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Effects of Different Sources of Probiotics on Performance, Carcass, Intestinal Morphology and Bone Characteristics in Broiler Chickens

Research Article

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ABSTRACT

The type of microorganisms in probiotic products is the considerable factor on the effectiveness of probiotics. An experiment was conducted to investigate the influence of different sources of probiotics on broiler performance, carcass variables, intestinal morphology and bone indices. A total of two hundred and eighty-eight one-day-old Ross 308 male broiler chicks were allocated into the three experimental groups with eight replications of 12 chicks each. The treatments were a basal diet (control) and a control diet supplemented with lactic acid bacteria (LABP) or *Bacillus* strains (BPS) probiotics. The birds were fed the respective diets from 1 to 42 days of age. Dietary treatments had no significant effect on the performance variables. The jejunal villus length and surface area increased ($P<0.05$) in the birds which received LABP or BPS compared to the control group. The ileal villus length, width, surface area and villus length to crypt depth were greater ($P<0.05$) in broiler chicks fed with BPS than the other groups. Supplement to diet with LABP increased ($P<0.05$) tibia strength and phosphorous compared with BPS, LABP did not affect phosphorus compared with control, BPS reduced ($P<0.05$) phosphorus content in the tibia compared with the control. The serum concentration of glucose and total protein were greater ($P<0.05$) in the birds received BPS compared to control group. It can be concluded that supplementation of LABP or BPS probiotics in the diet had beneficial effects on Jejunum villi length and surface area and bone strength, but not performance variables in broiler chickens.

KEY WORDS *Bacillus* strains, broiler chickens, lactic acid bacteria, morphology, performance.

INTRODUCTION

In recent years, use of in-feed-antibiotics have been limited or not at all in the poultry diets. Antibiotics can have negative impacts on beneficial bacteria (such as *Lactobacilli*, *Bifidobacterium*) and harmful bacteria in the gut. Improper use of antibiotics will lead to pathogen resistance and the emergence of problems in treating animal infections and in human, too (Deraz *et al*. 2019). Therefore, antibiotics alternatives have been introduced, including probiotics, prebiotics, symbiotics, postbiotics, organic acids, natural antimicrobials, medicinal plants, and essential oils (El Jeni *et al*. 2021). Probiotics are beneficial microorganisms that, if properly included in the host animal, provide some beneficial organisms such as *lactobacilli*, *Bifidobacterium*, and *Streptococci*, strengthen the intestinal microflora and improves the animal's living conditions (Sharifi *et al*. 2012). Therefore, several probiotic products based on bacteria or yeast have been used as alternative to antibiotic growth promotor in poultry nutrition (Mohammed *et al*. 2021; Pour baba *et al*. 2022). According to literature, probiotic-supplemented diets may improve production performance,

as well as reducing chicken mortality and environmental pollution (Bai *et al.* 2018; Al-Khalaifa *et al.* 2019; El Jeni *et al.* 2021). In broiler chickens, contradictory results have been observed in terms of the effects of probiotic supplementation on productive performance (Khattab *et al.* 2021; Mohammed *et al.* 2021). It has been reported that various factors such as age and immune status of the bird, history of antibiotics consumption in birds, environmental conditions, probiotic dosage, and the type and number of microorganisms in the probiotics are involved in these contradictory results (Aliakbarpour *et al.* 2012; Makled *et al.* 2019; Mohammed *et al.* 2021; Salehizadeh *et al.* 2019). According to previous studies, probiotics based on *Lactobacillus* and *Bacillus* species are most used in poultry feeding (Liang *et al.* 2021; Sandvang *et al.* 2021). Due to the existence of probiotic diversity in terms of providing microorganism strains, some researchers emphasized the comparison of their effectiveness (Reis *et al.* 2017; Rahmani *et al.* 2023). Therefore, this experiment was performed to compare the effect of two different probiotics in terms of bacterial constituents on performance variables, carcass traits, intestinal morphology and bone characteristics in broiler chickens.

