Evaluation of the Boron and Phytase, Alone or in Combination, in Broiler Diets

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A total of 800 day-old broiler chicks were assigned to four test diets to assess the efficacy of phytase, fermented from Aspergillus niger, and boron, as boric acid, on the growth performance and mineral profile in chickens. The dietary treatments included the basal diet and the basal diet supplemented with boron (20mg/kg), phytase (500 phytase units/kg diet), or a combination of boron and phytase. The following parameters were measured: growth performance indices, serum biochemicals, ash and the mineral profile of the tibia, breast muscle, liver and excreta. Boron decreased the overall feed conversion ratio by 1.9% (P<0.05) but did not affect the body weight and feed consumption of chickens grown for 42 days (P>0.05). The performance indices were not significantly influenced by dietary regimens for the first 21 days of the experiment (P>0.05). The addition of boron alone and boron + phytase resulted in significant increases in the boron concentrations of serum, bone, breast muscle, liver and excreta (P<0.001). The serum alanine aminotransferase activity of chicks fed phytase was higher (P<0.05) than all other treatments. The ash content and mineral composition of the breast meat, liver and tibia did not respond to individual or combined dietary modifications (P>0.05). The chickens administered a diet with boron excreted less Cathrough excreta. However, the ash content and Fe and Cu concentrations in the excreta increased in response to phytase supplementation (P<0.05). In summary, supplementation with boron alone improved the feed conversion efficiency of broiler chickens fed diets containing adequate levels of nutrients. However, the combination of boron and phytase did not cause further improvements in broiler performance or the bioavailability of minerals.

Key words: boron, broiler, growth, mineral profile, phytase

Introduction

Boron is a trace element that plays a regulatory role in mineral and hormonal metabolism and is known to influence a variety of metabolic processes (Hunt, 1996; Devirian and Volpe, 2003). Boron has been examined as a possible essential nutrient in the metabolism and utilization of macro- and micro-minerals and vitamin D (Nielsen et al., 1987, 1990; McCoy et al., 1994). Modifications to the skeletal structure and biological function of mammals and chickens in response to boron include changes in nutritional variables involving Ca, P (Rossi et al., 1993; Armstrong and Spears, 2001; Bozkurt et al., 2012), vitamin D (Hegsted et al., 1991; Kurtoğlu et al., 2005), Mg, Al, Mo, Cu and Fe (Nielsen and Shuler, 1992; Hunt et al., 1997). Thus, it has been hypothesized that supplemental boron could increase bone mineral density, or affect bone development and normal growth.

Studies by Armstrong and Spears (2001) and Armstrong et al. (2000), undertaken to determine whether an interaction exists among boron, Ca and P on the skeletal development of gilts, indicated that supplementation with boron could improve the bioavailability of Ca and P. The increase in boron in bodily organs and bones may be a due to the reaction of the borate anion with the molybdate assay, which may result in an apparent increase in P (Armstrong and Spears, 2001). Thus, if boron enhances Ca and P retention in the bone, there should be a corresponding reduction of these elements in the excreta. Collectively, the nutritional role of boron in bone composition is unclear, and the relevant studies have yielded inconsistent results in chickens fed diets supplemented with boron for 21, 45 and 42 day periods (Rossi et al., 1993; Kurtoğlu et al., 2001, 2005; Bozkurt et al., 2012).
Numerous studies have shown that phytase can be used to increase the availability of P, Fe, Zn, and Cu and to reduce the excretion of those minerals in chickens fed diets containing phytase with low Ca and P compared with those fed at recommended levels (Sebastian et al., 1996; Viveros et al., 2002). The beneficial effect of phytase in chickens could be due to the release of minerals and trace elements from complexes containing phytic acid and to a possible increase in starch and protein digestibility (Selle and Ravindran, 2007).

Both boron and phytase have the potential to interact with similar macro- and micro-minerals. Therefore, we hypothesized that boron’s action may be enhanced by using a dietary regimen including both boron and phytase, thereby creating either synergistic or additive effects on the bioavailability of minerals. The goal of the present study was to examine the influence of boron and phytase, alone or in combination, on growth performance, mineral composition of the serum and excreta, and mineralization of bone, liver and breast muscle in broilers grown for 42 days and fed on a corn-wheat-soybean-based diets.

**Materials and Methods**

**Birds and Housing**

A total of 800 1-day-old broiler chicks (Ross 308) of mixed sex (with a BW 43.7 ± 0.89 g) were used. At arrival to the house, after weighing, chicks were randomly allotted to floor pens, each representing a replicate. Birds were vaccinated against infectious bursal disease virus (Gallivac, Merial® Animal Health, Lyon-France) and Newcastle disease virus (Avinew, Merial® Animal Health, Lyon-France) via drinking water at 10 and 14 days of age, respectively. Each replicate was assigned to a clean floor pen (2.4 × 1.6 m) equipped with one hanging bell drinker, which was cleaned daily, two tube-type feeders and electrical heaters. Birds were reared in pens (13 birds per m² floor space) provided with litter (pine wood shavings) to a depth of 5–6 cm. The room temperature was gradually decreased from 33 °C at chick arrival to 23 °C on day 22, and thereafter was kept constant until trial termination on d 42. Chickens received 23 h light/d during the first 3 d of life and then 18 h light/d until the end of the experiment. The house was ventilated with adjustable windows and a tunnel ventilation fan, and efforts were made to reproduce commercial conditions as much as possible. The experimental diets and drinking water were available ad libitum. All of the following procedures were approved by the Animal Care and Use Committee of the Ministry of Agriculture, Directorate of Research.

