Growth performance, nutrient digestibility, bone mineralization, and hormone profile in broilers fed with phosphorus-deficient diets supplemented with butyric acid and *Saccharomyces boulardii*

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ABSTRACT The present study evaluated the effects of butyric acid supplementation and *Saccharomyces boulardii* (alone or in combination) on growth performance, nutrient digestibility, bone mineralization, and blood hormones of male broiler chickens fed a diet including reduced levels of nonphytate phosphorus (**NPP**). The chickens were allocated to 6 dietary treatments: 1) positive control diet with adequate amounts of NPP (**PC**; 0.48, 0.43, and 0.39% in the starter, grower, and finisher period, respectively); 2) negative control diet with low amounts of NPP (**NC**; 0.38, 0.33, and 0.29% in the starter, grower, and finisher period, respectively); 3) NC plus 500 FTU/kg microbial phytase (**PHY**); 4) NC plus 0.2% butyric acid (**BA**); 5) NC plus 1×10^8 cfu/kg *S. boulardii* (**SB**); 6) NC plus butyric acid and *S. boulardii* (**BA+SB**). Each treatment had 5 pen replicates of 25 birds. After 6 wk, the body weight and ADG in birds fed with any of the diets were higher ($P < 0.001$) than those in birds fed with the NC diet, where the birds fed with the PHY and BA+SB diets had the highest values. However, only the PHY diet improved ($P = 0.041$) overall F:G. All diets, except the SB diet, resulted in the increased apparent ileal digestibility coefficient (**AIDC**) of CP, AME_n, and tibia ash content and decreased serum alkaline phosphatase level compared with the NC diet ($P < 0.05$). Broiler chickens fed with the PHY, SB, and BA+SB diets also had increased AIDC of phosphorus ($P = 0.017$) than those fed with the NC and PC diets. Feeding PC, PHY, and BA+SB diets increased ($P = 0.007$) the tibia phosphorus content but decreased ($P = 0.033$) serum parathyroid hormone concentration. Overall, the present data indicate that the simultaneous inclusion of butyric acid plus *S. boulardii* in the low-NPP diets was beneficial for improving growth rate and bone mineralization, but not for feed efficiency.

Key words: broiler performance, butyric acid, live yeast, nonphytate phosphorus, nutrient bioavailability

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INTRODUCTION

Phytic acid or phytate (myo-inositol hexakisphosphate) is the primary storage compound of phosphorus (P) in animal feeds of plant origin, and it is mainly abundant in many cereal grains, legumes, oilseeds, flours, and brans. Phytate has also a strong capacity to chelate minerals and protein, and its degradation is a major metabolic process in biological systems (Selle and Ravindran, 2007; Walk and Rama Rao, 2019). As a result, inorganic P, an expensive and nonrenewable mineral, is added to the poultry diet to meet their nonphytate P (**NPP**) requirements. It is reported that dietary low NPP intake could induce related biochemical and physiological changes to regulate the metabolism of P and calcium (**Ca**) such as lowered parathyroid hormone (**PTH**) secretion (Almaden et al., 1998) and increased alkaline phosphatase (**ALP**) activity (Liu et al., 2017).

The application of microbial phytase in poultry diets to decrease the need for supplementation with inorganic P is common (Shang et al., 2015). The efficacy of supplemental phytase in improving the bioavailability of phytate-bound minerals, energy metabolizability and digestibility of amino acids, and bone quality has been shown by several studies in poultry (Pieniazek et al., 2017; Leyva-Jimenez et al., 2019; Siegert et al., 2019). Vieira et al. (2015) reported that average bioequivalence NPP for microbial phytase supplementation depended on the evaluated response and that the mean NPP

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relative equivalence in chickens fed with 500 FTU phytase/kg was 0.10%. El-Sherbiny et al. (2010) also reported the broilers fed with 500 FTU/kg of phytase in low NPP diets had similar body weight gain, feed intake, and nutrient digestibility coefficients to those fed the adequate-P diet. In general, it is recognized that only 0.05 to 0.1% reduction of NPP content can be achieved with phytase supplementation (Slominski, 2011; Shang et al., 2015). It is also reported that phytase has limitations for use in poultry diets because it is heat-labile and is not always cost-effective (Brenes et al., 2003). Hence, additional strategies may be needed to further improve phytate-P utilization in poultry diets.

Numerous approaches have been proposed to improve dietary phytate-P availability to broiler chickens. Recent studies indicated that organic acids could increase the utilization of phytate-P in poultry (Liem et al., 2008; Vieira et al., 2018). Although the organic acid supplementation was initially targeted for improving performance in broiler chickens, there is growing evidence that dietary supplementation with organic acids may also contribute to reduced nitrogen and P excretion with lower environmental pollution (Khodambashi Emami et al., 2013). Among organic acids, butyric acid is reported to increase the absorptive surface of the small intestine by stimulation of epithelial cell proliferation and differentiation, leading to better nutrient utilization (Abdelqader and Al-Fataftah, 2016; Jazi et al., 2018). Similarly, Jahanian and Golshadi (2015) showed that dietary supplementation of the glyceride form of butyric acid could decrease intestinal pathogenic bacteria and increase digestibility coefficients of ether extract and total ash in laying hens. Therefore, it hypostasized that an increase in the absorptive surface of the small intestine, as well as development of beneficial microflora in the gastrointestinal tract, by butyric acid supplementation may result in improvements in the digestibility and availability of nutrients, such as Ca and P, which in turn may improve mineral retention and bone mineralization.

An enormous number of microorganisms, including fungi, yeast, and bacteria, produce different types of enzymes, especially phytase (Ruijuan et al., 2010). Among yeasts, production of phytase by *Saccharomyces cerevisiae* strains has been reported in previous studies (Haraldsson et al., 2005, Moslehi-Jenabian et al., 2010). In general, different yeast strains as probiotic sources are considered to be useful organisms that produce several positive effects, and *S. cerevisiae* var. *boulardii* (*Saccharomyces boulardii*) is one of the recently well-known probiotics which beneficially affect intestinal health by modulation in the intestinal ultrastructure (Rajput et al., 2013). In addition, it is reported that *S. boulardii* increases the bioavailability of the nutrients in food fermentation systems via reduction of the contents of phytic acid, polyphenols, and trypsin inhibitor activity (Sindhu and Khetarpaul, 2001; Ryan et al., 2011). Moré and Yvan (2018) also confirmed the capacity of *S. boulardii* to make phosphate available within the intestinal tract. Therefore, it seems that *S. boulardii* can be applied for pretreatment of broiler feed to reduce the phytate contents or that it can be used as a feed supplement to degrade the phytate complex after digestion.

To date, no studies have been performed investigating P utilization and bone mineralization in broilers fed with diets supplemented with *S. boulardii* alone or in combination with an organic acid. The hypothesis of this study was that butyric acid supplementation would increase phytase activity of *S. boulardii* and that the combination of the 2 would act synergistically to improve growth performance, nutrient digestibility, and bone quality in broiler chickens fed with low-NPP feeds. Therefore, the overall objective of the present experiment was to determine whether the addition of butyric acid and *S. boulardii*, either singly or in combination, to a NPP-deficient corn and soybean meal diet would improve the utilization of Ca, P, and other nutrients in broiler chicken diets when compared with a well-known phytase (Natuphos; BASF SE, D-67056, Ludwigshafen, Germany). Growth performance, nutrient digestibility, bone characteristics, and hormone profile were used as the response criteria.