MATERIALS AND METHODS

Birds and experimental diets

The experiment was performed in agreement with the institutional animal care and research advisory committee of Babol branch, Islamic Azad University, Babol, Iran (ID number: IR.IAU. BABOL.REC. 1399.051).

In order to eliminate the effect of gender on some biological indicators and performance variables (Livingston *et al.* 2020), a total of two hundred and eighty-eight Ross 308 male broiler chickens were provided from a commercial hatchery (Babol city, Iran). All birds were distributed into three experimental groups with eight replications (pens) of 12 chicks each.

Pens with dimentions of 1 × 1.5 m were used. In this experiment, the groups included: C) control diet (Unsupplemented corn-soybean based commercial), LABP) control diet supplemented with lactic acid bacteria probiotic (brand name Lactofeed from Tak Gen group Company, Tehran, Iran), BPS) control diet supplemented with *Bacillus* strains probiotic (the brand name Parsi lact from Pardis Roshd Mehregan Company, Shiraz, Iran).

According to the manufacturers, LABP containing: *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium*, *Enterococcus faecium*, genetically modified organisms (the minimum amount of each bacterium was reported to be 2.5×10^7 CFU per gram) and BPS containing *Bacillus subtilis* and *Bacillus coagulans* (the minimum amount of each bacterium was reported 4×10^9 and 2×10^{11} CFU per gram, respectively).

Probiotics were added 0.2 and 0.1 g/kg of feed from day 1 to day 24 and day 25 to the end of the experiment (day 42) according to the manufacturer instructions. The experimental diets formulated by the WUFFDA software (version 1-2002) and were fed from 1 to 10, 11 to 24 and 25 to 42 days as starter, grower and finisher phases, respectively (Table 1). Chickens were reared on paper litter based on the Ross breed management program's guidelines.

Table 1 Ingredients and chemical composition of the experimental diets

Ingredients	Starter (0 to 10 d)	Grower (10 to 24 d)	Finisher (24 to 42 d)
Corn grain	55.30	59.90	63.40
Soybean meal (CP=44%)	37.52	33.30	30.25
Soybean oil	2.50	2.60	2.79
Dicalcium phosphate	1.90	1.75	1.20
Limestone	1.05	0.90	1.02
DL-methionine	0.30	0.25	0.24
L-lysine	0.35	0.30	0.14
L-threonine	0.18	0.10	-
Common slat	0.22	0.22	0.20
$NaHCO_3$	0.18	0.18	0.20
Vitamin supplement[1]	0.25	0.25	0.25
Mineral supplement[2]	0.25	0.25	0.25
Calculated chemical composition			
Apparent metabolizable energy corrected for nitrogen AMEn (kcal/kg)	2900	2950	3000
Crude protein (%)	20.50	19.00	17.80
Calcium (%)	0.95	0.90	0.75
Available phosphorus (%)	0.40	0.38	0.35
Lysine (%)	1.39	1.25	1.05
Methionine + cystine (%)	0.92	0.85	0.80

[1] Vitamin supplement per kilogram of feed including: vitamin A: 9000 IU; vitamin B_1: 1.8 mg; vitamin B_2: 0.015 mg; Biotin: 0.1 mg; vitamin D_3: 2000 IU; vitamin E: 18 IU; K_3: 2 mg and Choline chloride: 500 mg.
[2] Mineral supplements per kilogram of feed include: Manganese (manganese oxide): 100 mg; Iron (iron sulphate H_2O_7): 50 mg; Zinc (zinc oxide): 100 mg; Copper (copper sulphate H_2O_5): 10 mg; Iodine (calcium iodate): 1 Mg and Selenium (sodium selenite): 0.2 mg.