**Experimental Design and Diets**

The experiment was performed as a completely randomized design with 4 treatments. Each treatment had four replicates of 50 broilers (25 males and 25 females). The basal diet was a typical corn-wheat-soybean diet that was formulated to meet or exceed all nutrient recommendations published in the Ross 308 rearing guidelines (Aviagen, 2012). Broilers were fed with starter (1 to 21 d) and finisher (22 to 42 d) diets in mash form. Corn, wheat and soybean meal were ground at the feed mill of the institute using a hammer mill equipped with a 5-mm screen, resulting in a final average particle size of 600 μm. Then, all of the ingredients were blended in a mixture for 4 minutes and stored in sacks in a cool place. These diets contained no antibiotics or growth enhancers and were isoenergetic and isonitrogenous. The ingredient composition and nutrient content of the basal diets on an as-fed basis; starter (d 1–21) and finisher (d 21–42) are given in Table 1.

### Table 1. Ingredient composition of experimental basal diets

| Ingredient, (g/kg) | Starter diet | Finisher diet |
|-------------------|--------------|---------------|
| Corn              | 372.7        | 432.0         |
| Wheat             | 200.0        | 200.0         |
| Soybean meal (48 %) | 354.3      | 285.0         |
| Soybean oil       | 33.6         | 48.6          |
| Calcium carbonate | 9.7          | 9.3           |
| Dicalcium phosphate | 18.1         | 14.7          |
| Sodium chloride   | 2.5          | 2.6           |
| Vit.-Min. premix² | 2.5          | 2.5           |
| L-Lysine HCL      | 1.0          | 0.0           |
| DL-Methionine     | 2.0          | 2.2           |
| L-Threonine       | 0.6          | 0.1           |
| Anticoccidial premix³ | 1.0       | 1.0           |
| Saw dust⁴,⁵,⁶      | 2.0          | 2.0           |
| Calculated nutrient (%) |             |               |
| ME (kcal/kg)      | 3061         | 3235          |

**Ingredient, (g/kg) | Starter diet | Finisher diet |
|-------------------|--------------|---------------|
| Ca (intended)     | 0.95         | 0.85          |
| P, total (intended) | 0.75       | 0.64          |
| P (available)     | 0.47         | 0.40          |
| Lysine            | 1.28         | 1.00          |
| Methionine        | 0.55         | 0.50          |
| Methionine + Cysteine | 0.90       | 0.84          |
| Threonine         | 0.89         | 0.73          |

**Analysed nutrient**

| Dry matter (%) | 88.97 | 88.89 |
| Crude protein (%) | 22.21 | 19.63 |
| Fat (ether extract) (%) | 5.71 | 8.03 |
| Crude cellulose (%) | 3.29 | 3.20 |
| Crude ash (%) | 6.16 | 5.49 |
| Ca (%) | 1.01 | 0.88 |
| Total P (%) | 0.77 | 0.66 |
| Mg (%) | 0.18 | 0.17 |
| Fe (mg/kg) | 145 | 168 |
| Cu (mg/kg) | 10.3 | 13.0 |
| Zn (mg/kg) | 74.3 | 72.9 |
| B (mg/kg) | 5.52 | 5.36 |

1Contains 18.2% P and 23.6% Ca.

2Provides per kg of diet: trans-retinol 12000IU; cholecalciferol 1500 IU; α-tocopherol acetate 75 mg; Vitamin K₃ 5 mg; Vitamin B₁ 3 mg; Vitamin B₂; 6 mg; Vitamin B₆ 5 mg; Vitamin B₉; 0.03 mg; nicotinamide 40 mg; pantothenic acid 10 mg; folic acid 0.75 mg; D-biotin 0.075 mg; choline 375 mg; Mn 80 mg; Fe 40 mg; Zn 60 mg; Cu 5 mg; I 0.5 mg; Co 0.2 mg; Se 0.15 mg.

3Provides 70 mg narasin per kg diet.

4Saw dust replaced for boron or phytase preparations.

5Boric acid of 0.109 g provides 20 mg boron per kg of diet.

6Phytase premix (Natuphos® 500) provides 500 FTU phytase-zyme per kg of diet.
diets are shown in Table 1.

The dietary treatments were as follows: 1) basal diet with no supplemental boron and phytase (Control), 2) Control + 20 mg/kg of boron, 3) Control + phytase, (1 g/kg diet), and 4) Control + 20 mg/kg of boron + phytase, (1 g/kg diet). Boric acid (H₃BO₃) was used as the boron source. It was provided by the Institute of Boron (BOREN, Ankara-Turkey). The boric acid used in this study contained 18.32% boron. Briefly; 109 g boric acid was included into per ton of basal diet to supply 20 mg/kg boron to the kg diet. Each preparation (i.e., 109 g boron; 1 kg phytase and 1 kg phytase + 109 g boron) was added to 2.0 kg of saw dust homogenized by mixer, and then the pre-mixture was added to the basal mixture. Three samples were tested to check the homogeneity of boric acid before the experimental diets were prepared. Phytase, in the form of a premix powder, was provided as a commercial preparation at 1 kg. The birds of treatment 3 and 4 were fed diets including 500 FTU phytase, which is a 3-phytase preparation of the group mean, were selected at random. Blood samples of each replicate (i.e., 12 birds per treatment), with body weights close to the mean, were selected randomly and transferred to an offsite cage facility. The birds were placed in colony cages as groups, allowing access to feed and water. The broilers were allowed to adjust to the cage conditions for 3 days. Excreta samples were collected randomly and transferred to an offsite cage facility. The birds were placed in colony cages as groups, allowing access to feed and water. The broilers were allowed to adjust to the cage conditions for 3 days. Excreta samples were collected for 3 consecutive days (days 39–42, minimum 500 g/day/treatment). The samples were homogenously mixed, stored at −20°C prior to further analysis, and then analyzed for ash and mineral contents to determine the level of mineral excretion.

