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 increased apparent ileal digestibility coefficient (AIDC) of CP, AMEn, 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

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...
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; Mosleh-Jenabian et al., 2010). In general, different yeast strains as probiotic sources are considered to be useful organisms that specifically 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 × 10⁸ 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.
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 × 10⁸ cfu/kg S. boulardii (SB); and 6) the NC diet plus 0.2% butyric acid and 1 × 10⁸ 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:

\[
\text{AIDC} = \frac{\text{Digestibility}}{\text{Ileal digesta}} \times 100
\]

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

| Ingredients (%) | Starter (Day 0 to 10) | Grower (Day 10 to 24) | Finisher (Day 24 to 42) |
|-----------------|-----------------------|-----------------------|-------------------------|
|                 | 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 | 1.48   | 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 premix² | 0.25      | 0.25      | 0.25      | 0.25      | 0.25      | 0.25      |
| Mineral premix² | 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).

¹Monocalcium phosphate contained 18% Ca, 21% P.

²Supplied per kg diet: 18 mg retinol, 4 mg cholecalciferol, 36 mg a-tocopherol acetate, 2 mg vitamin K₃, 1.75 mg vitamin B₁₂, 0.1 mg biotin, 250 mg choline chloride, and 1 mg ethoxyquin.

³Supplied 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.
AIDC = 1 − \left[ \frac{\text{marker}_{\text{diet}}}{\text{marker}_{\text{id}}} \right] \times \left[ \frac{\text{nutrient}_{\text{diet}}}{\text{nutrient}_{\text{id}}} \right] \]

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

The AMEn value was determined using the following equation (Tavernari et al., 2018):

\[
\text{AMEn} (\text{kcal/kg of diet}) = \text{GE}_{\text{diet}} - \left[ \left( \text{GE}_{\text{id}} - \text{IF} \right) \times 8.22 \times \left( \frac{\text{N}_{\text{diet}}}{\text{N}_{\text{id}}} \times \text{IF} \right) \right]
\]

where GE$_{\text{diet}}$ is gross energy value in diet (kcal/kg) and GE$_{\text{id}}$ is gross energy value in ileal digestibility (kcal/kg); indigestibility factor = marker$_{\text{diet}}$/marker$_{\text{id}}$, N$_{\text{diet}}$ is nitrogen concentration in diet (%), N$_{\text{id}}$ 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 (T3), thyroxine or tetraiodotrienin (T4), PTH, and calcitonin (CT). Serum T3 and T4 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 T3 and 4.7 and 6.4% for T4, 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 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 | 0.09 | 0.09 |
| DEB$^1$, mEq/kg | 250 | 250 | 240 | 240 | 225 | 225 |

**Analysed 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).
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 AMEn 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.

### Blood Metabolites and Hormone Profile

Serum concentrations of P, Ca, T4, 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

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**Table 3. Effect of experimental treatments on growth performance of broiler chickens.**

| Item               | PC     | NC     | PHY    | BA     | SB     | BA+SB  | SEM  | \(P\) value |
|--------------------|--------|--------|--------|--------|--------|--------|------|-------------|
| BW (g)             | 264    | 253    | 259    | 259    | 270    |        |      | 7.2         | 0.382     |
| 10 D               | 975    | 909    | 942    | 962    | 985    |        |      | 23.8        | 0.208     |
| 42 D               | 2,435<sup>a</sup> | 2,185<sup>ab</sup> | 2,506<sup>a</sup> | 2,324<sup>b</sup> | 2,397<sup>ab</sup> | 2,477<sup>ab</sup> | 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<sup>b</sup> | 72.1<sup>c</sup> | 83.5<sup>a</sup> | 77.1<sup>b</sup> | 79.6<sup>ab</sup> | 80.2<sup>a</sup> | 1.78 | 0.002       |
| 0-42 D             | 56.9<sup>b</sup> | 51.0<sup>ab</sup> | 58.6<sup>a</sup> | 54.3<sup>b</sup> | 56.0<sup>ab</sup> | 58.6<sup>a</sup> | 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<sup>a</sup> | 72.0<sup>a</sup> | 74.4<sup>a</sup> | 74.8<sup>b</sup> | 73.2<sup>b</sup> | 79.3<sup>a</sup> | 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<sup>b</sup> | 1.85<sup>a</sup> | 1.67<sup>a</sup> | 1.82<sup>b</sup> | 1.75<sup>b</sup> | 1.72<sup>c</sup> | 0.039| 0.041       |