Data and sample collection

To determine the performance variables, all chicks in each pen were weighed at 0, 10, 24, and 42 days of age. Feed intake based on each pen was recorded at the same times. Feed conversion ratio (FCR) was calculated by dividing the feed intake to body weight gain of each replicate. To determine the biochemical variables, at the end of the experiment, one bird per pen (eight birds from each experimental group) was randomly selected, and weighed, then, blood samples were taken from the wing vein. The serum samples were prepared using a centrifuge (Hermle, made in Germany) at 3600 × g for 15 minutes. The serum samples were stored at -24 ˚C until the relevant tests were performed (Aliakbarpour *et al.* 2013).

Biochemical analysis

Total protein, calcium, phosphorus, alkaline phosphatase (ALP), glucose, triglyceride, cholesterol, and high-density lipoprotein (HDL), were analyzed with Ra-xtau to analyzer (Technicon-USA) and specialized biochemistry kits of Pars Azmon (Pars Azmon Co. Tehran-Iran). Serum low-density lipoprotein (LDL) was calculated according to Friedewald *et al.* (1972). LDL (mg/dL)= cholesterol (mg/dL) − HDL (mg/dL) − triglyceride (mg/dL) / 5

The jejunum and ileum histomorphometry

At 42 d of age (the end of the experiment), one chicken per replicate (eight chickens per experimental group) with a body weight similar to the group's average were selected per treatment, slaughter, and after emptying the gastrointestinal tract, 1 cm was separated from the middle part of the jejunum and ileum according to Aliakbarpour *et al.* (2012). The contents of the isolated intestinal sections were drained with PBS solution. After fixation in 10% formalin, dehydration with ethanol, clarification with xylose, and placement in paraffin, tissue blocks were prepared. Serial sections were cut at 5 µm by microtome (Leica RM2125-Germany) and were fixed and stained with Hematoxylin and Eosin. The slides were imaged with a light microscope (OLYMPUS-Taiwan) equipped with a special camera (HD light) with a magnification of 4 and then with the software (ipTipcapture), the length of the villi (from the top of the villi to its base), the width of the villi (the distance between the bottom of the villi) and the crypt depth (from the base of the villi to the submucosa) were measured. After that, the ratios of the villus length to the crypt depth and the surface area of the villus were determined. Villus surface area= 2π × villus height × villus width / 2 (Geyra *et al.* 2001).

Carcass characteristics and bone biometry

Immediately after slaughter of one bird per replicate at the end of the experiment, de-feathering was performed for carcass analysis. Abdominal fat pad, thigh, breast, wings, and back were measured with a digital scale with an accuracy of ± 0.01 g (Centaurus scale-China). All carcass data from each bird were calculated as a percentage of the body weight. To determine the bone variables, the tibia of the left foot (Shim *et al.* 2012) was selected, and after removing the muscles and tissues, the sample was placed in a plastic bag and kept in the freezer at -20 ˚C until the further tests according to Shaw *et al.* (2010).

The biometric indices, composition, and strength of the tibia were examined. Tibia length and diameter were measured using a caliper (±0.01). The diameter of the tibia modular canal was calculated by subtracting the thickness of the inner and lateral walls from the diameter of the bone diaphysis, and the tibiotarsal and robusticity indices were calculated based on the following formula (Mutuş *et al.* 2006; Mohammed *et al.* 2021).

Tibiotarsal= diaphysis diameter – medullary canal diameter / diaphysis diameter × 100

Robusticity index= bone length / cube root of bone weight

A device for tension and pressure (STM_250- Code 1568-Centam Engineering Design Company-Tehran-Iran) was used to measure bone strength. The bone was placed in a fixed position inside the device by applying pressure at a speed of five mm per minute to its midpoint. Then resistance strength in Newtons was recorded by the device (Shaw *et al.* 2010). The peak point in Figures 1, 2, and 3 shows the force required for complete bone failure.

