health in broiler chickens. The exact interaction between postbiotics and phytobiotics as feed additives in broiler chicken diets remains unexplored. Therefore, this study was conducted to investigate the impact of feeding different levels of combined postbiotics and phytobiotics on carcass traits, lipid profile, meat quality, and tibia bone health in broiler chickens.

Materials and methods

1. Postbiotic and phytobiotic produce

The initial culture of *Lactobacillus plantarum* was prepared at the Laboratory of animal resources within the College of Agricultural Engineering Science at Salahaddin University-Erbil, Iraq figure (1,2,3, and 4). The stock cultures underwent two rounds of revival using de-Mann Rogosa Sharpe (MRS) broth. These cultures were incubated at a temperature of 30°C, initially for 48 hours and subsequently for 24 hours under static conditions. Following this, spread plates were prepared, and the incubation continued for an additional 48 hours at 30°C. A single colony was carefully selected and introduced into 10 mL of MRS broth, where it was left to incubate for 24 hours. This step was followed by transferring the culture into

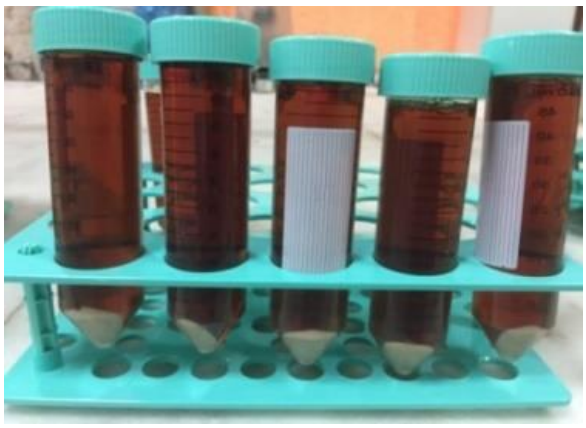
another 10 mL of MRS broth, allowing it to incubate for another 24 hours at 30°C. This prepared culture was now ready for use as an inoculum. To isolate the bacterial cells, a centrifugation process was carried out at a speed of 10,000 rpm (rotation per minute) for duration of 15 minutes [10].

The postbiotics, were collected and stored at a temperature of 4°C prior to the commencement of feeding trials. As for the phytobiotic component, extracts of thyme oil were locally obtained and purchased from Aram Factory in Duhok city.

Figure 1. Growth colony of *Lactobacillus plantarum* in MRS

Figure 2. LAB colony injected into MRS media

Figures 3. Centrifugation separate LAB from fermentation product



Figures 4. Postbiotics

2. Management and Experimental Design

The experiment took place at the farm under the Directorate of Agricultural Research in the Duhok government, spanning from November 10 to December 15, 2022. A total of 288 unsexed broiler chicks, aged one day, were procured from Evan Poultry hatchery situated in Erbil. A stocking density of 12 birds per square meter was maintained to ensure the welfare of the chicks. Each group of birds was equipped with an automated plastic bell-shaped drinker and a cylindrical feeder. The farm's temperature was set at 32°C during the initial week and was gradually reduced by 2°C each subsequent week. The chicks followed a conventional light schedule, receiving 24 hours of light during the first week and 23 hours of light with 1 hour of darkness in the following weeks. Adequate ventilation was maintained within the farm to dissipate heat and excess moisture, mitigate dust and odors, prevent the accumulation of harmful gases like ammonia and carbon dioxide, and provide ample oxygen for respiration.

In terms of nutrition, the chicks were fed a starter ration from day 1 to day 10, a grower ration from day 11 to day 24, and a finisher ration from day 25 to day 35. The basal diet was provided and produced by Bady Poultry Feed Company in Duhok (Table 2). An open-sided broiler house was divided into 24 equal-sized floor pens following proper cleaning, disinfection, and drying procedures. The experimental treatment groups included: T1 = Standard diet (negative control), T2 = Standard diet + 0.01% doxin200 (positive control), T3 (0.1%) = Standard diet + 0.05%

thyme oil + 0.05% postbiotic, T4 (0.2%)= Standard diet + 0.1% thyme oil + 0.1% postbiotic, T5 (0.3%) = Standard diet + 0.15% thyme oil + 0.15% postbiotic, T6 (0.4%) = Standard diet + 0.2% thyme oil + 0.2% postbiotic.

3. Bird Health and Vaccination Program:

According to the provided program below, the chicks received vaccinations following the recommendations of the local veterinarian's clinical advice (Table 1).

