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

Kamil Küçükyilmaz[1], Mehmet Bozkurt[2], Mustafa Çınar[2] and Ahmet E. Tüzün[3]

[1] Department of Animal Science, Faculty of Agriculture, Eskişehir Osmangazi University, Eskişehir, Turkey
[2] General Directorate of Research, Erbeyli Research Institute, Aydın, Turkey
[3] Adnan Menderes University, Koçarlı Vocational Scholl, Aydın, Turkey

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 (20 mg/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

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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).

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Correspondence: Kamil Küçükyılmaz, Department of Animal Science, Faculty of Agriculture, Eskişehir Osmangazi University, Kütahya yolu üzeri 26160 Odunpazar, Eskişehir, Turkey.
(E-mail: kamilkucukyilmaz@hotmail.com)

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-soy-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 m^2 floor space) provided with litter (pine wood shavings) to a depth of 5−6 cm. The room temperature was gradually decreased from 33℃ at chick arrival to 23℃ 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 hammermill 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

Table 1. **Ingredient composition of experimental basal diets on an as-fed basis; starter (d 1−21) and finisher (d 21−42)**

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[1]	18.1	14.7
Sodium chloride	2.5	2.6
Vit.−Min. premix[2]	2.5	2.5
L-Lysine HCL	1.0	0.0
DL-Methionine	2.0	2.2
L-Threonine	0.6	0.1
Anticoccidial premix[3]	1.0	1.0
Saw dust[4,5,6]	2.0	2.0
Calculated nutrient (%)		
ME (kcal/kg)	3061	3235
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

[1] Contains 18.2% P and 23.6% Ca.
[2] Provides per kg of diet: *trans*-retinol 12000 IU; cholecalciferol 1500 IU; α-tocopherol acetate 75 mg; Vitamin K_3 5 mg; Vitamin B_1 3 mg; Vitamin B_2 6 mg; Vitamin B_6 5 mg; Vitamin B_{12} 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.
[3] Provides 70 mg narasin per kg diet.
[4] Saw dust replaced for boron or phytase preparations.
[5] Boric acid of 0.109 g provides 20 mg boron per kg of diet.
[6] Phytase premix (Natuphos® 500) provides 500 FTU phytaseenzyme 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 (H_3BO_3) 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 (EC 3.1.3.8). Natuphos® 500 was purchased from BASF (BASF SE Nutrition Ingredients, Limburgerhof, Germany). The recommended dose for use in broiler chickens is 500 FTU. Natuphos® 500 is a preparation of 3-phytase produced by *Aspergillus niger* (CBS 114.94) with a minimum activity of 500 FTU/g. One unit phytase (FTU) is defined as the quantity of enzyme that liberates one micromole of inorganic phosphate per minute from sodium phytate at pH 5.5 and 37℃. Phytase activity analysis was not performed.

Broiler Performance Responses

Chickens were weighed on a pen basis on days 1, 21 and 42 to determine body weight (BW) through relevant experimental periods. Body weight gain (BWG) was determined as the difference between the initial and 21 d weights and the initial and 42 d of age weights. Feed intake (FI) within each replicate was calculated at d 21 and 42. The feed conversion ratio (FCR) was calculated as the ratio of FI to BWG (g feed/g gain). Mortality was recorded daily and was expressed as a percentage of the initial number of chicks. The FCR was adjusted for mortality and was calculated on a per pen basis. Any birds that died were weighed, and the FCR values were calculated by dividing the total FI by BWG of live plus dead birds.

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℃ after 1 hour of incubation at room temperature and were stored at −20℃ 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 Diagnostic End. LTD. (Istanbul, Turkey). The triiodothyronine (T_3) (Mybiosource, MBS269151, California, USA) and thyroxine (T_4) (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℃ 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℃ for 12 h, cooled in a desiccator, weighed, dry-ashed at 600℃ 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℃ 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 HNO_3 was added to each ash sample (i.e., 0.24 g) until it had completely dissolved. Then, 20 m*l* 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 m*l*. 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[1]		Control	Boron	Phytase	Boron + Phytase	SEM[2]	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[ab]	1.754[c]	1.764[bc]	1.800[a]	0.009	0.0129
	Mortality (%)	3.02	3.52	2.51	3.04	0.99	0.9129