To study the chemical composition of bone, the dried bone was first soaked in diethyl ether for 24 hours to remove the organic contents. Then it was dried in an oven at 105 ˚C for 24 hours, and bone weight was measured with a digital scale (±0.001). To measure bone ash, prepared bones were placed in an electric oven at 550 ˚C for 6 hours. The colorimetric method with molybdate-vanadate reagent at 420 nm and titration, was used to measure the amount of phosphorus and calcium in bone ash (Lv *et al.* 2019). Watkins and Southern (1991) methods' were used to calculate bone density.

Statistical analyses

The data analysis was conducted using SAS (2003). All data after ensuring the normal distribution by Shapiro–Wilk test were analysed by ANOVA using GLM procedure. The means were compared using Duncan's multiple range test, and the statements of statistical significance were based on $P < 0.05$.

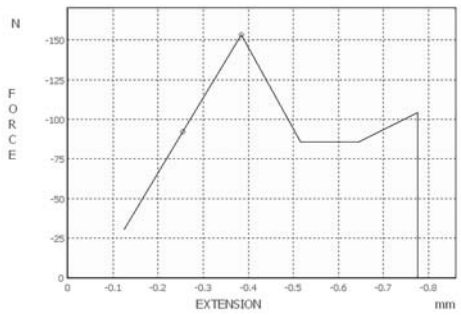

Figure 1 Resistance force recording diagram-tibia bone fracture of the control group (C)

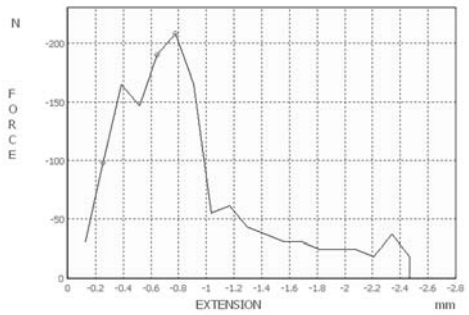

Figure 2 Resistance force recording diagram-tibia bone fracture of supplemented with probiotics based on lactic acid bacteria group (LABP)

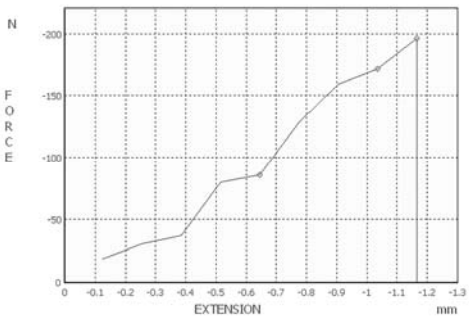

Figure 3 Resistance force recording diagram-tibia bone fracture of supplemented with *Bacillus* probiotic strains group (BPS)

RESULTS AND DISCUSSION

Table 2 shows the results of performance variables, including body weight gain, feed intake, and FCR of broiler chickens. Growth performance variables were not significantly different between experimental groups and the control in all phases of the experiment (P>0.05).

Tables 3 shows the results of histological parameters of the jejunum and ileum segments at 42 days of age, respectively. The jejunal villus length and surface area were greater (P<0.05) in probiotic groups (LABP or BPS) than the control group. Jejunal villus width and crypt depth in the LABP group were significantly higher (P<0.05) than in the control group. The thickness of the muscle layer of the jejunum in BPS was less (P<0.05) than in LABP. The ratio of villus length to crypt depth in the jejunal of the experimental groups was not significantly different (P>0.05). The villi length and ratio of villus length to crypt depth in the ileum of in the LABP group were less (P<0.05) than BPS and control group. The largest (P<0.05) ileal villus area belonged to BPS group although the difference of ileal villus width in LABP and control group was not significantly different, the villus width of BPS was significantly higher (P<0.05) than the other groups. There was not significant difference between the thickness of the muscle layer and the depth of the ileal crypt in experimental groups.