Table 1. Vaccination program for broiler

Vaccines name	Birds age/days	Ways
Newcastle disease (Ma5+clon30)	7	Drinking water
Infectious bronchitis bursal disease(gumboro)	14	Drinking water
Newcastle disease (lasota)	21	Drinking water

4. Sampling and Data Collection

At the conclusion of the experiment, on day 35 following a 12-hour fasting period, a total of eight chickens were chosen from each treatment group, four males and four females from each group. An average body weight close to the groups. These selected chickens were individually weighed, then subjected to the process of slaughter AL-Halal, feather removal, and evisceration. Blood samples were collected, with a volume of 5 ml, from which plasma was subsequently separated via centrifugation. This harvested plasma was employed for the analysis of the lipid profile. Furthermore, the mass of the cooled carcasses and relative carcass parts of edible giblets were estimated and calculated by using sensitive balance, and the right leg bone was collected from each treatment group for tibia bone characteristics. The percentage of meat cuts and organs weight was calculate according to [47]:

1- Relative carcass cuts % = (weight of cut/ Empty body weight) × 100.

2- Edible Organs % = (weight of organs / live body weight) × 100.

Meat Quality Characteristics Method

1. Drip Loss of Breast Meat

Drip loss was measured for each breast part sampled from eight broiler chickens for each experimental treatment. After slaughtering, approximately 30 g of fresh meat samples from the pectoralis major (breast) muscle were collected, individually weighed, and recorded as the initial weight (W1). The plastic bags used to pack each sample were sealed, and the vacuum packages were stored at (-20) °C in the refrigerators. Then, according to [38], after 7 days of storage, the final weight (W2) was measured immediately. After 24 hours after thawing from freezing, the meat samples were taken out of the bag and re-weighed, after drying them from moisture thoroughly with soft tissues Kleenex. The following formula by [11] was used to determine the percentage drip loss: Drip loss (%) = (W1−W2)/W1) ×100, whereby W1 is the muscle sample weight before storage (g) and W2 is the muscle sample weight after storage (g).

2. Breast Meat Cooking Loss

According to [12], the determination of cooking loss was conducted on each sample for eight broiler chickens in each experimental treatment. Approximately 30 g of each breast muscle sample was placed in polyethylene bags and vacuum-packed (W1). The samples were cooked by submerging them in a pre-heated water bath set at 80 oC for 20 minutes. Upon reaching the preferred internal temperature of 78 °C, the samples were allowed to cook for another 10 minutes. After cooling for 30 minutes under running water, the meat samples were removed from the bags, dried well from the water residue using soft paper towels without pressure, and weighed again (w2). The cooking loss percentage was then determined using the following equation: Cooking loss (%) = ((W1−W2) / W1) × 100, where W1 is the muscle sample weight before cooking in the water bath (g) and W2 is the muscle sample weight after cooking in the water bath (g).

Table 2: Composition of experimental standard diet (day 1–35th). Starter diet (1-10 days of age), (11-24 days of age), (25-35 days of age).

Ingredient (%)	Starter diet	Grower diet	Finisher diet
Broiler concentrate (5%)	5	5	3
Corn	45	50	49
Soybean (48%)	31	27.4	23
Wheat	14.1	12	19.2
Veget. 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	0.00
Anti-coccidiostat	0.05	0.05	0.00
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

3. Breast Meat Color

The methodology employed by [2] was adopted to assess the characteristics of meat color. Color values pertaining to the breast muscle sample, including L* (lightness), a* (redness), and b* (yellowness), were quantified utilizing the ColorFlex® system (NR20XE Precision Colorimeter). The light source was based on illuminant D65, and a 10° standard observer was employed, with an aperture size of 5 cm. To ensure accurate measurements, the color meter underwent calibration against black and white tiles initially. Subsequently, the frozen meat samples were thawed gradually over a span of 24 hours in a chiller set at a temperature of 4°C. Following the thawing process, the meat samples were allowed to bloom for duration of 30 minutes. They were then placed into the ColorFlex sample cup, with the surface of the meat facing the base of the cup. Measurements of meat color were taken at three distinct points, with the cup rotated by 90° in the second and third readings. The average values of the L*, a*, and b* readings were derived from these measurements, offering insights into the lightness, redness, and yellowness attributes of the meat sample.

4. pH Value of Breast Meat

The pH value of each sample of breast muscle was assessed following the procedure outlined by [2]. An indirect method was employed for determining the pH of the breast muscles, utilizing a portable pH meter (Eutech pH 700 pH/mV/°C/°F Bench Meter). Prior to usage, the pH meter underwent calibration using a standard buffer solution at pH 4.0 and subsequently at pH 7.0.