**Serum Biochemical Analysis**

At the end of the experiment, three birds per experimental unit (i.e., 12 birds per treatment), with body weights close to the group mean, were selected at random. Blood samples of 1 mL were collected into non-additive blood collection tubes by cardiac puncture. The sera were separated by centrifugation at 1800×g at 8°C after 1 hour of incubation at room temperature and were stored at −20°C until further analysis. After the collection of blood samples, the birds were anesthetized with carbon dioxide, bled by severing the jugular vein, and then euthanized for the ash and mineral analysis of the tibia, breast muscle and liver. The concentrations of Ca, inorganic P, Mg, and Zn, and the activities of alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were measured with a spectrophotometer (Shimadzu UV 1601) using commercial kits purchased from Archem Diagnostics End. LTD. (Istanbul, Turkey). The triiodothyronine (T₃) (Mybiosource, MBS269151, California, USA) and thyroxine (T₄) (Mybiosource, MBS881213, California, USA) concentrations were measured by an ELISA reader (Optic Ivymen System 2100-C) using commercially available kits. The boron concentration in the blood was measured using ICP-OES (Perkin Elmer Optima 2100 DV) (Laakso et al., 2001). The calibrations for the boron assays were conducted with a series of mixtures containing graded concentrations of standard solutions (CGB-1 B ICP Standard, Inorganic Ventures, Virginia, USA).

**Preparation of Tibia, Breast Muscle and Liver for Mineral Analysis**

Right tibias with some attached flesh were collected to measure the bone ash and bone mineral content. The tibias were excised, and all flesh and proximal cartilage was removed. The breast muscles (Pectoralis major) and one lobe of the livers were immediately detached, and samples of approximately 50 g wet weight were removed using stainless steel scalpels. The bone, breast muscle and liver samples were sealed individually in plastic bags and stored at −20°C until the analysis, which was performed within 2 months of the day of sampling.

The samples (i.e., tibia, breast meat and liver) were thawed at room temperature for 6 h in an air conditioned room before the analysis began. Each sample was broken into small pieces, weighed, oven-dried at 105°C for 12 h, cooled in a desiccator, weighed, dry-ashed at 600°C for 12 h, cooled in a desiccator, and weighed (AOAC, 1990). The ash content of the samples was expressed as a percentage of the dry sample weight.

**Excreta Collecting**

Following the 36-day growth period, 12 birds per treatment (one male and one female bird per replicate) were selected randomly and transferred to an offsite cage facility. The birds were placed in colony cages as groups, allowing for the collection of excreta. The birds were maintained in their respective experimental treatments and had ad libitum access to feed and water. The broilers were allowed to adjust to the cage conditions for 3 days. Excreta samples were collected for 3 consecutive days (days 39–42, minimum 500 g/day/treatment). The samples were homogenously mixed, stored at −20°C prior to further analysis, and then analyzed for ash and mineral contents to determine the level of mineral excretion.

**Chemical Analysis**

The chemical composition, crude ash content and mineral contents of the diets were determined according to the methods of AOAC (1990). All of the feed samples were analyzed for dry matter (934.01), ash (942.05), nitrogen (Kjeldahl procedure: 988.05), ether extracts (920.39), crude fiber (962.09), Ca (927.02) and total P (965.17). The experimental diets were also analyzed to guarantee that they were identical to the control with respect to chemical composition, with the exception of the supplements. Ground samples were dry-ashed (AOAC, 1990), and the concentrations of elements, including boron, Ca, P, Mg, Fe, Cu and Zn, were...
measured at a specific wavelength (nm) (boron, 249.677, Ca, 315.9; P, 214.9, Mg, 285.2; Fe, 248.3; Cu, 324.8; Zn, 213.9) using an ICP (Perkin Elmer Optima 2100 DV). The calibrations for the mineral assays are explained in detail below in the mineral analysis section.

**Mineral Analysis**

The mineral contents of the basal diets, tibias, breast muscles, livers and excreta were analyzed using twelve samples per treatment. The concentrations of Ca, P, Mg, Fe, Zn, Cu and boron were determined for each sample. Five mL of ultrapure HNO₃ was added to each ash sample (i.e., 0.24 g) until it had completely dissolved. Then, 20 mL of de-ionized water was added to each sample, and samples were filtered using WH 42 filter paper. The obtained solutions were diluted with de-ionized water to a final volume of 100 mL.