MATERIALS AND METHODS

Enzyme, Organic Acid, and Yeast

The phytase enzyme used in this study was Natuphos 5,000. The butyric acid used in this study was provided in the butyric acid glycerides form as mono-, di-, and tri-acyl glycerol (Baby C4; Silo Industria Zootecnica, Florence, Italy) and was mixed with the feed at 2 g/kg on top. The probiotic *S. boulardii* (yeast) was isolated and identified by the Institute of Animal Nutrition, Zist Darman Mahan Co. (Tehran, Iran), for the trial. *S. boulardii* was cultured in yeast peptone dextrose in aerobic conditions for 24 h (at 30°C). After cultivation, yeast pellets were separated by centrifugation at 6,000 rpm for 5 min at 4°C and washed twice with PBS (pH 7.3). The pellets were then suspended in skimmed milk powder, and the prepared mixture was added at 1×10^8 cfu/kg into the diet (Rajput et al., 2013; Sun et al., 2017).

Animals and Diets

All the procedures involving the birds used in this study were approved by the Arak University Institutional Animal Care and Use Committee. A total of 750 one-day-old male broiler chickens (Ross 308) were purchased from a local commercial hatchery. On arrival, all chickens were weighed (44 ± 0.5 g) and randomly allocated to a completely randomized design experiment with 6 treatments and 5 replicate pens (25 birds per pen with the size of 2 m × 1.75 m). The experiment lasted for 6 wk (from Day 0 to 42), and chickens had *ad libitum* access to water and feed during this period. The experimental house was equipped with automated gas heating

Table 1. Ingredient composition of starter, grower, and finisher diets.

	Starter (Day 0 to 10)		Grower (Day 10 to 24)		Finisher (Day 24 to 42)	
Ingredients (%)	PC	NC	PC	NC	PC	NC
Corn	55.07	55.14	59.17	59.24	63.80	63.87
Soybean meal, 44%	33.07	33.72	28.05	28.69	23.55	24.21
Corn gluten meal, 60%	4.71	4.27	5.42	4.98	4.80	4.35
Soybean oil	2.40	2.40	3.00	3.00	3.80	3.80
Monocalcium phosphate[1]	1.48	1.03	1.32	0.86	1.17	0.72
Limestone	1.58	1.78	1.45	1.65	1.34	1.54
Salt (NaCl)	0.25	0.27	0.17	0.19	0.17	0.19
Sodium bicarbonate	0.07	0.04	0.18	0.15	0.18	0.15
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[3]	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.27	0.27	0.21	0.22	0.20	0.20
L-Lysine HCl	0.40	0.38	0.37	0.36	0.35	0.33
L-Threonine	0.20	0.20	0.16	0.16	0.14	0.14
Total	100	100	100	100	100	100

Abbreviations: NC, low phosphorous diet (negative control); PC, normal phosphorous diet (positive control).
[1]Monocalcium phosphate contained 18% Ca, 21% P.
[2]Supplied per kg diet: 18 mg retinol, 4 mg cholecalciferol, 36 mg a-tocopherol acetate, 2 mg vitamin K_3, 1.75 mg vitamin B_1, 6.6 mg vitamin B_2, 9.8 mg niacin, 29.65 mg pantothenic acid, 2.94 mg vitamin B_6, 1 mg folic acid, 0.015 mg vitamin B_{12}, 0.1 mg biotin, 250 mg choline chloride, and 1 mg ethoxyquin.
[3]Supplied per kg diet: 99.2 mg Mn, 50 mg Fe, 84.7 mg Zn, 10 mg Cu, 0.99 mg I, 0.2 mg Se.

and artificial programmable lights. Lighting program was 24 h a day for the first 3 D and then reduced to 23 h of light afterward. The temperature program was adjusted as follows: 0 to 3 D: 33 to 34°C; 4 to 7 D: 31 to 33°C; second wk: 28 to 31°C; third wk: 25 to 28°C; fourth wk: 22 to 25°C; fifth and sixth wk: 22 to 23°C. Relative humidity, which was provided using a humidifier inside the rooms, was 55 ± 5% during the experimental period. All birds were vaccinated as routinely performed against infectious bronchitis, Newcastle, and Gumboro disease.

From Day 0, broiler chickens were fed with one of 6 experimental diets: 1) a positive control diet with sufficient levels of NPP (**PC**; 0.48, 0.43, and 0.39% in the starter, grower, and finisher period, respectively); 2) negative control diet with a low amount of NPP (**NC**; 0.38, 0.33, and 0.29% in the starter, grower, and finisher period, respectively); 3) the NC diet plus 500 FTU/kg phytase (**PHY**); 4) the NC diet plus 0.2% butyric acid (**BA**); 5) the NC diet plus 1×10^8 cfu/kg *S. boulardii* (**SB**); and 6) the NC diet plus 0.2% butyric acid and 1×10^8 cfu/kg *S. boulardii* (BA+SB). No medication was used during the whole experimental period. Tables 1 and 2 show the feed ingredients and chemical compositions of the NC and PC diets. Except for NPP, the PC and NC diets (in mash form) were formulated to possess the similar chemical composition according to the nutrient requirements suggested by the Ross 308 broiler nutrient specifications (Aviagen, 2014). The phytase activity of the PHY diet was measured according to the method of Slominski et al. (2007), and 1 phytase unit or FTU was described as the amount of phytase required to generate 1 μmol of inorganic P per min from sodium phytate at 37°C and pH 5.5.

Performance Data

Body weight and feed intake were measured at the end of the starter (0–10 D), grower (10–24 D), and finisher (24–42 D) periods. The ADG, ADFI, and F:G were calculated for each feeding phase and for the overall (0-42 D) experiment. All mortality was recorded daily in each group, and F:G was adjusted for mortality.

Sample Collection

To determine apparent ileal digestibility coefficients (**AIDC**) of nutrients, 3 D before ileal digesta collection (Day 39), chromic oxide (3 g/kg) was added to the diet as an exogenous marker. At the end of the experiment (on Day 42), 2 chickens per replicate pen were selected according to the average body weight (**BW**) of the pen. Blood samples were collected from the wing vein of each bird and then centrifuged (2,500 × g, 15 min) at 4°C to obtain serum samples for blood chemical analysis. The aforementioned 2 birds from each pen were then killed by cervical dislocation followed by blood sampling, and the contents of the ileum (from Meckel's diverticulum to the ileo-cecal junction) were collected and frozen immediately (−20°C) until further analysis. In addition, left tibia of each bird was excised, cleaned of adhering tissues, and kept frozen at −20°C until analysis.