Around 0.5 grams of each crushed muscle sample were homogenized for duration of 20 seconds in 10 milliliters of distilled water. The pH of the breast meat was promptly recorded within 24 hours postmortem of the breast muscle. This measurement provides valuable information about the acidity or alkalinity of the meat sample, contributing to our understanding of its quality attributes.

Tibia Bone Parameter Determination

Right tibia bones were obtained from the eight slaughtered birds within each treatment group. Muscle tissue was meticulously excised using a scalpel blade subsequent to boiling the bones in water at 100°C for duration of 8 minutes. Afterward, the bones were dry at oven 38 temperature for a period of 48 hours. The weight of the bones was recorded using a digital balance, measured in grams. In addition, a digital caliper was employed to measure various parameters including bone length (mm), medullary canal diameter (mm), and the maximum and minimum bone diameter (mm) (Figures 5, 6, and 7).

Figure 5. Tibiotarasal Length Measurement

Figure 6. Diaphysis Diameter Measurement(B)

Figure 7. Medullary Canal Measurement

Following this, they were ashed to determine calcium and phosphorus values as well as the percentage of ash content. This was achieved using the PerkinElmer DA 7250 NIR (PerkinElmer DA 7250, 2020), a third-generation diode array NIR instrument tailored for swift analysis and parameter determination in the food and agricultural sectors, yielding results within a mere 6 seconds. Tibiotarsal and robusticity indexes were computed using the subsequent formulas:

Tibiotarsal index = (diaphysis diameter - medullary canal diameter) / diaphysis diameter) x 100

Robusticity index = bone length / cube root of bone weight [13], [14], [15].

Statistical Analysis

The statistical analysis was performed using the SAS package version 9.1 software, utilizing a completely randomized design (CRD) model [16]. The data collected for various parameters including growth performance, carcass yield, meat quality, gut parameters, and tibia bone characteristics were subjected to the generalized linear model within SAS. To assess significant differences between treatment mean, Duncan's multiple range test was employed. This comparison was conducted at a significance level ($P < 0.05$).

Results

1. Effects of Different Levels of Feed Additives on Carcass Percentage

The outcomes of the experiment regarding the impact of diverse levels of a combination of postbiotics and phytobiotics on carcass characteristics in broiler chicken diets are summarized in Table 3. The effects of the experimental diets did not exhibit a notable significance ($P<0.05$) across all carcass parameters, with the exception of breast and back measurements. No substantial variations were observed in the dressing percentage, drumstick, thigh, wing, heart, liver, and gizzard among broiler chickens that were fed varying levels (0.1, 0.2, 0.3, and 0.4%) of postbiotic and phytobiotic feed additives, across all experimental treatment groups. A significant increase ($P>0.05$) in the percentage of breast weight was observed within all experimental treatment groups T3, T4, T5, and T6 in comparison to the negative control. Conversely, a substantial reduction in the percentage of back weight was evident in T3, T4, T5, and T6 where various levels of postbiotics and phytobiotics (0.1, 0.2, 0.3, and 0.4%) with compared to the negative control. However, no significant differences were noted among the different bird treatment groups that received varying levels of the experimental diet in terms of the percentage of breast and back weight, when compared to the bird group that received antibiotics.

Table 3: Effects of a combination of different levels of postbiotics and phytobiotics on broiler chicken's carcasses (%).

Treatments	Dressing	Breast	Drumstick	Thigh	Wings	Back	Heart	Liver	Gizzard
T1	77.15	31.4[b]	14.3	12.7	10.2	24.7[a]	0.54	2.4	1.4
T2	78.03	38.8[a]	13.8	11.4	9.4	20.7[b]	0.65	2.9	1.3
T3	79.3	36.9[a]	13.7	12.6	10.1	21.2[b]	0.61	2.6	1.3
T4	79.91	37.8[a]	14.2	11.8	10.0	20.4[b]	0.63	2.5	1.4
T5	80.0	36.9[a]	14.3	12.3	9.8	20.9[b]	0.69	2.8	1.3
T6	80.21	37.7[a]	13.7	11.4	10.9	20.5[b]	0.64	2.5	1.6
P-Value	0.393	0.001	0.843	0.349	0.374	0.035	0.397	0.637	0.776
SEM	0.489	0.634	0.168	0.215	0.188	0.463	0.020	0.081	0.054

a, b, Means with different superscripts within columns indicate significant differences (P<0.05).
Treatment groups: T1 = Standard diet (negative control), T2 = Standard diet + 0.01% Doxin200 WS (w/w) (positive control), T3 = Standard diet + 0.05% thyme oil (v/w) + 0.05% postbiotic, T4 = Standard diet + 0.1% thyme oil (v/w) + 0.1% postbiotic, T5 = Standard diet + 0.15% thyme oil (v/w) + 0.15% postbiotic, T6 = Standard diet + 0.2% thyme oil (v/w) + 0.2% postbiotic. SEM: Standard Error of the Mean (pooled).