The mineral concentrations were measured at specific wavelengths for each element with an ICP-OES (Perkin Elmer Optima 2100 DV, Perkin Elmer, Inc., Massachusetts, USA). The calibrations for the mineral assays were conducted with a series of mixtures containing graded concentrations of ICP standards (170308 Ca, 170340 P, 170331 Mg, 170326 Fe, 170314 Cu, 170369 Zn, and 170307 B, Merck, Darmstadt, Germany). The detection and quantification limits of the analytical methodology were determined on the basis of 10 measurements of the element concentrations in a reagent blank solution taken during the sample preparation procedure. The spike recoveries varied between 92% and 106%. The limits of detection were as follows: Ca, 0.55 mg/kg; P, 0.01 mg/kg; Mg, 0.01 mg/kg; Fe, 0.20 mg/kg; Zn, 0.05 mg/kg; Cu, 0.02 mg/kg; and boron, 0.01 mg/kg. The quantification limits for Ca, P, Mg, Fe, Zn, Cu and boron were 0.68, 0.01, 0.01, 0.21, 0.05, 0.02 and 0.01 mg/kg, respectively. The data regarding the minerals in the bone were described as their proportional weight in ash, and the corresponding values in breast muscle, liver and excreta were based on dry matter weight.

**Statistical Analyses**

The experiment was performed as a completely randomized design with 4 treatments. Data were analysed by ANOVA using the GLM procedure of JMP (SAS Institute, 2009). Pen served as the experimental unit for the growth performance parameters (i.e., BWG, FI, FCR and mortality) and other parameters (i.e., serum biochemicals, ash and mineral content of tibia, breast muscle and liver). An arc-sin transformation was applied to the percentage values (i.e., mortality and ash, Ca, P and Mg levels in tissues) before testing for differences. Tukey’s multiple range test was performed to detect differences among the treatments. All differences were considered significant at $P<0.05$.

**Results**

The supplementation of the diet with boron, phytase, or both, did not affect the performance indices of chickens during the course of the experiment (i.e., 1 to 42 days of age), except for the FCR value (Table 2). Boron supplementation tended to decrease the FCR of chickens between 1 and 21 days ($P=0.09$) compared to those receiving control, phytase and boron + phytase diets. Additionally, addition of boron decreased FCR for the entire experimental period, with significant differences between the boron and control and boron and phytase treatments ($P<0.05$). However, the dietary combination of boron with phytase worsened the FCR of chickens compared to supplementation with the mineral and enzyme alone ($P<0.05$). The mortality was unaffected by dietary treatment and was generally low, with a rate of approximately 2–3%.

Supplemental boron, alone or in combination with phytase, increased the concentrations of boron in the serum, tibia, liver, breast muscle and excreta in comparison with the control and phytase treatments ($P<0.01$; Table 3). The serum biochemical constituents are shown in Table 4. None of these compounds were affected ($P>0.05$) by supplemental boron, phytase, or both, with the exception of ALT. The serum ALT activity of chickens fed phytase was significantly higher ($P<0.05$) than for all other treatments.

The ash content and mineral profile of the breast muscle,

### Table 2. Effects of individual or combined supplementation of boron and phytase to nutritionally adequate diet on the performance indices of broiler chickens

| Diet | Control | Boron | Phytase | Boron + Phytase | SEM | P-value |
|------|---------|-------|---------|----------------|-----|---------|
| d1–21 | Body weight gain (g) | 757  | 766  | 776  | 762  | 6.26 | 0.1858 |
|       | Feed intake (g) | 1100 | 1099 | 1125 | 1116 | 10.7 | 0.2738 |
|       | Feed conversion ratio | 1.452 | 1.434 | 1.450 | 1.464 | 0.007 | 0.0959 |
|       | Mortality (%) | 0.50 | 2.00 | 1.00 | 1.00 | 0.75 | 0.5698 |
| d1–42 d. | Body weight (g) | 2280 | 2294 | 2303 | 2270 | 20.9 | 0.7009 |
|       | Feed intake (g) | 4079 | 4024 | 4063 | 4088 | 30.3 | 0.4809 |
|       | Feed conversion ratio | 1.788 | 1.754 | 1.764 | 1.800 | 0.009 | 0.0129 |
|       | Mortality (%) | 3.02 | 3.52 | 2.51 | 3.04 | 0.99 | 0.9129 |

* Values within a column not sharing the same superscript are different at $P<0.05$.

1 The broilers were fed a control diet that was formulated to meet the requirements for the strain (Ross 308) recommended by the breeder or a diet supplemented with preparations of boron (20 mg/kg), phytase (500 FTU g/kg phytase), or boron + phytase (20 mg/kg boron + 500 FTU g/kg phytase).

2 Data are the means of 4 replicate pens with 50 chicks each per treatment.
### Table 3. Boron concentrations in the serum, tibia, liver and breast muscle in broiler chickens fed diets with supplemental boron and phytase, individually or in combination

| Diet          | Control | Boron | Phytase | Boron + Phytase | SEM | P-value |
|---------------|---------|-------|---------|----------------|-----|---------|
| Serum (mg/L)  | 0.37b   | 0.84a | 0.37b   | 0.86c          | 0.004 | 0.0001 |
| Tibia (μg/g)  | 0.18b   | 0.36a | 0.17b   | 0.33c          | 0.019 | 0.0001 |
| Liver (μg/g)  | 0.21b   | 0.36a | 0.17b   | 0.36c          | 0.004 | 0.0078 |
| Breast muscle | 0.16b   | 0.26a | 0.14b   | 0.25c          | 0.014 | 0.0001 |

a-b Values within a column not sharing the same superscript are different at P<0.05.