Nutrient Digestibility

At the time of analysis, the samples were dried in an oven at 65°C for 24 h and then ground into fine powder for chemical analysis. After that, the amount of DM (method 930.15), CP (N × 6.25; method 984.13), crude fat (method 920.39), ash (method 942.05), Ca (method 968.08), and P (method 964.06) were measured in the diet and ileal digesta samples according to the methods of the AOAC (2005). Gross energy was also determined in an automatic adiabatic oxygen bomb calorimeter (Parr Instrument Company, Moline, IL). The amount of chromic oxide in the diet and ileal digesta was measured according to Mueller (1956).

The AIDC of nutrients in diets was calculated using the following equation:

Table 2. Calculated and analyzed nutrient contents of basal starter, grower, and finisher diets (as fed basis).

Calculated nutritive value (% unless stated otherwise)	Starter (Day 0 to 10)		Grower (Day 10 to 24)		Finisher (Day 24 to 42)	
	PC	NC	PC	NC	PC	NC
Metabolizable energy, kcal/kg	3,000	3,000	3,100	3,100	3,200	3,200
Crude protein	23	23	21.5	21.5	19.5	19.5
Calcium	0.96	0.96	0.87	0.87	0.79	0.79
Nonphytate phosphorus	0.48	0.38	0.43	0.33	0.39	0.29
Sodium	0.16	0.16	0.16	0.16	0.16	0.16
Digestible lysine	1.28	1.28	1.15	1.15	1.03	1.03
Digestible methionine	0.62	0.62	0.55	0.55	0.51	0.51
Digestible TSAA	0.95	0.95	0.87	0.87	0.80	0.80
Digestible threonine	0.86	0.86	0.77	0.77	0.69	0.69
DEB[1], mEq/kg	250	250	240	240	225	225
Analyzed nutritive value[2] (%)						
Crude protein	22.4	22.2	21.0	20.9	18.9	18.8
Total lysine	1.42	1.43	1.35	1.34	1.19	1.19
Total methionine	0.68	0.67	0.60	0.60	0.56	0.55
Total cysteine	0.38	0.38	0.36	0.37	0.32	0.33
TSAA	1.06	1.05	0.96	0.97	0.88	0.88
Total threonine	1.04	1.03	0.93	0.92	0.84	0.85
Total valine	1.15	1.17	1.04	1.06	0.94	0.93
Total tryptophan	0.26	0.26	0.23	0.22	0.19	0.20
Total arginine	1.44	1.45	1.29	1.28	1.15	1.15
Total phosphorus	0.73	0.65	0.67	0.58	0.62	0.52
Calcium	0.99	0.97	0.90	0.88	0.83	0.82

Abbreviations: DEB, dietary electrolyte balance; NC, low phosphorous diet (negative control); PC, normal phosphorous diet (positive control).
[1]DEB = (Na^+, mEq/kg + K^+, mEq/kg) – CL^-, mEq/kg.
[2]Mean of 2 samples per diet (Evonik Industries, Evonik Degussa GmbH, Hanau-Wolfgang, Germany).

$$\text{AIDC} = 1-[(\text{marker}_{\text{diet}} / \text{marker}_{\text{id}}) \times (\text{nutrient}_{\text{id}} / \text{nutrient}_{\text{diet}})]$$

where $\text{nutrient}_{\text{diet}}$ and $\text{marker}_{\text{diet}}$ are the concentrations of nutrient and chromic oxide in the diet (%) and $\text{nutrient}_{\text{id}}$ and $\text{marker}_{\text{id}}$ represent the concentrations of the same nutrient and chromic oxide in the ileal digesta (%).

The AME_{n} value was determined using the following equation (Tavernari et al., 2018):

$$\text{AME}_{\text{n}}\ (\text{kcal / kg of diet}) = \text{GE}_{\text{diet}} - [(\text{GE}_{\text{id}} - \text{IF}) + 8.22 \times (\text{N}_{\text{diet}} - \text{N}_{\text{id}} \times \text{IF})]$$

where GE_{diet} is gross energy value in diet (kcal/kg) and GE_{id} is gross energy value in ileal digestibility (kcal/kg); indigestibility factor = $\text{marker}_{\text{diet}}/\text{marker}_{\text{id}}$, N_{diet} is nitrogen concentration in diet (%), N_{id} is nitrogen concentration in ileal digesta (%), and 8.22 is the energy equivalent (kcal/g) of uric acid (0.034 MJ/kg uric acid nitrogen).

Bone Mineralization

The tibia samples were crushed and defatted by the Soxhlet apparatus using petroleum ether for 24 h and oven-dried at 100°C for 24 h. Dried samples were then burned in a muffle furnace at 600°C for 24 h to determine the percentage of bone ash (fat-free, dry weight basis). The solubilization of the ash from the tibia samples was performed with a nitric and perchloric acid mixture, and the P and Ca contents of these samples were determined by the same methods as those used for the samples from feed and ileal digesta.

Serum Metabolites and Hormone Profile

The Ca and P concentrations and ALP activity in serum were determined by using an automatic biochemical analyzer (Clima, Ral. Co, Spain) with commercial kits (Pars Azmoon Company, Tehran, Iran).

Hormonal profile investigation included triiodothyronine (T_3), thyroxine or tetraiodotrienin (T_4), PTH, and calcitonin (**CT**). Serum T_3 and T_4 levels were measured by ELISA using a commercial ELISA kit (Pishtaz Teb, Tehran, Iran). The mean inter- and intra-assay CV were 3.4 and 5.1% for T_3 and 4.7 and 6.4% for T_4, respectively. Serum concentrations of PTH and CT were determined using commercial kits (Immutopics, Inc., San Clemente, CA), the immunoradiometric assay method, and a gamma counter (BioSource International, Camarillo, CA).

Statistical Analysis

Data were analyzed in a completely randomized design using the GLM procedures of SAS (version 9.0; SAS Institute Inc., Cary, NC). Data on growth performance parameters were analyzed on a pen basis, whereas data on nutrient digestibility, bone characteristics, and blood constituents were based on individual birds. Percentage data were tested for normality using the Univariate Procedure of SAS, then transformed to arcsine values before analysis if normality was not met. The results are presented as the least-square means with SEM. All statements of significance were considered as $P < 0.001$ or $P < 0.05$.

