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Effect of Boron Supplementation on Bone Mineralization and Antioxidant Status in Broiler Chicken

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ABSTRACT

Background: Functionality and requirement of Boron as a trace element in livestock feeding has not been well established. Limited research conducted worldwide suggests B is a trace element known to influence various physiological functions specifically the metabolism of minerals, hormones, immunity and antioxidant defense mechanism; thereby the performance of the birds. The whole grains widely used in poultry diets contain very little boron and currently, there is no definitive information regarding the boron requirement for any class of poultry and inclusion levels are far from standardized. Therefore, the present experiment has been conducted to study the influence of boron on bone mineralization and antioxidant status in broiler.

Methods: A total of 240 day-old broiler chicks (Vencob) of mixed sex (avg. BW 47.50±0.26 g) were distributed in a completely randomized design into five treatments each with four replicates of 12 birds (6 of each sex). The dietary treatments involved supplementation of boron at 0 (B-0), 25 (B-25), 50 (B-50), 75 (B-75) and 100 (B-100) mg/kg diet. The birds were offered starter (d 1 to 21) and finisher (d 22 to 42) diet in mash form. At d 42, Whole blood (2 ml) sample was collected for the estimation of total antioxidant status and reduced glutathione by FRAP and DTNB method, respectively. Two birds per replication were selected randomly; sacrificed and right femur bone was collected to measure the bone ash and mineral content.

Result: Boron supplementation enhanced the bone ash, calcium and phosphorus content but decreased the manganese and iron content in bone. Supplementation of Boron significantly enhanced (P<0.05) the total antioxidant capacity but lowered the plasma reduced glutathione level.

Key words: Boron, Broiler chicken, Bone mineralization, Lipid peroxidation, Total Antioxidant.

INTRODUCTION

Nutritional supplement and biological significance of Boron as a micronutrient in livestock feeding is not fully explored (NRC, 1994). However, the distinctive chemical properties of B, allow it to form complex with organic molecules containing hydroxyl group; thereby influence cellular activity by interacting with various metabolites (Park *et al.,* 2005). Limited research conducted worldwide suggests B as a trace element known to influence various physiological functions specifically the metabolism of minerals, hormones, immunity and antioxidant defense mechanism (Hunt, 1998; Devirian and Volpe, 2003; Bhasker *et al*., 2016). For poultry, 2 ppm of B was recommended by NRC (1984) but this recommendation has not been made in the latest feeding standards for poultry (NRC, 1994; ICAR, 2013). Dietary B supplementation has been reported to improve the performances of broiler (Pradhan *et al*., 2020; Bozkurt *et al*., 2012; Kucukyilmaz *et al*., 2017) in terms of body weight gain, feed intake and feed conversion ratio. Moreover, supplemental B improve bone calcium content in rat (Nielsen, 2004), laying hens (Mizrak *et al*., 2010) and broiler (Bozkurt *et al*., 2012). Studies also suggested the influence of boron on metabolism of Ca and P, improved their bioavailability thereby the skeletal development (Armstrong and Spears, 2001; Armstrong *et al*., 2000). Additionally, boron deficiency caused insufficient growth and abnormal bone development (Naghii, 1999) in poultry. Besides studies also confirmed the ameliorative effect of boron on oxidative stress (Zafar and Ali, 2013; Ince *et al*., 2010; Coban *et al*., 2015) by increasing the antioxidant activities. Albeit several studies conducted with broiler chickens (Eliot and Edwards, 1992; Rossi *et al*., 1993; Kurtoglu *et al*., 2001; Fassani *et al*., 2004; Bozkurt *et al*., 2012; Eren *et al*., 2012; Cinar *et al*., 2015) there is no current definitive information regarding the boron requirement for any class of poultry and inclusion levels are far from standardized and ranged between 5 to 400 mg/kg diet (Bozkurt and Kucukyilmaz *et al*., 2015)

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supplementation. Further, the whole grains widely used in poultry diets (WHO, 1998) contain very little boron unlike that of roughages (Bhasker *et al*., 2015). Therefore, the present experiment has been conducted to study the influence of boron on bone mineralization and antioxidant status in broiler.