1 The broilers were fed a control diet that was formulated to meet the requirements for the strain (Ross 308) recommended by the breeder or a diet supplemented with preparations of boron (20 mg/kg), phytase (500 FTU g/kg phytase), or boron + phytase (20 mg/kg boron + 500 FTU g/kg phytase).

2 Data are the means of 12 measurements per treatment.

### Table 4. Serum triiodothyronine (T3), thyroxine (T4), Ca, P, Mg, and Zn concentrations and alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities in broiler chickens fed a nutritionally adequate diet with boron and phytase, individually or in combination

| Diet          | Control | Boron | Phytase | Boron + Phytase | SEM | P-value |
|---------------|---------|-------|---------|----------------|-----|---------|
| T 3 (ng/mL)   | 1.17    | 1.19  | 1.19    | 1.19           | 0.02 | 0.8377 |
| T 4 (μg/dL)   | 1.96    | 1.95  | 2.19    | 2.08           | 0.07 | 0.1100 |
| Ca (mg/dL)    | 5.22    | 5.91  | 5.08    | 6.02           | 0.35 | 0.1037 |
| P (mg/dL)     | 5.34    | 5.11  | 6.02    | 5.11           | 0.25 | 0.8974 |
| Mg (mmol/L)   | 0.61    | 0.53  | 0.61    | 0.53           | 0.03 | 0.1664 |
| Zn (μg/dL)    | 133     | 125   | 145     | 126            | 8.31 | 0.3441 |
| ALP (U/L)     | 2864    | 2627  | 2502    | 2493           | 278 | 0.7630 |
| ALT (U/L)     | 8.02b   | 8.15b | 9.71a   | 8.64ab         | 0.40 | 0.0188 |

a-b Values within a column not sharing the same superscript are different at P<0.05.

1 The broilers were fed a control diet that was formulated to meet the requirements for the strain (Ross 308) recommended by the breeder or a diet supplemented with preparations of boron (20 mg/kg), phytase (500 FTU g/kg phytase), or boron + phytase (20 mg/kg boron + 500 FTU g/kg phytase).

2 Data are the means of 12 measurements per treatment.

### Table 5. Effects of dietary modifications with boron and phytase on the ash and mineral content of breast muscle (Pectoralis major) and liver in broiler chickens

| Diet          | Control | Boron | Phytase | Boron + Phytase | SEM | P-value |
|---------------|---------|-------|---------|----------------|-----|---------|
| Ash (%)       | 4.18    | 4.33  | 4.32    | 4.26           | 0.07 | 0.4108 |
| Ca (μg/g)     | 173     | 178   | 163     | 164            | 6.6 | 0.3488 |
| P (mg/g)      | 9.59    | 9.92  | 9.91    | 9.80           | 0.23 | 0.7572 |
| Mg (mg/g)     | 1.18    | 1.16  | 1.17    | 1.18           | 0.02 | 0.8995 |
| Fe (μg/g)     | 42.0    | 44.3  | 37.9    | 35.0           | 5.8 | 0.6901 |
| Cu (μg/g)     | 4.93    | 4.54  | 3.40    | 3.84           | 0.66 | 0.3703 |
| Zn (μg/g)     | 22.2    | 21.2  | 22.9    | 22.5           | 0.50 | 0.1327 |

| Diet          | Control | Boron | Phytase | Boron + Phytase | SEM | P-value |
|---------------|---------|-------|---------|----------------|-----|---------|
| Liver         | 4.67    | 4.61  | 4.43    | 4.43           | 0.12 | 0.3760 |
| Ash (%)       | 4.31    | 4.36  | 4.33    | 4.31           | 0.33 | 0.1939 |
| Ca (μg/g)     | 1.99    | 1.06  | 1.03    | 1.03           | 0.96 | 0.3520 |
| P (mg/g)      | 0.53    | 0.48  | 0.49    | 0.44           | 0.44 | 0.0374 |
| Mg (mg/g)     | 21.4    | 21.9  | 20.8    | 18.8           | 1.62 | 0.5429 |
| Fe (μg/g)     | 95.9    | 97.4  | 95.1    | 96.6           | 2.70 | 0.9424 |

1 The broilers were fed a control diet that was formulated to meet the requirements for the strain (Ross 308) recommended by the breeder or a diet supplemented with preparations of boron (20 mg/kg), phytase (500 FTU g/kg phytase), or boron + phytase (20 mg/kg boron + 500 FTU g/kg phytase).

2 Data are means of 12 measurements each per treatment.
control and phytase and between control and boron concentration in the excreta differed significantly between patterns was observed for the Cu concentration in excreta. Cu receiving phytase and boron supplementation was not significant (P < 0.05; Table 2). However, it is not clear whether the improvement in the FCR was due to the increased nutrient use promoted by boron or by a direct impact of the reduced feed intake.

Discussion

Here, the most pronounced beneficial effect of dietary boron supplementation was the marked improvement (P < 0.05) in the FCR by 1.91% between 1 and 42 days of age. The regulatory role of boron in the metabolism of Ca, P, and Mg (Chapin et al., 1998; Nielsen, 1990), energy-substrate metabolism, steroid hormones, immune system function, and antioxidant defense systems may be partly responsible for this improvement (Hunt, 1998). The 54 g reduction in feed consumption by chickens fed boron compared with controls was accompanied by a significant reduction in the FCR (P < 0.05; Table 2). However, it is not clear whether this increase in the FCR was due to the increased nutrient use promoted by boron or by a direct impact of the reduced feed intake.