[a−c] 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[1]	Control	Boron	Phytase	Boron + Phytase	SEM[2]	P-value
Serum (mg/L)	0.37[b]	0.84[a]	0.37[b]	0.86[a]	0.004	0.0001
Tibia (μg/g)	0.18[b]	0.36[a]	0.17[b]	0.33[a]	0.019	0.0001
Liver (μg/g)	0.21[b]	0.36[a]	0.17[b]	0.36[a]	0.004	0.0078
Breast muscle (μg/g)	0.16[b]	0.26[a]	0.14[b]	0.25[a]	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 (T_3), thyroxine (T_4), 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[1]	Control	Boron	Phytase	Boron + Phytase	SEM[2]	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)	8.02[b]	8.15[b]	9.71[a]	8.64[ab]	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**

	Breast muscle						Liver					
Diet[1]	Control	Boron	Phytase	Boron + Phytase	SEM[2]	P-value	Control	Boron	Phytase	Boron + Phytase	SEM[2]	P-value
Ash (%)	4.18	4.33	4.32	4.26	0.07	0.4108	4.67	4.61	4.43	4.43	0.12	0.3760
Ca (μg/g)	173	178	163	164	6.6	0.3488	0.36	0.38	0.33	0.31	0.02	0.1939
P (mg/g)	9.59	9.92	9.91	9.80	0.23	0.7572	11.7	11.8	11.8	11.7	0.26	0.9970
Mg (mg/g)	1.18	1.16	1.17	1.18	0.02	0.8995	0.99	1.06	1.03	0.96	0.04	0.3520
Fe (μg/g)	42.0	44.3	37.9	35.0	5.8	0.6901	0.53	0.48	0.49	0.44	0.03	0.4187
Cu (μg/g)	4.93	4.54	3.40	3.84	0.66	0.3703	21.4	21.9	20.8	18.8	1.62	0.5429
Zn (μg/g)	22.2	21.2	22.9	22.5	0.50	0.1327	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.

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**

	Tibia						Excreta					
Diet[1]	Control	Boron	Phytase	Boron + Phytase	SEM[2]	P-value	Control	Boron	Phytase	Boron + Phytase	SEM[1]	P-value
Ash (%)	37.4	37.5	36.8	36.6	0.48	0.4809	13.0^{bc}	12.5^{c}	13.8^{a}	13.4^{ab}	0.17	0.0001
Ca (%)	35.6	35.2	33.9	34.0	1.8	0.0719	2.76^{a}	2.49^{b}	2.96^{a}	2.96^{a}	0.086	0.0008
P (%)	13.5	13.4	13.0	13.1	0.88	0.7596	1.70	1.60	1.58	1.68	0.046	0.1860
Mg (%)	0.40	0.40	0.39	0.39	0.01	0.9245	0.29	0.29	0.29	0.29	0.007	0.8833
Fe (μg/g)	153	152	144	148	4.2	0.4501	628^{c}	724^{b}	806^{a}	804^{a}	18.3	0.0001
Cu (μg/g)	10.5	10.6	10.2	10.1	0.86	0.5980	44.4^{b}	49.5^{ab}	53.3^{a}	51.3^{a}	2.03	0.0216
Zn (μg/g)	159	158	157	159	10.1	0.9895	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.

liver, tibia and excreta are presented in Tables 5 and 6. Supplementation with boron and phytase, alone or in combination, did not influence the breast meat, liver and tibia ash content or the mineral composition of chickens following a 42-day feeding period ($P>0.05$). However, following a 3-d sample collection period, the ash content and the Ca, Fe and Cu concentrations in the excreta were significantly influenced by the supplemental regimens. The excreta ash content increased significantly in diets supplemented with phytase ($P<0.01$) but increased only slightly in boron + phytase diets and was slightly reduced by boron supplementation. Compared with other treatments, boron supplementation significantly decreased Ca concentration in the excreta ($P<0.01$). Fe concentrations in the excreta in the control were significantly lower ($P<0.01$) than for all other treatments. The chickens fed boron excreted less Fe compared with those receiving phytase and boron + phytase ($P<0.01$). A similar pattern was observed for the Cu concentration in excreta. Cu concentration in the excreta differed significantly between control and phytase and between control and boron + phytase treatments ($P<0.05$); the difference between the control and boron treatments was not significant ($P>0.05$).

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 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.

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 fulfil 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, tibia, breast muscle, liver and excreta were closely related to dietary supplementation. The findings related to the boron content of the serum, bone and bodily organs were in accordance with other reports (Rossi *et al*., 1993; Kurtoğlu *et al*., 2005; Küçükyılmaz *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 breast meat (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

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*., 1998; 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 phytaseresulted in an oversupply of minerals, with the eventual excretion of unabsorbed portions through the excreta.

Several authors (Sebestian *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 unresponsiveness 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.

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