Table 4 shows the results of biometric indices, composition, and bone strength of the tibia. Based on the results, the difference in the length, diaphysis diameter, density, ash, and calcium, as well as indices, tibiotarsal, and robusticity of tibia bone between the groups studied in this experiment, was not significant (P>0.05). The average thickness of the modular canal in probiotic groups (LABP and BPS) was higher (P<0.05) than the control group. The mean of bone phosphorus of LABP was significantly higher (P<0.05) than the BPS group. The mean of strength of tibia bone due to fracture force in LABP was higher (P<0.05) than the control group, but compared to the BPS group, there is no significant difference (P>0.05).

The results of mean of blood biochemical parameters concentration are shown in Table 5. There was not significant difference (P>0.05) for the HDL, LDL, triglyceride, cholesterol, calcium, and blood ALP concentrations among experimental treatments. The amount of glucose and total protein in BPS group was higher (P<0.05) than those in the control group (P<0.05) but the amount of blood phosphorus in the LABP group was higher (P<0.05) than the BPS group.

Table 6 shows the results of the effect of probiotics used in this experiment on carcass components at the last stage of the experimental period (42 days). No significant differences (P>0.05) were observed between the experimental groups for carcass breast, thigh, wing, and back and abdominal fat percentage in this study.

Consumption of probiotics may improve growth and FCR by enhancing quail and broiler microbiological conditions, in the gastrointestinal ecosystem, increasing the efficiency of nutrient uptake and production of exogenous enzymes (Hazrati *et al.* 2020; Ramlucken *et al.* 2020). However, some authors, without mentioning the reason, reported that growth decreased with probiotic consumption, and the FCR was impaired (Sharifi *et al.* 2012) or the addition of the probiotic had no effect on performance (Bai *et al.* 2018; Shokrinejad *et al.* 2023).

Table 2 Effects of dietary treatments on performance variables of broiler chickens (Mean±SD)

Performance	Age (day)	Treatments			P-value
		C	LABP	BPS	
Feed intake (g)	1 to 10	190.84±9.49	181.07±10.63	187.59±9.00	0.1484
	10 to 24	1279.62±54.26	1275.62±59.82	1287.09±35.22	0.9875
	24 to 42	2587.02±117.11	2632.19±155.96	2603.58±184.37	0.8415
	1 to 42	4057.48±123.77	4088.89±147.40	4069.26±191.77	0.9217
Body weight gain (g)	1 to 10	215.25±16.32	208.3±4.88	217.04±9.31	0.2854
	10 to 24	916.18±61.60	886.75±39.81	933.41±29.62	0.1431
	24 to 42	1041.42±59.45	1035.86±53.09	1056.01±92.83	0.8418
	1 to 42	2172.85±75.06	2130.99±64.84	2206.47±82.98	0.1535
Feed conversion ratio	1 to 10	0.89±0.10	0.87±0.06	0.87±0.06	0.7868
	10 to 24	1.40±0.08	1.44±0.10	1.37±0.07	0.2631
	24 to 42	2.49±0.24	2.55±0.27	2.48±0.26	0.8425
	1 to 42	1.87±0.09	1.92±0.11	1.85±0.10	0.3363

C: control diet without probiotics (control); LABP: control diet supplemented with probiotics based on lactic acid bacteria and BPS: control diet supplemented with *Bacillus* probiotic strains.
n= 8.

Table 3 Effect of dietary treatments on the histological parameters of jejunum and ileum at 42 days of age (Mean±SD)