2. Effects of Different Levels of Feed Additives on Serum Lipid Profile

The impact of different levels of postbiotic and phytobiotic combinations on the serum lipid profiles of broiler chickens at the conclusion of the study (day 35) is presented in Table 4. Notably, varying levels of dietary supplementation 0.1, 0.2, 0.3, and 0.4% exhibited a significant reduction (P<0.05) in the average concentration of total cholesterol within treatment groups T4, T5, and T6 that received 0.2, 0.3, and 0.4% supplementation, when compared to group T3 fed 0.1% supplementation, as well as the positive and negative control. Additionally, the average concentration of triglycerides demonstrated a significant decrease (P<0.05) across all treatment groups that received different levels of supplementation compared to the negative control. Particularly noteworthy was the lower concentration of triglycerides observed in the high-level supplementation group 0.4% within treatment T6, recording a reduced value of 38.0 mg/dl. This reading displayed no significant differences in comparison to all treatment groups as well as the positive control. No significant variations were noted in the levels of LDL and VLDL among all treatment groups T2, T3, T4, T5, and T6 in contrast to the control group (P>0.05).

However, the average levels of HDL exhibited a significant increase in birds across various diet levels 0.1%, 0.2%, 0.3%, and 0.4% within treatment groups T3, T4, T5, and T6, in comparison to birds fed antibiotics T2, and the negative control. Remarkably, the birds fed in the T2 displayed a lower level of HDL (44.5 mg/dl) in comparison to all other experimental treatment group.

Table 4: Effects of different levels of postbiotics and phytobiotics combination on broiler chicken's serum lipid Concentration (mg/dl).

Treatments	Cholesterol	Triglyceride	HDL	LDL	VLDL
T1	121.0[a]	60.3[a]	50.5[b]	21.0	10.0
T2	123.0[a]	49.8[b]	44.5[c]	24.0	11.8
T3	124.0[a]	51.0[b]	58.0[a]	26.5	12.3
T4	104.0[b]	52.8[b]	56.3[a]	23.5	12.3
T5	106.3[b]	49.3[b]	55.7[a]	24.3	13.7
T6	101.0[b]	38.0[c]	55.8[a]	22.7	12.5
P-value	0.002	0.0001	0.0001	0.522	0.764
SEM	2.574	1.579	1.118	0.784	0.659

a, b, Means with different superscripts within columns indicate significant differences (P<0.05).
Treatment groups: T1 = Standard diet (negative control), T2 = Standard diet + 0.01% Doxin200 WS (w/w) (positive control), T3 = Standard diet + 0.05% thyme oil (v/w) + 0.05% postbiotic, T4 = Standard diet + 0.1% thyme oil (v/w) + 0.1% postbiotic, T5 = Standard diet + 0.15% thyme oil (v/w) + 0.15% postbiotic, T6 = Standard diet + 0.2% thyme oil (v/w) + 0.2% postbiotic. SEM: Standard Error of the Mean (pooled).

3. Effects of Different Levels of Feed Additives on Meat Quality

Table 5 presents the outcomes regarding the influence of various feed additives on meat quality attributes in broiler chickens, encompassing drip loss, and cooking loss, as well as the attributes of lightness (L*), redness (a*), and yellowness (b*). Across all experimental treatment groups, no significant variances were observed in terms of drip loss rate in broiler chickens (P<0.05). However, significant marginal changes were noted in cooking loss rates among all experimental treatment groups that received varying levels of the experimental diet. Notably, the meat from birds in treatment group T6, which was provided with a 0.4% combination of postbiotics and phytobiotics, exhibited a significantly reduced cooking loss rate when compared to all other experimental treatment groups. Furthermore, no siginificant differences were evident among the various experimental treatment groups that received levels of 0.1, 0.2, 0.3, and 0.4% of the combination of postbiotics and phytobiotics, in relation to breast meat pH values. These values remained within the normal pH range, highlighting the stability of pH under the influence of the experimental additives.