Table 3. Effect of experimental treatments on growth performance of broiler chickens.[1]

Item	Experimental treatments						SEM	*P* value
	PC	NC	PHY	BA	SB	BA+SB		
BW (g)								
10 D	264	253	268	259	259	270	7.2	0.382
24 D	975	909	996	942	962	985	23.8	0.208
42 D	2,435^{a,b}	2,185^{c}	2,506^{a}	2,324^{b}	2,397^{a,b}	2,477^{a}	42.6	<0.001
ADG (g/bird/D)								
0-10 D	21.9	20.8	22.4	21.5	21.4	22.5	0.62	0.381
10-24 D	51.1	45.5	52.7	48.4	50.1	52.1	1.68	0.056
24-42 D	80.9^{a,b}	72.1^{c}	83.5^{a}	77.1^{b,c}	79.6^{a,b}	80.2^{a,b}	1.78	0.002
0-42 D	56.9^{a,b}	51.0^{c}	58.6^{a}	54.3^{b}	56.0^{a,b}	58.6^{a}	0.99	<0.001
ADFI (g/bird/D)								
0-10 D	26.4	25.7	26.3	27.2	26.4	26.7	0.309	0.054
10-24 D	78.4^{a}	72.0^{b}	74.4^{b}	74.8^{b}	73.2^{b}	79.3^{a}	1.17	0.001
24-42 D	157	150	155	157	157	158	3.5	0.598
0-42 D	99.8	94.1	97.7	98.7	97.9	100.8	1.62	0.112
F:G (g:g)								
0-10 D	1.20	1.25	1.20	1.27	1.22	1.19	0.026	0.533
10-24 D	1.53	1.59	1.41	1.57	1.53	1.52	0.064	0.498
24-42 D	1.94	2.08	1.87	2.04	1.98	1.93	0.060	0.184
0-42 D	1.75^{a,b}	1.85^{a}	1.67^{b}	1.82^{a}	1.75^{a,b}	1.72^{a,b}	0.039	0.041

[a-c]Means within each raw with no common superscript differ ($P < 0.05$).

Abbreviations: BA, NC + butyric acid (0.2%); BA + SB, NC + butyric acid (0.2%) + *Saccharomyces boulardii* (1×10^8 cfu/kg); NC, low phosphorous diet (negative control); PC, normal phosphorous diet (positive control); PHY, NC + phytase enzyme (500 FTU/kg); SB, NC + *Saccharomyces boulardii* (1×10^8 cfu/kg); SEM, standard error of mean.

[1]Values are means of 5 pens per treatment combination with 25 male broiler chickens.

RESULTS

Growth Performance

All diets, except the BA diet, increased ADG ($P = 0.002$) compared with the NC diet during the finisher (Day 24–42) period (Table 3). The BW at 42 D and ADG in the overall (Day 0–42) experimental period in the birds fed with any of the diets were higher ($P < 0.001$) than those in birds fed with the NC diet, where the birds fed with the PHY and BA+SB diets had the highest values. Although the PC and BA+SB groups had higher ADFI ($P = 0.001$) than other treatments in the grower period (Day 11–24), there were no significant differences among the experimental groups in overall ADFI. The addition of dietary supplements did not significantly affect F:G ($P > 0.05$) during the starter, grower, and finisher periods. In contrast, the PHY group exhibited lower F:G ($P = 0.041$) than NC and BA groups during the overall experimental period, whereas PC, SB, and BA+SB treatments were intermediate and not significantly different from the other treatments.

Nutrient Digestibility

The AIDC of DM and crude fat were not affected ($P > 0.05$) by diet (Table 4). However, the AIDC of CP, as well as AME_n value, were lower ($P = 0.041$ and $P = 0.021$, respectively) in broiler chickens fed with the NC diet than for chickens in the other groups, except those in the SB group. The AIDC of P in broiler chickens fed with the PHY diet was higher ($P = 0.017$) than that in birds fed with NC, PC, and BA diets but similar to the values in birds fed with SB and BA+SB diets. By comparison, birds fed with the SB and BA+SB diets also exhibited higher AIDC of P ($P = 0.017$) than birds fed with the PC and NC diets. Finally, broiler chickens fed with the PHY diet had higher AIDC of Ca ($P < 0.001$) than birds fed with the NC, BA, or SB diet but had similar AIDC of Ca compared with broiler chickens fed with the PC and BA+SB diets.

Bone Characteristics

The concentration of Ca in the tibia was not affected ($P > 0.05$) by dietary treatments (Table 5). In contrast, the tibia ash content in broiler chickens fed with the PC and PHY diets was higher ($P = 0.003$) than that in birds fed with NC and SB diets but similar to the values in birds fed with the BA and BA+SB diets. By comparison, the tibia ash content in birds fed with BA and BA+SB diets was also higher ($P = 0.003$) than that in birds fed with the NC diet. Tibia P concentration in birds fed with the PC, PHY, or BA+SB diet was also higher ($P = 0.007$) than that in birds fed with the NC diet, whereas BA and SB treatments were intermediate and not significantly different from the other treatments.

Blood Metabolites and Hormone Profile

Serum concentrations of P, Ca, T_4, and CT were not different ($P > 0.05$) when the supplemented groups were compared with the NC group (Table 6). In contrast, broilers fed with the PC, PHY, BA, and BA+SB diets had a lower ALP level ($P = 0.048$) than birds fed with the NC diet but had a similar ALP level compared with birds fed with the SB diet. With respect

Table 4. Effect of experimental treatments on apparent ileal digestibility coefficient (**AIDC**) of nutrients in broiler chickens on Day 42.[1]

	Experimental treatments							
Item	PC	NC	PHY	BA	SB	BA+SB	SEM	*P* value
Dry matter	0.750	0.742	0.746	0.736	0.744	0.742	0.007	0.826
Crude protein	0.781[a]	0.753[b]	0.783[a]	0.780[a]	0.767[a,b]	0.789[a]	0.008	0.041
Crude fat	0.868	0.826	0.868	0.852	0.858	0.872	0.012	0.109
Calcium	0.443[a,b]	0.389[b]	0.467[a]	0.394[b]	0.407[b]	0.444[a,b]	0.019	<0.001
Phosphorus	0.573[c]	0.576[c]	0.639[a]	0.588[b,c]	0.625[a,b]	0.626[a,b]	0.015	0.017
AMEn	3,130[a]	3,027[b]	3,145[a]	3,121[a]	3,082[a,b]	3,157[a]	26.4	0.021

[a-c]Means within each raw with no common superscript differ ($P < 0.05$).

Abbreviations: BA, NC + butyric acid (0.2%); BA + SB, NC + butyric acid (0.2%) + Saccharomyces boulardii (1 × 108 cfu/kg); NC, low phosphorous diet (negative control); PC, normal phosphorous diet (positive control); PHY, NC + phytase enzyme (500 FTU/kg); SB, NC + Saccharomyces boulardii (1 × 108 cfu/kg); SEM, standard error of mean.

[1]Values are means of 5 pens per treatment combination with 2 male broiler chickens per pen selected for ileal digesta collection.

to the hormone levels, broiler chickens fed with the diet PC exhibited the highest serum T_3 concentration, which was higher ($P = 0.048$) than that in birds fed with NC, BA, and SB diets. Serum T_3 level was also higher ($P = 0.048$) in broiler chickens fed with the BA+SB diet than that in birds fed with the NC and BA diets but similar to the level in birds fed with other experimental diets. Serum PTH concentration was also lower ($P = 0.033$) in the PC, PHY, and BA+SB groups than that in the NC group.