Villi characteristics	Experimental groups			P-value
	C	LABP	BPS	
Jejunum				
Villi length (µm)	1074.37± 152.59^{b}	1203.31±207.87^{a}	1164.51±23.02^{a}	0.0081
Villi width (µm)	216.53±51.77^{b}	250.19±51.84^{a}	235.33±2.08^{ab}	0.0219
Crypt depth (mm)	231.37±57.54^{b}	279.12±43.05^{a}	242.82±30.46^{b}	< 0.0001
Muscle thickness (mm)	192.94±55.49^{ab}	221.44±47.00^{a}	183.59±23.57^{b}	0.0469
Villi length/crypt depth	4.88±1.18	4.37±0.97	4.83±1.05	0.0923
Villi surface area (mm^2)	0.12±0.03^{b}	0.15±0.05^{a}	0.14±0.05^{a}	0.0003
Ileum				
Villi length (µm)	1051.53±175.83^{a}	764.31±113.90^{b}	1047.45±182.42^{a}	< 0.0001
Villi width (µm)	171.76±31.67^{b}	184.81±49.78^{b}	214.00±53.50^{a}	0.0005
Crypt depth (µm)	206.02±56.40	199.61±55.08	223.00±52.43	0.1925
Muscle thickness (µm)	221.08±63.32	227.67±54.18	203.00±18.80	0.6418
Villi length/crypt depth	5.45±1.63^{a}	3.94±1.13^{b}	4.85±0.94^{a}	< 0.0001
Villi area (mm^2)	0.09±0.02^{b}	0.07±0.02^{c}	0.11±0.03^{a}	< 0.0001

C: control diet without probiotics (control); LABP: control diet supplemented with probiotics based on lactic acid bacteria and BPS: control diet supplemented with *Bacillus* probiotic strains.
n= 8.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 4 Effects of dietary treatments on the tibia variables in broiler chickens (Mean±SD)

Indices	Experimental groups			P-value
	C	LABP	BPS	
Tibia length (mm)	90.70±2.74	90.55±2.14	92.36±3.72	0.4092
Tibial diaphysis diameter (mm)	11.93±0.87	12.03±1.14	12.35±1.02	0.6877
Bone dry weight (g)	5.58±0.56	5.51±0.43	5.76±0.56	0.6277
Modular channel thickness (mm)	8.28±0.85^{b}	9.79±0.90^{a}	9.60±0.62^{a}	0.0078
Tibiotarsal index	27.10±6.26	23.67±4.77	25.73±4.96	0.4514
Tibia Robusticity index	51.21±1.39	51.33±1.52	51.60±1.88	0.8866
Bone strength (Newton)	149.20±19.64^{b}	213.58±40.20^{a}	195.31±34.56^{ab}	0.0102
Ash (%)	45.98±4.05	46.60±2.45	46.75±2.57	0.8715
Density (g/cm^3)	0.91±0.14	0.86±0.09	0.96±0.13	0.3422
Calcium (%)	31.74±0.89	31.13±0.81	31.90±0.61	0.1376
Phosphorus (%)	19.72±0.70^{a}	20.44±0.76^{a}	18.40±0.77^{b}	< 0.0001

C: control diet without probiotics (control); LABP: control diet supplemented with probiotics based on lactic acid bacteria and BPS: control diet supplemented with *Bacillus* probiotic strains.
n= 8.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 5 Effects of dietary treatments on the serum biochemical parameters concentrations of broiler chickens at 42 days of age (Mean±SD)

Parameters	Experimental groups			P-value
	C	LABP	BPS	
Glucose (mg/dL)	131.29±26.59^{b}	180.57±48.27^{ab}	199.14±42.90^{a}	0.0156
Cholesterol (mg/dL)	125.29±22.96	121.35±14.87	125.66±1142	0.8495
Triglyceride (mg/dL)	103.73±9.47	112.36±21.98	103.11±14.88	0.4589
HDL (mg/dL)	78.50±8.91	79.38±7.69	77.13±7.40	0.8534
Total protein (g/dL)	2.88±0.99^{b}	3.73±1.15^{ab}	4.47±0.46^{a}	0.0158
ALP (IU/L)	2150.75±281.52	2046.88±231.93	2170.13±195.77	0.5500
Calcium (mg/dL)	11.17±2.65	9.63±2.53	10.08±1.77	0.4567
Phosphorus (mg/dL)	4.58±0.28^{ab}	5.55±0.95^{a}	4.32±0.61^{b}	0.0071
LDL (mg/dL)	25.38±16.10	22.41±14.62	27.92±13.13	0.7672