The findings additionally indicate that the different levels of the combination of postbiotic and phytobiotic experimental diets did not significant effects on the attributes of meat color, encompassing (L*), (a*), (b*), across all treatment groups (P<0.05).

Table 5: Effects of a combination of different levels of postbiotics and phytobiotics on broiler chicken's meat quality

Treatments	Drip loss%	Cooking loss%	Lightness	Redness	Yellowness	pH
T1	6.53	35.88[a]	57.158	8.395	8.656	6.047
T2	5.82	33.92[a]	55.577	9.585	9.673	6.087
T3	6.04	33.88[a]	58.110	8.395	9.462	6.135
T4	6.86	35.04[a]	57.693	7.869	9.100	6.105
T5	7.78	34.43[a]	55.648	8.570	9.137	6.105
T6	7.64	31.17[b]	54.471	9.505	8.755	6.067
P-value	0.436	0.031	0.590	0.264	0.838	0.327
SEM	0.329	0.449	0.666	0.245	0.245	0.012

a, b, Means with different superscripts within columns indicate significant differences (P<0.05).
SEM: Standard Error of the Mean (pooled).

4. Effects of Different Levels of Feed Additives on Tibia Bone Quality

Table 6 provides insights into the influence of varying levels of feed additives on the tibia bone characteristics of broiler chickens. The effects were evaluated across tibiotarsal weight, tibiotarsal length, medullary canals, and tibiotarsal index. Notably, no significant distinctions (P<0.05) were observed in tibiotarsal weight, tibiotarsal length, medullary canals, or tibiotarsal index among broiler chickens that were subjected to different levels of 0.1%, 0.2%, 0.3%, and 0.4% of the postbiotic and phytobiotic combination within all experimental treatment groups T3, T4, T5, and T6, in comparison to the negative and positive control However, data analysis revealed that the diaphysis diameter and robusticity index of the right tibia in broiler chickens exhibited a significant decrease across all treatment groups that were provided varying levels of the experimental feed additives, as opposed to the negative control group. Nevertheless, no significant differences were evident when compared to the positive control (antibiotics). Notably, the treatment group T6, which received 0.4% of the combined phytobiotics and probiotics, recorded the lowest diaphysis diameter (9.8 mm) and robusticity index (52.2 mm), displaying a significant difference when compared to other treatment groups (P>0.05).

Table 6: Effects of a combination of different levels of postbiotics and phytobiotics on tibia bone quality.

Tibiotarsal	weight(g)	Tibiotarsal Length(m)	W/L Index (mg/mm)	Diaphysis Diameter (m)	Medullary Canal(m)	Tibiotarsal Index	Robusticity Index
T1	5.3	85.9	58.1	11.2^{a}	5.3	48.1	5.2^{a}
T2	4.9	84.6	58.1	10.4^{ab}	5.2	51.1	4.9^{bc}
T3	5.2	89.7	57.7	10.9^{ab}	5.1	53.3	5.2^{ab}
T4	4.9	86.7	56.2	9.9^{ab}	5.0	50.3	5.1^{ab}
T5	5.1	88.7	57.2	10.9^{ab}	5.2	53.5	5.2^{ab}
T6	5.4	85.4	62.9	9.8^{b}	5.0	52.2	4.9^{c}
P-value	0.467	0.227	0.311	0.126	0.672	0.357	0.009
SEM	0.207	1.683	2.150	0.456	0.158	1.901	0.089

a, b, Means with different superscripts within columns indicate significant differences (P<0.05).

Discussion

1. Impact on Carcass Cuts and Edible Organs Percentage

The findings of this study reveal that the inclusion of various levels of postbiotics and phytobiotics in the diet of broiler chickens led to a notable increase in the percentage of breast and back cuts, while no significant differences were observed in the percentages of dressing, wings, drumsticks, thighs, liver, gizzards, or hearts. This contrasts with the observations of [2], who did not find any significant differences in breast percentage when utilizing different strains of 0.3% postbiotics and inulin. Nevertheless, their findings align with our results in terms of dressing percentage, liver, and gizzard percentages, which showed no significant differences compared to the control group. Similarly, [9], support our outcomes, stating that there were no significant variations in the percentages of carcass, legs, wings, liver, gizzard, and heart, although they diverge from our study regarding breast and back percentages. They reported that dietary supplements containing postbiotics did not affect breast and back percentages, and there were no significant differences between treatment groups fed 0.3% postbiotics of various strains compared to the negative control and a positive control containing 0.02% oxytetracycline. Additionally, the results of a study by [17] corroborate our findings, indicating that breast percentage increased and back percentage decreased in broiler chickens fed 0.3% postbiotic dietary supplements. No significant differences were observed in the percentages of wings, thighs, and drumsticks compared to the control group. This concurs with the findings of [18], who reported that carcass yield, including dressing, thighs, wings, liver, heart, and gizzard percentages, remained unaffected by a diet supplemented with 0.075% thyme essential oil. Their results showed no significant differences compared to the control group, except for breast percentage, which differed from our observations.