Similarly, Fassani et al. (2004) reported that chickens fed with a diet of 30 ppm boron consumed 140 g less feed than controls while exhibiting lower FCR and without compromising weight gain and livability. In contrast, Bozkurt et al. (2012) reported that 30 and 60 ppm boron supplementation tended to increase feed intake. More research is needed to verify the assumption that chickens fed a diet with supplemental boron could fulfill their nutrient requirements on a lower feed intake and reach a similar body weight to those given a diet containing no boron.

The result of the present experiment showed that boron and phytase did not significantly affect the growth rate of broilers (Table 2). The studies with boron show substantial inconsistencies. As in this study, boron acted as a “growth promoter” in several initial evaluations (Hunt and Nielsen, 1981; Rossi et al., 1989) and in more recent works (Fassani et al., 2004; Bozkurt et al., 2012). However, this was not the case in previous feeding studies (Eliot and Edwards, 1992; Rossi et al., 1993; Kurtoğlu et al., 2001). The results of these studies are difficult to evaluate because of the different protocols used, including differences in breed, age and performance of the chickens, and differences in the composition and nutritive value of the diets, including inherent boron concentrations in the basal diet.

The boron contents differed between various bodily organs, with the highest accumulation found in bone, which suggests that boron has specific functions in each organ (Moseman, 1994). Here, the boron concentrations in the serum, liver, bone, muscle, and excreta were closely related to dietary supplementation. The findings related to the correlation of serum, bone, and bodily organs were in accordance with other reports (Rossi et al., 1993; Kurtoğlu et al., 2005; Küçükýılmaz et al., 2014). In this study, the ingestion of 25.4 mg of boron per kg induced a 0.6-0.7-fold increase in the boron content of the liver (0.36 μg/g) and the breast (0.25 μg/g) compared with the unsupplemented group. As demonstrated previously by Litovitz et al. (1988), acute-duration oral exposures of humans to high levels of 

### Table 6. Ash content and mineral composition of tibia and excreta in broiler chickens fed on nutritionally adequate diet with boron and phytase, individually or in combination

| Diet1       | Control | Boron | Phytase | Boron + Phytase | SEM2 | P-value |
|-------------|---------|-------|---------|-----------------|------|---------|
| Ash (%)     | 37.4    | 37.5  | 36.8    | 36.6            | 0.48 | 0.4809  |
| Ca (%)      | 35.6    | 35.2  | 33.9    | 34.0            | 1.8  | 0.0719  |
| P (%)       | 15.3    | 13.4  | 13.0    | 13.1            | 0.88 | 0.7596  |
| Mg (%)      | 0.40    | 0.40  | 0.39    | 0.39            | 0.01 | 0.9245  |
| Fe (μg/g)   | 153     | 152   | 144     | 148             | 4.2  | 0.4501  |
| Cu (μg/g)   | 10.5    | 10.6  | 10.2    | 10.1            | 0.86 | 0.5980  |
| Zn (μg/g)   | 159     | 158   | 157     | 159             | 10.1 | 0.9895  |
| Tibia       |         |       |         |                 |      |         |
| Control     | 13.0\(^+\) | 12.5\(^+\) | 13.8\(^+\) | 13.4\(^+\)      | 0.17 | 0.0001  |
| Boron       | 2.76\(^+\) | 2.49\(^+\) | 2.96\(^+\) | 2.96\(^+\)      | 0.086 | 0.0008 |
| Phytase     | 1.70    | 1.60  | 1.58    | 1.68            | 0.046 | 0.1860 |
| Boron + Phytase | 628\(^+\) | 724\(^+\) | 806\(^+\) | 804\(^+\)      | 0.8833 |
| Excreta     |         |       |         |                 |      |         |
| Control     | 44.4\(^+\) | 49.5\(^+\) | 53.3\(^+\) | 51.3\(^+\)      | 0.0216 |
| Boron       | 233     | 237   | 239     | 242             | 8.05 | 0.8905  |

1 The broilers were fed a control diet that was formulated to meet the requirements for the strain (Ross 308) recommended by the breeder or a diet supplemented with preparations of boron (20 mg/kg), phytase (500 FTU g/kg phytase), or boron + phytase (20 mg/kg boron + 500 FTU g/kg phytase).

2 Data are the means of 12 measurements per treatment.
boron (as boric acid) have resulted in little or no observable toxicity; for example, 88% of accidental poisonings of 10–88 g were asymptomatic. In addition, the minimal risk to humans (MRL) of 0.2 mg/kg/day has been determined for oral exposure to boron. A daily boron intake of 1–13 mg has been reported to be typical for humans (World Health Organization, 1996). Therefore, a daily intake of 50 μg boron per day by humans via consumption of 200 g chicken breast meat should not result in toxic levels of boron.

The combined evidence indicates that boron can interact with the metabolism of some macro-minerals, such as Ca, P and Mg, and ALP, thereby modifying their concentrations in the serum of broiler chickens (Kurtoğlu et al., 2001, 2005; Bozkurt et al., 2012). However, here, the serum biochemical constituents were not affected by supplemental boron (P > 0.05). Likewise, dietary boron supplementation did not improve mineral retention in soft tissues and bone, except for the boron concentration. Our results are in accord with several authors who reported no benefits in the bone ash and Ca concentration of broilers in response to boron supplementation of 20 to 150 mg/kg (Fassani et al., 2004; Çınar et al., 2015). However, others have reported significant increases in tibia Ca concentration (Armstrong et al., 2000; Kurtoğlu et al., 2005; Bozkurt et al., 2012).