MATERIALS AND METHODS

Birds and housing

The experiment was conducted at Navsari Agricultural University, Navsari, Gujarat during the month of March and April in the year 2020. A total of 240 one-day old commercial broiler chicks (Vencob) of mixed sex (mean BW 47.50±0.26 g) were used for this experiment. Upon arrival, the chicks were weighed and randomly allotted to floor pens, each representing a replication. Birds were vaccinated against infectious bursal disease virus (GUMBORO I$^+$, Haster Biosciences Limited, Mehsana, India) and Newcastle disease virus (LaSota Strain, Venkateshwara Hatcheries Pvt. Ltd, Pune, India) via drinking water at 10 and 14 d of age, respectively. Each replica was supplied with a floor space of 1.5 m^2 (1.5×1.0 m) along with the provision of hanging feeder and waterer. Birds were reared in pens provided with litter material (rice husk and saw dust) to a depth of 5-6 cm. The house was well-ventilated with adjustable windows and every effort was made to reproduce the commercial condition as much as possible. The room temperature was maintained at 33±1°C up to 7 d and gradually decreased to 26±1°C by 21 d. Thereafter, the birds were kept at room temperature up to 6 weeks of age.

Experimental design and diets

The experiment was performed 240 one-day old chicks distributed in completely randomize design with five treatments. Each treatment comprised of four replication with 12 birds (6 males and 6 females) per replicate. The basal diet was a corn-rice-soya based diet formulated to meet or exceed the nutrient requirement of broiler as per the ICAR (2013) recommendations. The birds were offered starter (d 1 to 21) and finisher (d 22 to 42) diet in mash form. The chicks received feed within 12 h of hatching. The ingredient and nutrient composition of basal diet are given in Table 1.

The five dietary treatment were comprised of the basal di*et al*one (B-0) or with additional boron supplemented at 25 (B-25), 50 (B-50), 75 (B-75) and 100 (B-100) mg/kg. Boric acid (Loba Chemie Pvt. Ltd, Mumbai, India) with 17.48% elemental boron was used as a source of boron. Accordingly, the dietary groups B-0, B-25, B-50, B-75 and B-100 were supplemented with 0, 0.143, 0.286, 0.429 and 0.572 g of boric acid per kg basal diet, respectively. Respective amount

Table 1: Ingredients and nutrient content of basal diet fed to broiler chicken (g/kg feed).

Ingredients	Starter	Finisher	Calculated nutrient content	Starter	Finisher
Maize	525.000	545.000	ME (Kcal/kg)	3100.0	3200.0
Soya DOC 45%	364.000	311.000	Crude protein (%)	22.0	20.0
Rice polish	36.100	56.700	Lysine (%)	1.20	1.05
Vegetable oil	35.250	46.300	Methionine (%)	0.50	0.45
Corn starch	3.150	4.700	Threonine (%)	0.8	0.8
Salt	2.000	2.000	Calcium (%)	1.0	1.0
Sodium bi-carbonate	1.200	1.200	Phosphorous (%)	0.45	0.45
Dicalcium phosphate	9.000	9.000	Fat (%)	6.5	8.0
Lime stone powder	13.500	13.500	Crude fiber (%)	4.9	4.8
Enzyme	0.300	0.300			
DL-Methionine	2.300	2.000			
L-Lysine HCL	1.400	1.500			
L-Threonine	0.300	0.300			
Vitamin premix[a]	2.000	2.000			
Trace mineral mixture[b]	1.000	1.000			
Toxin binder	1.000	1.000			
Choline chloride, 60%	0.500	0.500			
Acidifier[c]	1.000	1.000			
Liver tonic hepatocare	1.000	1.000			

[a]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.

[b]Contained (per kg) manganese 40 g; Iron 40 g; zinc 60 g; copper 5 g; cobalt, 0.2 g (all as sulfate salt); Iodine 0.5 g (as potassium iodide); selenium 0.15 g (as sodium selenite).

[c]Acidifier contains (per kg) ortho-phosphoric acid (400 g), formic acid (150 g), propionic acid (15 g) and calcium propionate (15 g) mixed with a carrier.

of boric acid for each treatment was mixed with the basal diet as a premix prior to feeding the birds.

Sample collection

Whole blood (2 ml) sample was collected in vials with anticoagulant, acid citrate dextrose (300 µl/2 ml blood) and centrifuged at 2000 rpm for 15 min at 4°C with separation of plasma and kept at -40°C and used for the estimation of total antioxidant capacity (TCA), reduced Glutathione and lipid peroxidation. Two birds per replication were selected randomly and sacrificed as per the standard protocol. Thereafter the right femur bone was collected to measure the bone ash and mineral content. The femur bone was excised, all flesh and proximal cartilages were removed. The bone samples were sealed individually in plastic bags and stored at -20°C until the analysis, which was performed within one month after sample collection.