C: control diet without probiotics (control); LABP: control diet supplemented with probiotics based on lactic acid bacteria and BPS: control diet supplemented with *Bacillus* probiotic strains.
HDL: high density lipoprotein; LDL: low density lipoprotein and ALP: alkaline phosphatase.
n= 8.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 6 Effect of dietary treatments on carcass composition (% of body weight) at 42 days of age (Mean±SD)

Carcass composition	Experimental groups			P-value
	C	LABP	BPS	
Breast	24.01±1.83	23.75±2.45	23.91±2.03	0.9686
Thigh	19.18±0.81	19.37±1.06	19.36±0.95	0.9003
Wing and back	19.62±2.46	20.04±1.25	20.35±1.35	0.7104
Abdominal fat	0.73±0.43	0.70±0.49	0.82±0.20	0.8360

C: control diet without probiotics (control); LABP: control diet supplemented with probiotics based on lactic acid bacteria and BPS: control diet supplemented with *Bacillus* probiotic strains.
n= 8.

In the present study, no effect on performance was observed with probiotics addition to diet. Authors have indicated that, probiotic consumption is essential in reducing muscle myopathy and impaired collagen storage of muscle tissue by increasing protein availability (Rehman *et al.* 2020) and preventing oxidative stress and its damage to muscles (Paz *et al.* 2019). No changes was observed in carcass characteristics with probiotics in the present study. These results are consistent with the results by authors that reported using probiotics based on *Bacillus subtilis* or lactic acid-producing bacteria did not cause significant changes in carcass characteristics (Sarangi *et al.* 2016; Wang *et al.* 2016; Aziz *et al.* 2020). According to available reports, the difference between the published results on performance variables and carcass characteristics during probiotic consumption may be due to differences in the type of probiotics, bird age, dietary composition, and physical form of diet (Bai *et al.* 2018; Abou-Kassem *et al.* 2021).

Intestinal morphological status plays an essential role in the uptake of nutrients (Seifi *et al.* 2017). The greater length of the villus and less crypt depth in the small intestine are important indicators of intestinal development and health because as the cells originated by mitosis in the crypts, migrate steadily toward the top of villi. Crypt depth represents the amount of cell renewal in crypt. Increasing the length of villi provide a greater surface area and improving the nutrient transport system at the small intestinal tract and increases the efficiency of digestion and absorption of nutrients (Aliakbarpour *et al.* 2012; Sharifi *et al.* 2012; Nabizadeh *et al.* 2018). Intestinal histological changes such as villus length, crypt depth, or increase in intestinal muscle layer thickness, are influenced by the diverse and modifiable population of microorganisms present in the intestine (Reis *et al.* 2017; Al-Khalaifa *et al.* 2019).

Some researchers have shown that modification of the intestinal microbiota due to probiotic consumption and the increased presence of short-chain fatty acids synthesized by beneficial intestinal bacteria can be a factor in greater villi length (Sharifi *et al.* 2012) and changing intestinal histometric parameters (Khattab *et al.* 2021). However, Wang *et al.* (2016) did not detect change in the structure and morphology of the jejunum villi during probiotic addition in diet. Sharifi *et al.* (2012) reported that probiotic consumption increased villi length and crypt depth of the duodenum, jejunum, and ileum. Some researchers have reported that the effect of probiotics on the structure of different parts of the intestine is not the same, and the effects of *Bacillus subtilis* based probiotic decreases from the beginning of the small intestine to the end (Elhassan *et al.* 2019). In this study, probiotics based on lactic acid bacteria or *Bacillus* probiotic strains had a similar impact on the length and width of the jejunal villi, however, in the group of probiotics based on lactic acid bacteria (LABP), the width of the ileum villi was shorter than in the *Bacillus* probiotic strains group (BPS). According to these results, adding probiotics based on lactic acid bacteria or *Bacillus* probiotic strains

could affect some indicators of intestinal morphology, but their effects on different parts of the intestine were not the same.