The rationale behind the observed increase in breast meat percentage and decrease in back meat percentage may stem from the improved health and growth performance of the birds due to the combined effect of phytobiotics and postbiotics in their diet. These compounds are recognized for being rich sources of nutrients and active substances with a crucial role in reducing pathogenic bacteria in the bird's body. [19] Emphasized that the composition of phytobiotic feed additives plays a pivotal role in determining their efficacy when combined, as the behavior of bioactive molecules can differ when used individually versus in combination. Additionally, [20] identified thyme as a valuable source of protein, fat, fiber, minerals, and essential vitamins that contribute to bird health. Furthermore, [20] highlighted the positive impact of postbiotic feed additives on broiler growth and health, citing improvements in immune status and gut health through enhanced gut morphology, increased lactic acid bacteria populations, and a reduction in Enterobacteriaceae populations and fecal pH under normal conditions.

2. Serum Lipid Profile Concentration

The results of the current study indicate significantly improved levels of cholesterol, triglycerides, and LDL among all treatment groups, while no significant differences were observed in LDL and VLDL. These findings align with [22], who reported a significant reduction in serum triglyceride and cholesterol levels in broiler chickens supplemented with different combinations of 0.3% postbiotic strains (RI11, RS5, UL4). However, they differ from our results regarding lower LDL and VLDL levels compared to controls. Similar outcomes were observed by [10], who noted decreased serum triglyceride and cholesterol levels following supplementation of postbiotics and inulin in broiler diets, yet disagreed with our observation of reduced LDL compared to antibiotic and negative control groups. These findings agree with [21], who found that supplementation with three different strains of 0.6% postbiotic significantly lowered serum cholesterol concentrations compared to the control group. Similarly, [23] reported that probiotic and herb plant supplementation reduced serum triglyceride levels compared to the control group. [24] Supported our findings of significantly decreased triglycerides and cholesterol levels, with no significant difference in LDL, but disagreed on VLDL, reporting significant reduction due to probiotic supplementation. Conversely, [25] observed increased levels of total cholesterol, total triglyceride, and LDL concentrations, as well as reduced HDL, in broiler chickens' blood serum fed with 300 mg/kg thyme oil compared to the control group.

It is important to note that cholesterol comes in two types: LDL often referred to as "bad" cholesterol, carries cholesterol to the body's tissues and blood vessels, while HDL, often referred to as "good" cholesterol, transports excess cholesterol from the tissues and blood vessels to the liver for removal. Triglycerides, on the other hand, are a type of fat that stores unused calories and provides energy to the body. Cholesterol is used to build cells and certain hormones [26].

The improvement in lipid profile concentration in blood serum may be attributed to active compounds in the plant extract stimulating liver and intestine cells to form HDL, while other compounds reduce cholesterol concentration in the blood. Flavonoids, acting as antioxidants, may contribute to normal LDL levels and increased HDL. Furthermore, the presence of normal LDL levels might be due to flavonoids' ability to enhance lipoprotein lipase activity, which breaks down triglycerides into fatty acids and glycerol, consequently reducing VLDL levels [27]; [28]; [29]; [26]. The presence of vitamins, minerals, phosphates, lecithin, and choline in herbal extracts could also interfere with cholesterol biosynthesis in the liver, reducing cholesterol levels [15].

Furthermore, the improved lipoprotein profile might be attributed to the postbiotics' potential to increase lactic acid bacteria population and enzyme production for bile salts in the gut. This leads to enhanced cholesterol breakdown and reduced absorption due to decreased gut pH, creating an unfavorable environment for bile salt dissolution. Additionally, intestinal LAB can convert primary bile acids into secondary bile acids through deconjugation [30]; [31]; [21]; [10].