DISCUSSION

The objective of this study was to examine whether supplementing low-NPP diets with butyric acid and live yeast (*S. boulardii*) could have additive or synergistic effects on growth performance, nutrient digestibility, bone mineralization, and hormone profile of broiler chickens. The results of the present study showed that all dietary supplementation had a positive effect on BW and ADG during the entire experimental period. Moreover, broilers fed with the PHY and BA+SB diets had the highest BW (at 42 D) and ADG (in the overall experimental period) compared with all other groups, indicating a synergistic effect from the combination of butyric acid and *S. boulardii* during the overall phase. Besides having probiotic properties, *S. boulardii* has been reported to be beneficial for the breakdown of dietary phytate to improve the nutritional value of food (Moslehi-Jenabian et al., 2010). Similar to our results, Rajput et al. (2013) reported a higher live BW for broilers supplemented with 1×10^8 cfu/kg of *S. boulardii* in the diet, which is in agreement with the findings of Sun et al. (2017) who reported that broiler supplemented with 1×10^8 cfu/kg of *S. boulardii* in the diets had significantly higher BW in the whole 72-Day period. This improvement may be associated with the more balanced microflora provided by *S. boulardii* in this study, which has an important role in overall health and growth performance (Czerucka et al., 2007). It has been also reported that a low gastric pH because of feeding organic acid increases the transformation rate of pepsinogen to pepsin and enhances pepsin activity (Park et al., 2009), as well as prevents the formation of mineral–phytate complexes (Khodambashi Emami et al., 2013), hence improving the absorption rate of proteins and minerals. These findings may contribute to better growth performance in broiler chickens fed with the BA+SB diet than that in chickens fed with either *S. boulardii* or butyric acid alone.

The present findings also indicate that only supplementation of phytase in the diet could induce a positive effect on F:G of broilers compared with the NC treatment. This improvement in F:G was as a result of an increased ADG at an unaffected ADFI (Table 3). According to Selle and Ravindran (2007), a complex mixture of mineral and phytate can contribute to the formation of water-insoluble metallic compounds in the gastrointestinal tract, and addition of phytase to the diet can overcome this adverse effect and increase nutrient utilization. Improvements made in feed efficiency of the phytase group in the present study could be explained by a higher AIDC of energy, CP, Ca, and P in the respective group than those in all other groups.

Table 5. Effect of experimental treatments on the bone mineralization of broilers on Day 42.[1]

	Experimental treatments							
Item	PC	NC	PHY	BA	SB	BA+SB	SEM	*P* value
Tibia ash, % (fat-free dry basis)	47.7[a]	42.3[c]	47.4[a]	45.3[a,b]	44.7[b,c]	46.5[a,b]	0.93	0.003
Tibia Ca, % (fat-free dry tibia weight)	33.7	29.3	34.1	31.8	33.7	33.7	1.32	0.114
Tibia P, % (fat-free dry tibia weight)	17.9[a]	15.2[b]	17.9[a]	16.4[a,b]	16.3[a,b]	17.2[a]	0.51	0.007

[a-c]Means within each raw with no common superscript differ ($P < 0.05$).

Abbreviations: BA, NC + butyric acid (0.2%); BA+SB, NC + butyric acid (0.2%) + *Saccharomyces boulardii* (1×10^8 cfu/kg); NC, low phosphorous diet (negative control); PC, normal phosphorous diet (positive control); PHY, NC + phytase enzyme (500 FTU/kg); SB, NC + *Saccharomyces boulardii* (1×10^8 cfu/kg).

[1]Values are means of 5 pens per treatment combination with 2 male broiler chickens per pen selected for tibia collection.

Table 6. Effect of experimental treatments on concentrations of serum metabolites and hormones in broilers on Day 42.[1]

Item		Experimental treatments						SEM	*P* value
		PC	NC	PHY	BA	SB	BA+SB		
Calcium	mg/dL	9.60	11.18	9.78	9.88	10.28	10.00	0.364	0.076
Phosphorus	mg/dL	6.90	6.13	7.13	5.85	6.13	6.80	0.401	0.192
ALP	U/L	4,135^{b}	5,891^{a}	4,175^{b}	4,686^{b}	4,931^{a,b}	4,589^{b}	384	0.048
T_3	ng/mL	2.69^{a}	2.03^{c}	2.34^{a,b,c}	2.10^{c}	2.18^{b,c}	2.55^{a,b}	0.140	0.022
T_4	ng/mL	14.63	11.94	12.87	12.49	12.69	13.75	0.688	0.129
CT	pg/mL	123.2	76.1	117.8	88.6	99.2	113.5	10.9	0.085
PTH	pg/mL	26.8^{b,c}	37.5^{a}	24.7^{c}	34.8^{a,b}	32.3^{a,b,c}	25.3^{b,c}	3.04	0.033

$^{a-c}$Means within each raw with no common superscript differ ($P < 0.05$).

Abbreviations: ALP, Alkaline phosphatase; BA, NC + butyric acid (0.2%); BA+SB, NC + butyric acid (0.2%) + *Saccharomyces boulardii* (1×10^8 cfu/kg) ; CT, calcitonin; NC, low phosphorous diet (negative control); PC, normal phosphorous diet (positive control); PHY, NC + phytase enzyme (500 FTU/kg); PTH, parathyroid hormone; SB, NC + *Saccharomyces boulardii* (1×10^8 cfu/kg); SEM, standard error of mean; T_3, triiodothyronine; T_4, thyroxine or tetraiodotrienin.

[1]Values are means of 5 pens per treatment combination with 2 male broiler chickens per pen selected for blood collection.

Similar to the results of the present study, improvements in AME value and ileal P and CP digestibility have been reported in different studies when phytase was supplemented to low P diets (Pieniazek et al., 2017; Leyva-Jimenez et al., 2019; Siegert et al., 2019). The mechanism of phytase to increase the AME value in broiler chickens is largely unknown, but improved CP digestibility in this study could be responsible, at least partially, for this response. These positive effects of phytase may be associated with phytate destruction and provision of nutrients beyond P that would allow the host to use more nutrients (Walk and Rama Rao, 2019). Liu and Ru (2010) also reported that dietary supplementation with *Escherichia coli*–derived phytase (500 FTU/kg) decreases the endogenous losses of amino acids and minerals in the intestine of broiler chickens.

It is also hypothesized that the phytate-degrading activities of both *S. boulardii* (Ryan et al., 2011) and organic acid (Vieira et al., 2018) have evolved toward the digestive benefits of these supplements. Therefore, they can be considered advantageous in enhancing digestion and absorption of minerals and proteins, which is normally achieved by the degradation of phytate in the gastrointestinal system. In the present study, the addition of butyric acid in the diets was found to increase the ileal CP digestibility and, consequently, diet AME on Day 42. Our results partially agree with those of Sileikiene et al. (2005), who reported that higher activities of trypsin and amylase and higher secretion of pancreatic fluid were observed when the diets were supplemented with butyric acid. It was also suggested by Sileikiene et al. (2008) that butyrate may increase the cellular Ca^{2+} concentration and membrane conductance in the pancreatic acinar cells and subsequently activate the processes involving fluid and enzyme secretion. Therefore, it is reasonable to assume that an increase in the absorptive surface of the small intestine and exocrine pancreatic secretion by butyric acid supplementation may lead to improvements in the digestibility of CP and other nutrients, which in turn may contribute to enhanced dietary AME content.