Bone mineralization plays a vital role in skeletal health and in preventing some metabolic disorders. Bone weight and strength against fracture force, robusticity, and tibiotarsal indices are the most critical indicators of mineralization, bone health, and quality (Mohammed *et al.* 2021). Probiotics can make an important contribution to bone health (Yan *et al.* 2020). Some researchers have reported that bone strength increased with the consumption of probiotics based on *Bacillus subtilis* (Mohammed *et al.* 2021) or *Bacillus amyloliquefaciens* (Tellez Jr *et al.* 2020), or lactic acid bacteria. In this experiment, a diet supplemented with probiotic based on lactic acid bacteria (LABP) increased bone resistance. Although the bone strength in the BPS group was numerically higher than the control, the differences are not statistically significant. According to available reports, the amount of probiotic consumption and the type of beneficial microorganisms play an essential role in the effect of this additive on bone indices (Mohammed *et al.* 2021). Researchers believe that the beneficial effects of probiotics on improving bone growth and strength can be due to increased digestibility and absorption of calcium and phosphorus in the intestine, as well as their deposition in bone (Yan *et al.* 2020; Mohammed *et al.* 2021). In this experiment, the blood and bone phosphorus in chickens supplemented with probiotics based on lactic acid bacteria were significantly higher than those supplemented with *Bacillus* probiotic strains. This study shows that two different types of probiotics based on bacterial strains may affect blood and bone phosphorus concentrations differently. It should be noted that, during the implementation of separate experiments by different researchers, contradictory results have been reported when using different types of probiotics concerning the concentration of calcium and phosphorus in the blood or bone. Fuentes *et al.* (2013) reported that the amount of calcium and phosphorus in bones increased during the consumption of probiotics based on lactic acid bacteria. However, in Nari and Ghasemi's (2020) study, probiotic consumption based on *Saccharomyces boulardi* increased blood phosphorus, do not affect blood calcium levels, and do not change the amount of calcium and bone phosphorus. In this study, although blood and bone phosphorus levels were affected by probiotics (LABP or BPS) supplementation, the blood and bone calcium levels were not affected by probiotic treatments. The absorption of some nutrients in the intestine varies according to the type and amount of probiotic consumption (Seifi *et al.* 2017; Mohammed *et al.* 2021). However, the concentration of some nutrients in the blood is also related to their absorption (Seifi *et al.* 2017). In this study, the blood glucose and total protein concentrations in the *Bacillus* probiotic strains group were higher than that of the control group, as was the case with phosphorus. Biochemical variables of blood are unstable indicators whose changes, in addition to showing their absorption, also reflect the animal's health status and how the body responds to internal and external stimuli and stressors (Ciurescu *et al.* 2020; Khattab *et al.* 2021). According to previous investigations, probiotics can reduce blood cholesterol levels by increasing cholesterol uptake by bacteria in the gut, hydrolysis of bile salts, and furthermore, certain probiotics have been found to exert inhibitory effects on the activity of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, the enzyme responsible for the rate-limiting step in cholesterol synthesis. The decrease in bile salt concentration can also reduce the absorption of lipids from the gastrointestinal tract (Seifi *et al.* 2017; Abou-Kassem *et al.* 2021). However, our results are in sync with those found by Deraz *et al.* (2019) who did not see any change in blood lipid metabolites. Similarly, in this study, the blood metabolites were not affected by inclusion of probiotics in broiler chick's diet.

CONCLUSION

In conclusion, the current study revealed that supplementation diet with probiotics based on lactic acid bacteria or *Bacillus* probiotic strains increase villi surface area, but they do not show same effects in different parts of the intestinal tract. Lactic acid bacteria probiotic supplementation increases the count of bone phosphorous and can be effective in improving bone strength. Supplemented probiotics not have any effect on productive variables.

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