3. Meat Quality

The findings of the present study reveal no significant differences in drip loss, lightness L*, redness a*, or yellowness b* of bird's breast meat in all treated groups compared to the negative and positive controls. However, a significant reduction in cooking loss was observed in birds fed a 0.4% combination of postbiotics and phytobiotics compared to the control and other treated groups 0.1, 0.2, and 0.3%. These results are consistent with [3], who found no significant differences in lightness, redness, or yellowness among all treatment groups compared to positive and negative controls after feeding phytobiotics and probiotics. In contrast, [2] reported that birds fed a combination of 0.3% postbiotic and 0.8-1% inulin exhibited lower drip loss, with no significant difference in cooking loss compared to controls. [32] Disagreed with our findings, reporting increased breast color L*, a*, and b* in treated groups fed probiotics (0.25-0.5 g/kg feed) compared to control. [17] Also presented opposing results, noting

significantly decreased drip loss, cooking loss, and pH value in the pectoralis major muscle of the treated group fed 0.3% postbiotic compared to controls. However, they agreed with us on the lack of significant differences in L* and a* compared to positive and negative controls, and b* compared to the negative control. [33] Reported similar results to our study when supplementing birds with phytobiotics alone or combined with Bacillus licheniformis (probiotic), finding no significant differences in breast meat drip loss, cooking loss, L* , a* , and b* compared to controls.

Meat color is a crucial factor in consumer preferences for purchasing poultry products, with some favoring white meat and others preferring yellow-skinned chickens or shades in between [34]. Factors such as breed, age of birds, diet composition, pH levels, processing methods, and lipid content can influence meat color, with higher pH and lipid content causing darker meat [35]. In the current study, meat color values were found within normal ranges, except for birds fed a 0.4% combination of postbiotics and phytobiotics, which fell into the "dark" color category according to [36]. Nevertheless, our results remained within the normal meat color range according to Petracci et al. (2004), who classify lightness (L*) values as dark (L* < 50), normal (50 ≤ L* ≤ 56), or pale (L* > 56).

Meat properties like drip loss, cooking loss, and pH value are crucial for producing value-added meat products [37]. Drip loss indicates water-holding capacity (WHC), where lower rates signify higher water-soluble nutrient content and juiciness [38]. Meat pH is also significant, influencing both bacterial decay susceptibility and shelf life. Normal pH values fall within the range of 5.3-6.5 [39]. Low pH values result in greater drip and cooking loss, while higher pH levels enhance water-holding capacity [34]. Furthermore, pH is correlated with meat color; lighter muscles (L* > 50) exhibit higher pH values, while darker muscles (L* < 45) have lower pH values, with negative correlations between pH, L*, and b*, and positive correlation between pH and a* values [40].

4. Tibia Bone Quality

The findings of this study indicated that the combination of postbiotics and phytobiotics at different levels had no impact on tibiotarsal weight, tibiotarsal length, W/L index, medullary canals, or tibiotarsal index. While improving the medullary canal and robusticity index. This observation agrees with the findings of [2], who reported that 0.3% postbiotic (RI11, RG14) alone or in combination with 0.8–1.0% inulin didn't improve the bone-breaking strength and tibiotarsal index when they reported there were no significant differences across all treatment groups compared to the positive and negative control, but regarding the robusticity index, they disagreed with our finding when they reported no significant differences among all treatment groups. Also, this finding is consistent with the results of [32], who suggested that 0.25 and 0.5 g/kg of probiotics (Bacillus subtilis) did not result in any significant changes in the tibiotarsal index and medullary diameter, compared to all treatment groups and the control group, but that they contrasted in diaphysis diameter and robusticity index, where no significant differences were found in comparison to the control. The results of the current research are similar to those reported by [41]; no significant differences in tibia-breaking strength were observed in broilers fed 50 mg/kg of thyme essential oil, rosemary, or French lavender alone or in combinations at a level of 25 mg/kg. While these data contradict the findings of [42], who showed a significant increase in the tibia weight and tibia length of broilers in the diet containing the phytogenic compound (thyme cm/liter of water) compared to treatment groups with (1cm/ litter of water and 1cm /15 litter) and to controls. The results of the present study also differ from those of [43], who found that birds fed 0.1% probiotic (lactobacillus) had significantly higher tibia weight, tibia length, tibiotarsal index, tibia weight/tibia length index, and robusticity, but no significant differences in bone ash % or diaphysis diameter compared to controls.