Birds receiving a diet with boron excreted less ash and Ca but had markedly increased Fe and Cu excretion compared with controls (Table 8). Boron plays a regulatory role in mineral metabolism, interacting with some macro- and micro-elements, but the mechanism has not yet been clearly established (Nielsen et al., 1987; Chapin et al., 1991; Hegsted et al., 1991). Limited scientific evidence confirms our findings that dietary boron supplementation decreased urinary Ca excretion in rats (Hegsted et al., 1991) and excreta ash and Ca in chickens (Bozkurt et al., 2012).

Here, phytase supplementation did not affect the growth performance parameters of the mineral contents of the serum, tibia, breast meat and liver (P > 0.05), but it did increase excreta ash, Ca, Fe and Cu. Phytase supplementation enhances phytic acid hydrolysis and increases the availability of minerals bound to phytic acid in broiler chickens (Sebastian et al., 1996; Viveros et al., 2002). However, the mineral composition of the excreta measured herein were not consistent with the results of earlier studies. In addition to the ingestion of an adequate amount of minerals via a balanced mineral premix, it is likely that the increased liberation of Ca, Fe and Cu, induced by phytase resulted in an oversupply of minerals, with the eventual excretion of unab sorbed portions through the excreta.

Several authors (Sebastian et al., 1996, 1997; Waldroup et al., 2000) have reported that phytase supplementation did not affect growth performance of broilers on standard diets but significantly increased the weight gain and feed efficiency, without affecting the toe ash content, in chickens fed diets with reduced Ca, P, protein, amino acids and energy densities. The basal diet used here was adequate in macro- and micro-minerals and could be the reason for the unresponsive ness to phytase in terms of growth performance and bioavailability of minerals. Therefore, it may be that phytase has a pronounced influence on mineral metabolism only when animals are partly deprived of P and Ca. In addition, micro-minerals (e.g., Fe, Cu, Zn and Mn) bound to phytic acid may interact with phytase.

Here, phytase supplementation increased serum ALT activity by 21% compared with controls (P < 0.01). A similar observation was reported by Brenes et al. (2003) that phytase linearly increased serum ALT activity by 40% in broiler chickens without sacrificing normal growth. In contrast, Viveros et al. (2002) showed that serum ALT activity was reduced by phytase supplementation in chickens. An increase in the activity of serum ALT is an indicator of hepatic toxicity and concurrent hepatic tissue damage. However, here, birds fed phytase exhibited a comparable performance and survival to those not treated with phytase, which indicates that such an increase in ALT activity is within biological limits. The specific reason for the increase in serum ALT is inconclusive, and no valid literature regarding any such mechanism was found.

In conclusion, the results presented herein do not support the conclusions that boron has growth-promoting effects in broiler chickens or that boron has the potential to regulate mineral metabolism, which in turn enhances tissue mineral accretion. The combination of boron and phytase caused no further improvements in bird performance and mineral composition in the soft tissues and bone. Additional studies, preferably using experimental diets deficient in minerals with the potential to interact with boron and phytase, are warranted to justify the nascent state of knowledge that these supplements have on the regulation of the metabolism of several macro- and micro-minerals affecting the productivity and bone mineralization of chickens.

References

AOAC. Official Methods of Analysis (15th ed.), Association of official analytical chemists, Washington, DC. 1990.

Armstrong TA, Spears JW, Crenshaw TD and Nielsen FH. Boron supplementation of a semi-purified diet for weanling pigs improved feed efficiency and bone strength characteristics and alters plasma lipid metabolites. Journal of Nutrition, 130: 2575–2581. 2000.

Armstrong TA and Spears JW. Effect of dietary boron on growth performance, calcium, and phosphorus metabolism, and bone mechanical properties in growing barrows. Journal of Animal Science, 79: 3120–3127. 2001.

Aviagen, T. (Ed.). Ross 308 Broiler Nutrition Specifications. Aviagen Group, Huntsville, Alabama, 2012.

Bozkurt M, Kâcıkölmac K, Çathi AU, Çınar M, Çabuk M and Bintaş E. Effect of boron supplementation to diets deficient in calcium and phosphorus on performance with some serum, bone and fecal characteristics of broiler chickens. Asian Australasian Journal of Animal Science, 25: 248–255. 2012.

Brenes A, Viveros A, Arij A, Centeno C, Pizarro M and Bravo C. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Animal Feed Science and Technology, 110: 201–219. 2003.