In this study, supplementary live yeast increased the ileal digestibility of P, and the combination of butyric acid and *S. boulardii* had a positive effect on AME and ileal CP digestibility. However, the combination of butyric acid and *S. boulardii* did not result in any extra beneficial effects on nutrient digestibility. In general, there is little information regarding the effect of live yeast supplementation on nutrient digestibility in broiler chickens. Giang et al. (2010) found that oral administration of yeast culture (0.2% of the diet) improved the apparent total tract digestibility of organic matter, CP, and crude fiber in weaned piglets. In a study on rats (Buts et al., 1999), the administration of *S. boulardii* CNCM I-745 enhanced the gene expression of digestive enzymes and nutrient uptake transporters in the small intestine. The increased availability of intestinal digestive enzymes seems to be one of the primary mechanisms by which *S. boulardii* enhances the digestive and absorptive capacity.

P is essentially transferred to the skeleton and other tissues in the fast-growing chickens. The improvements observed in tibia mineralization for broilers fed with phytase-supplemented diet agreed with those observed in other published studies (Chung et al., 2013; Pieniazek et al., 2017; Walk and Rama Rao, 2019). Dephosphorylation of myo-inositol hexaphosphate by the phytase used in the current experiment presumably led to increased bone mineralization via increasing the ileal digestibility of P and Ca.

Broiler chickens fed with the BA diet had a higher percentage of tibia ash than chickens fed with the NC diet, but their tibia ash and P contents were still lower than those of chickens fed with the PC diet, indicating that although butyric acid alone improved P utilization, chickens in the BA group still suffered from a deficiency of P compared with chickens in the PC group. Similarly, Khodambashi Emami et al. (2013) observed that supplementation of low-NPP diets (3.9 and 3.4% NPP in the starter and grower diets, respectively) with an organic acid mixture (0.2%) increased tibia ash content. Vieira et al. (2018) reported that any organic acid in the dissociated form was found to be beneficial for the increase in the phytate P availability, mainly because of their ability to reduce the gastrointestinal pH, generally leading to better conditions for phytate degradation.

Although butyric acid and *S. boulardii* did not show a synergistic effect on tibia ash content, their combination showed significant improvement in tibia P concentration of birds, particularly when compared with the NC diet. In a study under simulated digestive conditions, Haraldsson et al. (2005) showed a strong reduction of phytate (up to 60%) in the early gastric phase using *S. cerevisiae* strains. The phytase activity of *S. cerevisiae* strains has been shown to be high at pH 2.8-5.5 in the gastric phase (Haraldsson et al., 2005). It seems that the low pH environment in the gastrointestinal tract caused by BA intake affected positively phytase activity of *S. boulardii* and improved bone mineralization of broiler chickens in the present study. Thus, combining butyric acid with *S. boulardii* could further improve mineral utilization compared with their supplementation alone.

The final mineral concentrations of blood are influenced by mineral absorption and retention, bone resorption and deposition, as well as urinary and fecal mineral excretion (Namgung and Tsang, 2012). However, in the present study, a relationship among all of these parameters was not always indicated clearly. In the present study, low-P diet induced an increased blood ALP activity, which was reversed when PC, PHY, BA, or BA+SB diet was fed to broiler chickens. The ALP enzyme activity, which is attached to the plasma membrane of osteoblasts, associates with bone reabsorption activity and the release of minerals from the bone matrix (Saraç and Saygılı, 2007). The present study found that serum ALP activity was reduced as dietary NPP levels increased, which was in agreement with the previous studies on broilers (Jiang et al., 2013; Baradaran et al., 2017). The decrease in serum ALP activity related to the diets supplemented with phytase, butyric acid, or a combination of butyric acid and *S. boulardii* might reflect the downregulation of this enzyme, which could result from the increased bioavailability of P. In this study, changes in ALP activity were also consistent with changes in ash and P in the tibia.

The thyroid gland is responsible for the secretion of T_3, T_4, and CT, which provide mechanisms for controlling numerous metabolic pathways including Ca and P homeostasis. Thyroid hormone increases the genomic action of 1,25-dihydroxycalciferol (calcitriol) in the small intestine (Cross et al., 1990) and subsequently enhances P absorption by stimulation of a secondary active transport process through sodium-coupled phosphate cotransporters in the small intestine (Schröder et al., 1996). Indeed, very limited evidence links dietary P level to blood thyroid hormone levels in animals. In this study, feeding a low-NPP diet for 6 wk to broiler chickens reduced serum T_3 levels compared with PC diet, indicating P deficiency could adversely affect thyroid hormone function by reducing serum T_3 levels. Therefore, the increased ability to use phytate P could reflect the higher T_3 level in the BA+SB group. The principal mechanisms of thyroid hormone deficiency to induce bone resorption include increased catecholamine sensitivity of beta-adrenergic receptors, enhanced bone cell sensitivity to PTH, and interleukin 1–mediated osteoclastogenesis (Dhanwal, 2011). As T_3 hormone has an impact on growth rate via anabolic and catabolic processes (Oster et al., 2018), the reduced T_3 levels reflect the lower BW gain, which is in line with our findings in the NC group.

The endocrine factors such as PTH and CT in responses to diets varying in Ca and P concentrations could enable serum Ca and P homeostasis (Matsuda et al., 2006; Palmer et al., 2011). Our results also showed that the chronic low level of NPP increased the serum PTH level and partially decreased CT in broiler chickens. CT acts as an inhibitor of bone resorption, which could lead to a decreased plasma Ca concentration (Matsuda et al., 2006). PTH is considered the most important regulator of Ca and P concentrations within the bone and in the blood (Palmer et al., 2011). In previous studies, dietary higher NPP level (lower Ca/P ratio) increased PTH secretion and its gene expression level and then caused a continuous process in bone resorption in humans (Almaden et al., 1998) and rats (Hernandez et al., 1996). However, in the present study, an increase in serum PTH level was observed in response to decreasing supplementary NPP levels or increasing Ca:NPP ratios in the diet. It was believed that chicken PTH gene expression and secretion were predominantly regulated by modifications in ionized Ca and calcitriol (Liu et al., 1996). It seems that the lower serum Ca concentration in the PC group in the present study might directly activate renal 25-hydroxycalciferol-1-hydroxylase to produce calcitriol in the intestine, bones, and kidney (Delmez et al., 1989). Subsequently, the elevated calcitriol levels could inhibit PTH secretion in the parathyroid glands, hence reducing the blood PTH concentration through the negative feedback mechanism (Proszkowiec-Weglarz and Angel, 2013). In the present study, it is possible that the negative effect of the low level of dietary NPP on P and Ca metabolism and bone characteristics might be reversed by coordinated actions of the calcitropic hormones, PTH and calcitriol in the PHY and BA+SB groups.

In conclusion, the dietary applications of butyric acid and *S. boulardii*, singly or in combination, could exhibit differential effects on growth performance, nutrient digestibility, bone characteristics, and blood metabolic profile of the low-NPP–fed broilers. Butyric acid was advantageous compared with *S. boulardii* with respect to CP digestibility, AME_n, and tibia ash content. In contrast, the SB diet was advantageous over the BA diet only with respect to overall ADG, when compared with the NC diet. Interestingly, we observed a clear synergistic effect when we compared the combination feeding to feeding butyric acid and *S. boulardii* alone in terms of ADG, bone mineralization, and blood T_3 and PTH concentrations. These improvements were of similar magnitude to those determined for normal-P (positive control) and PHY groups. However, when compared with the NC diet, the PHY diet yielded better feed efficiency and Ca digestibility than the other experimental diets.