Mineral estimation

The bone samples were ashed in a muffle furnace at 650°C and a mineral extract was prepared from the ash samples for mineral estimation. Mineral content in samples were determined using the Microwave Plasma Atomic Emission Spectrometer (MP-AES) (MP-AES, Agilent, Santa Clara, California, USA) with operating condition (Table 2) suggested by the manufacturer.

Antioxidant status

Total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1996). Briefly, 100 µl of plasma sample was mixed with 3 ml of working FRAP reagent (Acetate buffer (300 mM pH 3.6), 2, 4, 6-tripyridyl-s- triazine (10 mM in 40 mM HCl) and FeCl3. 6H2O (20 mM) mixed in the ratio of 10:1:1) and absorbance (593 nm) was measured at 0 minute after vortexing. Thereafter, samples were placed at 37°C in water bath and absorption is again measured after 4 minutes. Ascorbic acid standards (100µM-1000µM) were processed in the same way.

$$\text{FRAP value of sample (µM)} = \frac{\text{Change in absorbance of sample from 0 to 4 minute}}{\text{Change in absorbance of standard from 0 to 4 minutes}} * \text{FRAP value of Standard (1000µM)}$$

Note: FRAP value of ascorbic acid is 2.

The concentration of reduced glutathione (GSH) in plasma was estimated by 5, 5-dithiobis-(2-nitro- benzoic acid; DTNB) method as per the procedure of Prins and Loos (1969). The lipid peroxides level in the plasma (malonlaldehyde (MDA) was determined by the method of Placer *et al.,* (1966). The concentration of MDA in nmol/ml plasma was calculated using the extinction coefficient of $1.56 * 10^{-5}$ L $mmol^{-1}$ cm^{-1} (Utley *et al.,* 1967) and expressed in nmol of MDA per ml: LPO (µmol MDA formed/ml) = $[(OD_T / \in) * (TV / VT) * df \times (1/ ml)] \times 10^6$; where, OD_T: Absorbance of test, $\in$: Molar extinction coefficient (1.56×10^5)/m/cm, TV: Total volume of reagent with sample taken, VT: Volume of sample taken and df: dilution factor.

Statistical analysis

Data generated in the study were analyzed using the SPSS v. 20.0 (SPSS Inc., Chicago, USA) by one-way ANOVA and comparison of means was tested using Duncan's multiple range tests (Duncan, 1955). The effects were considered to be significant at $P<0.05$.

RESULTS AND DISCUSSION

Supplementation of boron significantly increase the ash, Ca and P content of femur bone with a significant reduction in manganese and iron content (Table 3). Supplementation of boron has a significant ($P<0.05$) effect on the retention percentage of calcium, phosphorus, manganese, iron and boron (Pradhan *et al*., 2020). He reported an increased ($P<0.05$) retention of calcium and phosphorus and reduced ($P<0.05$) retention of manganese and iron in broilers fed a diet with supplemental boron at 25 to 100 mg/kg diet. Several researchers suggested the biological role of boron in the metabolism of various minerals in human and animals by interacting with Ca, P, Mg, Mn, Cu and iron (Kurtoglu *et al*., 2001, 2005; Bozkurt *et al*., 2012). It is well known that Ca and P are essential elements for normal skeletal growth and bone development. In addition, boron seems to have 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*., 1988; Brown *et al*., 1989; Hegsted *et al*., 1991). The particular mechanism through which boron influences bone development is described as enhancing the macro mineral content of normal bone (Hunt *et al*., 1994). Since the enhance concentration of these minerals were not measured in other soft tissues in this study, the increased Ca and P level in bones was assumed as an indicative for the regulatory role of supplemental boron on bone mineralization. Present finding is in agreement with those of Armstrong *et al*. (2000), Kurtoglu *et al*. (2005) and Bozkurt *et al*. (2012) who reported significant increase in the tibia bone Ca concentration of broilers in response to dietary boron supplementation. In another study, supplementation of boron at 200 mg/L through drinking water significantly increased the bone ash content (Cheng *et al.,* 2011). However, our results contradicts 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; Cinar *et al*., 2015). The contradictory results of these studies are difficult to evaluate because of the different protocols used, including differences in breed, their level of performance, differences in the composition and nutritive value of the diets, different source and form of boron supplementation, including inherent boron concentrations in the basal diet. Birds receiving a diet with supplemental boron excreted more iron (Kucukyilmaz *et al*., 2017; Pradhan *et al*., 2020) and manganese (Pradhan *et al*., 2020). The decreased femur iron concentration may be the reflection of its higher excretion. The negative interaction of