Chapin RE, Ku WW, Kenney MA and McCoy H. The effects of dietary boric acid on bone strength in rats. Biological Trace
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Element Research, 66: 395–399. 1998.
Çinar M, Küçükyılmaz K, Bozkurt M, Çatlı AU, Bintaş E, Akşit H, Konak R, Yamaner Ç and Seyrek K. Effects of dietary boron and phytase supplementation on growth performance and mineral profile of broiler chickens fed on diets adequate or deficient in calcium and phosphorus. British Poultry Science, 56: 576–589. 2015.
Devrian T and Volpe S. The physiological effects of dietary boron. Critical Reviews in Food Science and Nutrition, 2: 219–231. 2003.
Eliot MA and Edwards HM. Studies to determine whether an interaction exists among boron, calcium, and cholecalciferol on the skeletal development of broiler chickens. Poultry Science, 71: 677–690. 1992.
Fassani EJ, Bertechini AG, Brito JAG, Kato RK, Fialho ET and Eliot MA and Edwards HM. Studies to determine whether an interaction exists among boron, calcium, and cholecalciferol on the skeletal development of broiler chickens. Poultry Science, 71: 677–690. 1992.
Hunt CD and Nielsen FH. Interaction between boron and cholecalciferol in the chick. Pages 597–600 in Proc. Trace Element Metabolism in Man and Animals. J.M. Howell, J.M. Gawthorne and C.L. White, ed. Australian Academy of Science Press, Canberra, Australia. 1981.
Hunt CD. Biochemical effects of physiological amounts of dietary boron. The Journal of Trace Elements in Experimental Medicine, 9: 185–213. 1996.
Hunt CD, Herbel SL and Nielsen FH. Metabolic responses of postmenopausal women to supplemental dietary boron and aluminum during usual and low magnesium intake: boron, calcium and magnesium absorption and retention and blood mineral concentrations. The American Journal of Clinical Nutrition, 65: 803–813. 1997.
Hunt CD. One possible role of dietary boron in higher animals and humans, Biological Trace Element Research, 66: 205–225. 1998.
Kurtoğlu V, Kurtoğlu F and Coşkun B. Effects of boron supplementation of adequate and inadequate vitamin D3-containing diet on performance and serum biochemical characters of broiler chickens. Research in Veterinary Science, 71: 183–187. 2001.
Kurtoğlu F, Kurtoğlu V, Çelik I, Keçeci T and Nizamloğlu M. Effects of dietary boron supplementation on some biochemical parameters, peripheral blood lymphocyte, splenic plasma cell counts and bone characteristics of broiler chicks fed with adequate or inadequate vitamin D3 containing diet. British Poultry Science, 46: 87–96. 2005.
Küçükyılmaz K, Erkek R and Bozkurt M. The effects of boron supplementation of layer diets varying in calcium and phosphorus concentrations on performance, egg quality, bone strength and mineral constituents of serum, bone and faeces. British Poultry Science, 55: 804–816. 2014.
Laakso J, Martti K, Inkeri R, Jyrki V, Riitta Z, Markus F and Merja K. Atomic emission method for total boron in blood during neutron-capture therapy. Clinical Chemistry, 47: 1796–1803. 2001.
Litovitz TL, Klein-Schwartz, W, Oderda, GM and Schmitz, BF, Clinical manifestations of toxicity in a series of 784 boric acid ingestions. The American Journal of Emergency Medicine, 6: 209–213. 1988.
McCoy H, Montgomery C, Kenny MA, Irwin A, Williams L and Orrell R. Relation of boron to the compositional and mechanical properties of bone. Environmental Health Perspectives, 102: 1–10. 1994.
Moseman FF. Chemical deposition of B in animals and humans. Environmental Health Perspectives, 102 (Suppl 7): 5–11. 1994.
Nielsen FH, Hunt CD, Mullen LM and Hunt JR. Effect of dietary boron on mineral, estrogen, and testosterone metabolism in postmenopausal women. The FASEB Journal, 87: 394–397. 1993.
Nielsen FH. Studies on the relationship between boron and magnesium which possibly affects the formation and maintenance of bones. Magnesium and Trace Elements, 9: 61–69. 1990.
Nielsen FH and Shuler TR. Studies of the interaction between boron and calcium, and its modification by magnesium and potassium, in rats. Effects on growth, blood variables and bone mineral composition. Biological Trace Element Research, 35: 225–237. 1992.
Rossi AF, Miles RD and Damron BL. The effect of boron supplementation to broilers fed a practical corn-soybean meal diet. Poultry Science, 68 (Suppl.1): 202. (Abstr.) 1989.
Rossi AF, Miles RD, Damron BL and Flunker LK. Effects of dietary boron supplementation on broilers. Poultry Science, 72: 2124–2130. 1993.
SAS Institute. JMP. Version 8, User’s Guide. SAS Institute Inc. Cary, NC, USA. 2009.
Sebastian S, Touchburn SP, Chavez ER and Lague PC. The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper, and zinc in broiler chickens fed corn-soybean diets. Poultry Science, 75: 729–736. 1996.
Sebastian S, Touchburn SP, Chavez ER and Lague PC. Apparent digestibility of protein and amino acids in broiler chickens fed a corn–soybean diet supplemented with microbial phytase. Poultry Science, 76: 1760–1769. 1997.
Selle PH and Ravindran V. Microbial phytase in poultry nutrition. Animal Feed Science and Technology, 135: 1–41. 2007.
Viveros A, Brenes A, Arija I and Centeno C. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. Poultry Science, 81: 1172–1183. 2002.
Waldroup PW, Kersey JH, Saleh EA, Fritts CA, Yan F, Stilborn HL, Crum RC and Raboy V. Nonphytate phosphorus requirements and phosphate excretion of broiler chicks fed diets composed of normal high available phosphate corn with and without microbial phytase. Poultry Science, 79: 1451–1459. 2000.
WHO. Boron: In: TraceElements in Human Nutrition and Health. World Health Organization, Geneva 175–179 pp. 1996.
Wilson JH and Ruzzier L. Long term effects of boron on layer bone strength and production parameters. British Poultry Science, 39: 11–15. 1998.