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REFERENCES

Abdelqader, A., and A. Al-Fataftah. 2016. Effect of dietary butyric acid on performance, intestinal morphology, microflora composition and intestinal recovery of heat-stressed broilers. Lives. Sci. 183:78–83.

Almaden, Y., A. Hernandez, V. Torregrosa, A. Canalejo, L. Sabate, L. Fernandez Cruz, J. M. Campistol, A. Torres, and M. Rodriguez. 1998. High phosphate level directly stimulates parathyroid hormone secretion and synthesis by human parathyroid tissue *in vitro*. J. Am. Soc. Nephrol. 9:1845–1852.

AOAC 2005. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Aviagen 2014. Ross 308 Broiler: Nutrition Specification. Aviagen Limited, Newbridge Midlothian EH28 8SZ, Scotland, UK.

Baradaran, N., M. H. Shahir, and Z. Asadi Kermani. 2017. Subsequent bone and metabolic responses of broilers to high non-phytate phosphorus diets in the starter period. Br. Poult. Sci. 58:435–441.

Brenes, A., A. Viveros, I. Arija, C. Centeno, M. Pizarro, and C. Bravo. 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Anim. Feed Sci. Technol. 110:201–219.

Buts, J. P., N. De Keyser, S. Marandi, D. Hermans, E. M. Sokal, Y. H. E. Chae, L. Lambotte, H. Chanteux, and P. M. Tulkens. 1999. *Saccharomyces boulardii* upgrades cellular adaptation after proximal enterectomy in rats. Gut. 45:89–96.

Chung, T. K., S. M. Rutherfurd, D. V. Thomas, and P. J. Moughan. 2013. Effect of two microbial phytases on mineral availability and retention and bone mineral density in low-phosphorus diets for broilers. Br. Poult. Sci. 54:362–373.

Cross, H. S., H. Debie, and M. Peterlik. 1990. Mechanism and regulation of intestinal phosphate absorption. Miner. Electrolyte Metab. 16:115–124.

Czerucka, D., T. Piche, and P. Rampal. 2007. Yeast as probiotics - *Saccharomyces boulardii*. Aliment. Pharmacol. Ther. 26:767–778.

Delmez, J. A., C. Tindira, P. Grooms, A. Dusso, D. W. Windus, and E. Slatopolsky. 1989. Parathyroid hormone suppression by intravenous 1,25-dihydroxyvitamin D. A role for increased sensitivity to calcium. J. Clin. Invest. 83:1349–1355.

Dhanwal, D. K. 2011. Thyroid disorders and bone mineral metabolism. Indian J. Endocrinol. Metab. 15(Suppl. 2):107–112.

El-Sherbiny, A. E., H. M. A. Hassan, M. O. Abd-Elsamee, A. Samy, and M. A. Mohamed. 2010. Performance, bone parameters and phosphorus excretion of broilers fed low phosphorus diets supplemented with phytase from 23 to 40 days of age. Int. J. Poult. Sci. 9:972–977.

Giang, H. H., T. Q. Viet, J. E. Lindberg, and B. Ogle. 2010. Effects of microbial enzymes and a complex of lactic acid bacteria and *Saccharomyces boulardii* on growth performance and total tract digestibility in weaned pigs. Livest. Res. Rural Dev. 22:179.

Haraldsson, A. K., J. Veide, T. Andlid, M. L. Alminger, and S. A. Sandberg. 2005. Degradation of phytate by high-phytase *Saccharomyces cerevisiae* strains during simulated gastrointestinal digestion. J. Agric. Food Chem. 53:5438–5444.

Hernandez, A., M. T. Concepcion, M. Rodriguez, E. Salido, and A. Torres. 1996. High phosphorus diet increases preproPTH mRNA independent of calcium and calcitriol in normal rats. Kidney Int. 50:1872–1878.

Jahanian, R., and M. Golshadi. 2015. Effect of dietary supplementation of butyric acid glycerides on performance, immunological responses, ileal microflora, and nutrient digestibility in laying hens fed different basal diets. Lives. Sci. 178:228–236.

Jazi, V., A. D. Foroozandeh, M. Toghyani, B. Dastar, R. Rezaie Koochaksaraie, and M. Toghyani. 2018. Effects of *Pediococcus acidilactici*, mannan-oligosaccharide, butyric acid and their combination on growth performance and intestinal health in young broiler chickens challenged with *Salmonella Typhimurium*. Poult. Sci. 97:2034–2043.

Jiang, X. R., F. H. Luo, M. R. Qu, V. Bontempo, S. G. Wu, H. J. Zhang, H. Y. Yue, and G. H. Qi. 2013. Effects of non-phytate phosphorus levels and phytase sources on growth performance, serum biochemical and tibia parameters of broiler chickens. Ital. J. Anim. Sci. 12:375–380.

Khodambashi Emami, N., S. Zafari Naeini, and C. A. Ruiz-Feria. 2013. Growth performance, digestibility, immune response and intestinal morphology of male broilers fed phosphorus deficient diets supplemented with microbial phytase and organic acids. Livest. Sci. 157:506–513.

Leyva-Jimenez, H., A. M. Alsadwi, K. Gardner, E. Voltura, and C. A. Bailey. 2019. Evaluation of high dietary phytase supplementation on performance, bone mineralization, and apparent ileal digestible energy of growing broilers. Poult. Sci. 98:811–819.

Liem, A., G. M. Pesti, and H. M. Edwards. 2008. The effect of several organic acids on phytate phosphorus hydrolysis in broiler chicks. Poult. Sci. 87:689–693.

Liu, S. M., N. Koszewski, M. Lupez, H. H. Malluche, A. Olivera, and J. Russell. 1996. Characterization of a response element in the 5′-flanking region of the avian (chicken) PTH gene that mediates negative regulation of gene transcription by 1,25-dihydroxyvitamin D_3 and binds the vitamin D_3 receptor. Mol. Endocrinol 10:206–215.

Liu, S. B., X. D. Liao, L. Lu, S. F. Li, L. Wang, L. Y. Zhang, Y. Jiang, and X. G. Luo. 2017. Dietary non-phytate phosphorus requirement of broilers fed a conventional corn-soybean meal diet from 1 to 21 D of age. Poult. Sci. 96:151–159.

Liu, N., and Y. J. Ru. 2010. Effect of phytate and phytase on the ileal flows of endogenous minerals and amino acids for growing broiler chickens fed purified diets. Anim. Feed Sci. Technol. 156:126–130.

Matsuda, M., T. A. Yamamoto, and M. Hirata. 2006. Ca^{2+}-dependent regulation of calcitonin gene expression by the transcriptional repressor DREAM. Endocrinology. 147:4608–4617.