boron and manganese was also reported by Bhasker *et al.* (2016) who observed a lower serum manganese concentration in rat fed a diet supplemented with graded levels of boron at 5-40 mg/kg diet. The increased boron concentration in femur bone was related to the dietary supplementation and was in agreement with other findings (Rossi *et al*., 1993; Kurtoglu *et al*., 2005; Kucukyilmaz *et al*., 2014) who suggested an elevated boron concentration in bone of poultry birds fed a boron supplemented diet. However, increase in supplemental boron from 25 to 100 mg/kg diet increased the femur concentration up to 0.5 to 2.5 ppm suggesting that the boron is under homeostatic control (Vaziri *et al.,* 2001).

Data pertaining to the antioxidant status of plasma in broilers fed a boron supplemented diet was given in Table 4. Supplementing graded level of boron up to 75 mg/kg diet significantly increased (P<0.01) the TAC (FRAP value) as compared to control. Similar to the present findings, boron supplementation has resulted in significant (P<0.05) increase in the erythrocytic SOD activity and total antioxidant activities (Turkez *et al*., 2012, 2013; Bhasker *et al.,* 2016). Other studies have also indicated the role of dietary boron in ameliorating the toxicity induced by carbon tetrachloride (Ince *et al.,* 2010) and malathion (Coban *et al.,*2015), reducing the severity of hepatic cell carcinoma in rats by enhancing the SOD activity in liver and improving the antioxidant defense mechanism under oxidative stress condition. Supplementation of boric acid at higher dose (100 mg/kg) is toxic to the cell (Fort *et al*., 2000) and associated with decreased TAC. In agreement to our assumption, Hu *et al*. (2014) suggested that 40 mg/L of boron through drinking water increased the antioxidant capacity and that

Table 2: Operating condition for MP-AES.

Element*	Emission wave length (nm)	Viewing position	Nebulizer gasflow rate (L/min)	MDL (µg g^{-1})	LOQ (µg g^{-1})
P (I)	214.915	-10	0.65	1.800	5.900
Ca (II)	396.847	0	0.80	0.010	0.040
Mg (II)	279.553	10	0.60	0.002	0.008
B (I)	249.772	0	0.55	0.009	0.030
Cu (I)	324.754	-10	0.85	0.005	0.020
Fe (II)	259.940	0	0.60	0.020	0.050
Mn (II)	257.610	0	0.65	0.020	0.060
Zn (I)	213.857	0	0.65	0.002	0.006

*I. Atomic line; II. Ionic line; MDL-Method detection limits; LOQ-Limit of quantification; P-Phosphorus; Ca-Calcium; Mg-Mangnesium; B-Boron; Cu-Copper; Fe-Iron; Mn-Manganese; Zn-Zinc.

Table 3: Effects of graded levels of boron supplementation on ash and mineral content of femur bone in broiler chicken.