<sup>a</sup><sup>b</sup>Means within each row with no common superscript differ \((P < 0.05)\).

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

<sup>1</sup>Values are means of 5 pens per treatment combination with 25 male broiler chickens.

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### 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 AMEn 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.
to the hormone levels, broiler chickens fed with the diet PC exhibited the highest serum T₃ level, which was higher \( P = 0.048 \) than that in birds fed with NC, BA, and SB diets. Serum T₄ 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 \( 1 \times 10^8 \) 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 \( 1 \times 10^8 \) 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.

| 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           | 0.753           | 0.783           | 0.780           | 0.767           | 0.789           | 0.008| 0.041   |
| Calcium                     | 0.443           | 0.389           | 0.467           | 0.394           | 0.407           | 0.444           | 0.019| <0.001  |
| Phosphorus                  | 0.573           | 0.576           | 0.639           | 0.588           | 0.625           | 0.626           | 0.015| 0.017   |
| AMEn                        | 3.130           | 3.027           | 3.145           | 3.121           | 3.082           | 3.157           | 26.4 | 0.021   |

**Table 4.** Effect of experimental treatments on apparent ileal digestibility coefficient (AIDC) of nutrients in broiler chickens on Day 42.¹

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

**Table 5.** Effect of experimental treatments on the bone mineralization of broilers on Day 42.¹

¹Means within each raw with no common superscript differ \( P < 0.05 \).
Sileikiene et al. (2005), who reported that higher activity of trypsin and amylase and higher secretion of butyric acid in the diets was found to increase the ileal CP digestibility and, consequently, diet AME content. It was also suggested by Sileikiene et al. (2008) that butyrate may increase the AME value in broiler chickens. 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). Dethorphanization 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.

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, 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.

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| Item | PC | NC | PHY | BA | SB | BA+SB | SEM | P value |
|------|----|----|-----|----|----|-------|-----|--------|
| 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\(^ab\) | 4,175\(^b\) | 4,686\(^b\) | 4,931\(^a\) | 4,589\(^b\) | 384 | 0.048 |
| T\(_3\) ng/mL | 2.69\(^a\) | 2.03\(^c\) | 2.34\(^b\) | 2.10\(^e\) | 2.18\(^b\) | 2.55\(^a\) | 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 (g/dL) | 123.2 | 76.1 | 117.8 | 88.6 | 99.2 | 113.5 | 10.9 | 0.085 |
| PTH (U/L) | 26.8\(^bc\) | 37.5\(^e\) | 24.7\(^c\) | 34.8\(^b\) | 32.3\(^a\) | 25.3\(^b\) | 3.04 | 0.033 |

\(^{1}\)Values are means of 5 pens per treatment combination with 2 male broiler chickens per pen selected for blood collection.
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 Saygıli, 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.

Thyroid hormone increases the genomic action of 1,25-dihydroxyvitamin D3 (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 (Schroder 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 T3 levels compared with PC diet, indicating P deficiency could adversely affect thyroid hormone function by reducing serum T3 levels. Therefore, the increased ability to use phytate P could reflect the higher T3 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 T3 hormone has an impact on growth rate via anabolic and catabolic processes (Oster et al., 2018), the reduced T3 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 response to diets varying in Ca and P concentrations could enable serum Ca and P homeostasis (Matsuda et al., 2006; 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-hydroxyvitamin D3-1-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, AMEn, 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 T3 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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