Moré, M. I., and V. Yvan. 2018. *Saccharomyces boulardii* CNCM I-745 improves intestinal enzyme function: a trophic effects review. Clin. Med. Insights Gastroenterol 11:1–14.

Moslehi-Jenabian, S., L. L. Pedersen, and L. Jespersen. 2010. Beneficial effects of probiotic and food borne yeasts on human health. Nutrients 2:449–473.

Mueller, W. 1956. Feasibility of the chromic oxide and lignin indicator methods for metabolism experiments with chickens. J. Nutr. 58:29–36.

Namgung, R., and R. C. Tsang. 2012. Perinatal calcium and phosphorus metabolism. In: W. Oh, J. P. Guignard and S. Baumgart (Eds.), Nephrology and Fluid/Electrolyte Physiology. Neonatology Questions and Controversies, 2nd ed. Elsevier Saunders, Philadelphia, pp. 85–103.

Oster, M., C. Gerlinger, K. Heide, F. Just, L. Borgelt, P. Wolf, C. Polley, B. Vollmar, E. Muráni, S. Ponsuksili, and K. Wimmers. 2018. Lower dietary phosphorus supply in pigs match both animal welfare aspects and resource efficiency. Ambio. 47:20–29.

Palmer, S. C., A. Hayen, P. Macaskill, F. Pellegrini, J. C. Craig, G. J. Elder, and G. F. Strippoli. 2011. Serum levels of phosphorus parathyroid hormone, and calcium and risks of death and cardiovascular disease in individuals with chronic kidney disease: a systematic review and meta-analysis. JAMA. 305:1119–1127.

Park, K. W., A. R. Rhee, J. S. Um, and I. K. Paik. 2009. Effect of dietary available phosphorus and organic acids on the performance and egg quality of laying hens. J. Appl. Poult. Res. 18:598–604.

Pieniazek, J., K. A. Smith, M. P. Williams, M. K. Manangi, M. Vazquez-Anon, A. Solbak, M. Miller, and J. T. Lee. 2017. Evaluation of increasing levels of a microbial phytase in phosphorus deficient broiler diets via live broiler performance, tibia bone ash, apparent metabolizable energy, and amino acid digestibility. Poult. Sci. 96:370–382.

Proszkowiec-Weglarz, M., and R. Angel. 2013. Calcium and phosphorus metabolism in broilers: effect of homeostatic mechanism on

calcium and phosphorus digestibility. J. Appl. Poult. Res. 22:609–627.

Rajput, I. R., L. Y. Li, X. Xin, B. B. Wu, Z. L. Juan, Z. W. Cui, D. Y. Yu, and W. F. Li. 2013. Effect of *Saccharomyces boulardii* and *Bacillus subtilis* B10 on intestinal ultrastructure modulation and mucosal immunity development mechanism in broiler chickens. Poult. Sci. 92:956–965.

Ruijuan, L. I., J. Zhao, C. Sun, W. Lu, C. Guo, and K. Xiao. 2010. Biochemical properties, molecular characterizations, functions, and application perspectives of phytases. Front. Agric. China 4:195–209.

Ryan, E. P., A. L. Heuberger, T. L. Weir, B. Barnett, C. D. Broeckling, and J. E. Prenni. 2011. Rice bran fermented with *Saccharomyces boulardii* generates novel metabolite profiles with bioactivity. J. Agric. Food Chem. 59:1862–1870.

Saraç, F., and F. Saygılı. 2007. Causes of high bone alkaline phosphatase. Biotechnol. Biotec. Eq. 21:194–197.

Schröder, B., G. Breves, and M. Rodehutscord. 1996. Mechanisms of intestinal phosphorus absorption and availability of dietary phosphorus in pigs. Deut. Tierarztl. Woch. 103:209–214.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41.

Shang, Y., A. Rogiewicz, R. Patterson, B. A. Slominski, and W. K. Kim. 2015. The effect of phytase and fructooligosaccharide supplementation on growth performance, bone quality, and phosphorus utilization in broiler chickens. Poult. Sci. 94:955–964.

Siegert, W., T. Zuber, V. Sommerfeld, J. Krieg, D. Feuerstein, U. Kurrle, and M. Rodehutscord. 2019. Prececal amino acid digestibility and phytate degradation in broiler chickens when using different oilseed meals, phytase and protease supplements in the feed. Poult. Sci. 98:5700–5713.

Sileikiene, V., R. Mosenthin, E. Bauer, H. P. Piepho, M. Tafaj, D. Kruszewska, B. Weström, C. Erlanson-Albertsson, and S. G. Pierzynowski. 2008. Effect of ileal infusion of short-chain fatty acids on pancreatic prandial secretion and gastrointestinal hormones in pigs. Pancreas 37:196–202.

Sileikiene, V., R. Mosenthin, M. Tafaj, D. Kruszewska, B. Weström, I. Mattsson, and S. G. Pierzynowski. 2005. Effect of short chain fatty acids infused intraileally on interdigestive exocrine pancreatic secretions in growing pigs. J. Anim. Physiol. Anim. Nutr. 89: 253–259.

Sindhu, S. C., and N. Khetarpaul. 2001. Probiotic fermentation of indigenous food mixture: effect on antinutrients and digestibility of starch and protein. J. Food Compos. Anal. 14:601–609.

Slominski, B. A. 2011. Recent advances in research on enzymes for poultry diets. Poult. Sci. 90:2013–2023.

Slominski, B. A., T. Davie, M. C. Nyachoti, and O. Jones. 2007. Heat stability of endogenous and microbial phytase during feed pelleting. Livest. Sci. 109:244–246.

Sun, Y., I. R. Rajput, M. A. Arain, Y. Li, and D. M. Baloch. 2017. Oral administration of *Saccharomyces boulardii* alters duodenal morphology, enzymatic activity and cytokine production response in broiler chickens. Anim. Sci. J. 88:1204–1211.

Tavernari, F. D. C., L. F. Roza, D. Surek, C. Sordi, M. L. B. D. Silva, L. F. T. Albino, M. J. Migliorini, D. Paiano, and M. M. Boiago. 2018. Apparent metabolisable energy and amino acid digestibility of microalgae *Spirulina platensis* as an ingredient in broiler chicken diets. Br. Poult. Sci. 59:562–567.

Vieira, S. L., D. L. Anschau, Stefanello, N. C. Serafini, L. Kindlein, A. J. Cowieson, and J. O. B. Sorbara. 2015. Phosphorus equivalency of a *Citrobracter braakii* phytase in broilers. J. Appl. Poult. Res. 24:335–342.

Vieira, B. S., J. G. Caramori, C. F. S. Oliveira, and G. S. S. Correa. 2018. Combination of phytase and organic acid for broilers: role in mineral digestibility and phytic acid degradation. Worlds Poult. Sci. J. 74:711–726.

Walk, C. L., and S. V. Rama Rao. 2019. Dietary phytate has a greater anti-nutrient effect on feed conversion ratio compared to body weight gain and greater doses of phytase are required to alleviate this effect as evidenced by prediction equations on growth performance, bone ash and phytate degradation in broilers. Poult. Sci. https://doi.org/10.3382/ps/pez469.