Our interpretation of the findings is that the decrease in tibia diaphysis diameter and robusticity index value indicates an improvement in bone quality, health, and

strength. This improvement may have been attributed to the fact that thyme is a rich source of minerals, especially calcium, phosphorus, and magnesium, which are essential for bone development, due to thymol and carvacrol, which have a beneficial function in enhancing digestive secretions in the gut. [44] Reported that a low value of the robusticity index indicates a strong bone structure and a high value of the tibiotarsal index indicates a high mineralization level of the bone. [41], reported that the bird's tibia contained a higher concentration of Ca 24.9, P 10.3, and Mg 0.32%, among all other treatments fed (50 mg/kg of rosemary and French lavender oil and their combination), and reported higher excreta mineral concentrations of Ca 3.1, p 1.4, Mg 0.32, Cu 34.5, Fe 560, Mn 3.7, and Zn 333 mg/l, among all other treatments. It has been reported that thyme essential oil, which contains the polyphenolic compounds thymol and carvacrol, may affect Ca and P metabolism and thus have positive effects on bone resorption [45]. The main functions of volatile oils are to control pathogens as antimicrobials and antioxidants, assist in digestion through enzyme stimulation activity, and absorb nitrogen [46].

Conclusion

In conclusion, the study suggests that the utilization of higher levels of combined postbiotics and phytobiotics, specifically at a concentration of 0.4% (basal diet + 0.2% postbiotic + 0.2% thyme oil), holds promise as a viable alternative to antibiotics in broiler diets. This approach exhibits the potential to not only enhance carcass yield, but also improve blood lipid profiles, elevate meat quality, and fortify tibia bone quality in broiler chickens.

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مجلة جامعة كركوك للعلوم الزراعية

تأثير مستويات مختلفة من مزيج البوستبايوتك والفايتوبايوتك كإضافات علفية على الذبيحة، ونسبة الدهون، ونوعية اللحوم، وعظام الساق في فروج اللحم

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- تاريخ استلام البحث2023/08/12 وتاريخ قبوله 2023/08/28.
- البحث مستل من اطروحة دكتوراه للباحث الاول.

الملخص

تعمل البوستبايوتك، على غرار البروبيوتيك، دون خلايا حية ، تتكون من البكتريوسينات ومنتجات ثانوية اخرى مضادة للميكروبات. إنها تعززمنتجات الدواجن عن طريق تقليل حموضة الأمعاء ومنع اصابتها بمسببات العدوى الانتهازية. تم تقسيم اجمالي 288 من طيور اللحم Ross308 غير مجنسة بعمر يوم واحد إلى 6 معاملات تجريبية، حيث تحتوي كل معاملة على 4 مكررات وتحتوي كل مكرر على 12 طائرا، وكانت على النحو التالي:

T1 = السيطرة السالبة (علف قياسي).

T2 = السيطرة الموجبة (علف قياسي + 0.01٪ مضاد حيوي دوكسين200.

T3 = 0.1 ٪ (علف قياسي + 0.05٪ زيت الزعتر + 0.05٪ بوستبايوتك).

T4 = 0.2 ٪ (علف قياسي + 0.1٪ زيت الزعتر + 0.1 ٪ بوستبايوتك).

T5 = 0.3٪ (علف قياسي + 0.15 ٪ زيت زعتر + 0.15 ٪ بوستبايوتيك).

T6 = 0.4٪ (علف قياسي + 0.2 ٪ زيت زعتر + 0.2٪ بوستبايوتك).

أظهرت النتائج بوجود زيادة معنوية ($P<0.05$) في قطع الصدر وانخفاضها في قطع الظهر للطيورالذي يتغذى على مستويات متفاوتة (0.4,0.3,0.2,0.1٪) من علف التجربة مقارنة بمعاملة السيطرة الموجبة والسالبة. انخفض إجمالي الكوليسترول والدهون الثلاثية في الدم بشكل ملحوظ، بينما زاد تركيز HDL في جميع المعاملات التي تلقت مستويات مختلفة من الإضافات الغذائية مقارنة بالسيطرة الموجبة والسالبة. كما انخفض معدل فقد اللحوم أثناء الطهي بشكل ملحوظ للطيور عند مستوى 0.4٪ مقارنة بالمستوى الاقل في المجموعات التجريبية الاخرى. كما لوحظ بعدم وجود تاثير معنوى على صفات جودة اللحوم ، في حين ظلت ضمن النطاق الطبيعي بالاخص صفة لون اللحم. كما لوحظ هناك تحسن في صحة وجودة عظام الساق بشكل ملحوظ و خاصة عند مستوى 0.4٪. خلصت الدراسة إلى أن أنسب نسبة استبدال للمضادات الحيوية في أعلاف دجاج التسمين كانت 0.4٪ (علف قياسي + 0.2٪ زيت زعتر + 0.2٪ بوستبيوتيك).

الكلمات المفتاحية: منتجات البروبيوتيك الثانوية، زيت الزعتر، بديل المضادات الحيوية، صحة الساق، منتجات اللحوم.