Parameters	Treatments*					SEM	P value
	B-0	B-25	B-50	B-75	B-100		
Bone ash %	46.24^{b}±0.22	47.62^{a}±0.15	47.66^{a}±0.11	47.41^{a}±0.15	47.23^{a}±0.34	0.211	0.001
Calcium % ash	36.58^{b}±0.53	38.42^{a}±0.36	37.61^{ab}±0.19	37.60^{ab}±0.38	38.36^{a}±0.35	0.378	0.023
Calcium % bone	16.91^{b}±0.32	18.29^{a}±0.12	17.93^{a}±0.12	17.83^{a}±0.24	18.12^{a}±0.22	0.216	0.004
Phosphorus % ash	13.23^{c}±0.08	15.10^{a}±0.16	14.17^{b}±0.23	13.92^{b}±0.24	14.40^{b}±0.30	0.217	0.001
Phosphorus % bone	6.12^{c}±0.08	7.19^{a}±0.09	6.75^{b}±0.11	6.60^{b}±0.13	6.80^{b}±0.17	0.114	0.001
Magnesium % ash	0.44±0.02	0.43±0.01	0.42±0.02	0.42±0.02	0.44±0.01	0.015	0.664
Magnesium % bone	0.20±0.01	0.21±0.01	0.20±0.01	0.20±0.01	0.21±0.00	0.007	0.766
Manganese (µg/g) ash	36.13^{a}±0.70	30.33^{b}±1.31	28.11^{bc}±0.83	25.84^{cd}±0.64	22.86^{d}±1.41	1.028	0.001
Manganese (µg/g) bone	16.71^{a}±0.35	14.44^{b}±0.58	13.39^{bc}±0.39	12.25^{cd}±0.33	10.81^{d}±0.72	0.498	0.001
Copper (µg/g) ash	21.36±0.54	21.92±0.77	21.64±0.80	21.66±0.67	21.90±1.00	0.770	0.985
Copper (µg/g) bone	9.87±0.23	10.44±0.38	10.32±0.40	10.27±0.33	10.34±0.46	0.367	0.839
Zinc (µg/g) ash	151.78±5.81	149.96±5.31	147.68±3.34	155.85±6.40	153.89±2.91	4.949	0.791
Zinc (µg/g) bone	70.16±2.54	71.39±2.32	70.38±1.57	73.90±3.11	72.66±1.98	2.234	0.737
Iron (µg/g) ash	182.67^{a}±3.36	152.58^{b}±4.75	151.75^{b}±3.51	145.37^{b}±3.47	142.84^{b}±2.31	3.566	0.001
Iron (µg/g) bone	84.46^{a}±1.62	72.65^{b}±2.19	72.31^{b}±1.57	68.93^{b}±1.85	67.48^{b}±1.50	1.763	0.001
Boron (µg/g) ash	0.38^{d}±0.03	1.02^{c}±0.05	1.63^{c}±0.08	3.36^{b}±0.27	5.03^{a}±0.46	0.243	0.001
Boron (µg/g) bone	0.18^{d}±0.01	0.49^{c}±0.02	0.78^{c}±0.04	1.59^{b}±0.12	2.38^{a}±0.22	0.116	0.001

*Basal diet (B-0); Basal diet supplemented with boron @ 25 mg/kg diet (B-25), 50 mg/kg diet (B-50), 75 mg/kg diet (B-75), 100 mg/kg diet (B-100).

^{abcd}Mean with different superscript in a row differ significantly; SEM-Standard error of mean.

Table 4: Effects of graded levels of boron supplementation on plasma antioxidant status in broiler chicken.

Parameters	Treatments*					SEM	P value
	B-0	B-25	B-50	B-75	B-100		
TAC mM/ml	0.92^{d}±0.01	1.78^{a}±0.07	1.48^{b}±0.01	1.15^{c}±0.01	0.88^{d}±0.01	0.034	0.001
GSH (µmol/ml)	0.76^{a}±0.003	0.69^{c}±0.004	0.73^{b}±0.005	0.73^{b}±0.005	0.72^{b}±0.004	0.005	0.001
MDA (nmol/ml)	1.20±0.004	1.21±0.003	1.20±0.01	1.21±0.004	1.20±0.005	0.005	0.239

*Basal diet (B-0); Basal diet supplemented with boron @ 25 mg/kg diet (B-25), 50 mg/kg diet (B-50), 75 mg/kg diet (B-75), 100 mg/kg diet (B-100).

^{abcd}Mean with different superscript in a row differ significantly; SEM-Standard error of mean.

of 80 mg/L decreased the antioxidant capacity of spleens in rat. In the present study, it was found that addition of boron to the diet did not change plasma MDA levels but significantly lowered ($P<0.05$) plasma reduced glutathione (GSH) levels (Table 4). This shows that the GSH is effective in preventing lipid peroxidation caused by boron supplementation (Sizmaz and Yildiz, 2014). Earlier studies also suggested that boron supplementation enhanced antioxidant defense mechanisms through decreasing lipid peroxidation (Ince *et al*., 2014).

CONCLUSION

The results of the present experiment showed that supplementation of boron at 25 mg/kg basal diet in broiler is beneficial in term of bone mineralization and total antioxidant status during six weeks of rearing.

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