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Calcium and boron alone or in combination influence performances and mineral metabolism in broilers

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ABSTRACT

An experiment was conducted to study the effect of dietary boron supplementation on performance and mineral metabolism in broilers fed a diet with optimal or sub-optimal levels of calcium. A total of 216 one-day old broiler chicks (Vencob) of mixed sex (mean BW 43.79±0.83 g) were distributed in completely randomized design into six treatments each with three replicates of 12 birds (6 of each sex). The six dietary treatment groups comprised of B_0C_0 (basal diet with normal calcium (10 g Ca/kg)), B_0C_1 (Basal diet of 20% calcium deficiency (8 g Ca/kg)), B_0C_2 (Basal diet of 40% calcium deficiency (6 g Ca/kg)), B_1C_0 (Basal diet with normal calcium + 25 mg/kg boron), B_1C_1 (Basal diet of 20% calcium deficiency + 25 mg/kg boron) and B_1C_2 (Basal diet of 40% calcium deficiency + 25 mg/kg boron). The performance indices like body weight (BW), weekly body weight gain, feed intake (FI) and feed conversion ratio (FCR) were recorded till 42 d of age. A metabolism trial was conducted at the end to determine the balance of minerals. Lower than the recommended level of calcium alone or with supplemental boron significantly increased the BW, feed intake, and FCR in commercial broilers at six weeks of age. Low dietary calcium significantly increased the retention of calcium, phosphorous, magnesium, iron and manganese. Dietary boron supplementation significantly increased the excreta concentration of iron and manganese. However, the absolute and relative retention of manganese and iron were unaffected when boron was supplemented into a low calcium diet; indicating the negative effect of boron on manganese, and iron metabolism can be compensated by low dietary Ca concentration.

Keywords: Boron, Broiler chicken, Calcium deficient, Performances, Mineral balance

Bioactive role of boron (B) influencing the metabolism of various minerals, vitamins and hormones has been studied (Devirian and Volpe 2003) but the essentiality of B as a trace element in animal production is still awaiting (NRC 1994). Dietary B intake has been reported to improve the skeletal calcium deposit in rats (Neilsen 2004) and poultry birds (Mizrak *et al.* 2010, Cufadar *et al*. 2011, Bozkurt *et al*. 2012) as well as improved the bone breaking strength in rats (Naghii *et al*. 2006), rabbits (Hakki *et al.*2013) and poultry birds (Fassani *et al.* 2004, Mizrak *et al.* 2010, Cufadar *et al.* 2011). One of our earlier study revealed that supplementation of B at 25 mg/kg diet improved gain in body weight, feed intake and reduced FCR in broilers (Pradhan *et al.* 2020). B plays a role in either hydroxylating or extending the half-life of vitamin D_3, which in turn would most likely enhances the metabolism of calcium and phosphorus and their content of normal bone (Hunt *et al.* 1994) and the interaction among boron, calcium and phosphorous on the skeletal development has been reported in gilts (Armstrong *et al.* 2000, Armstrong and Spears 2001) and suggested that supplementation with boron could improve the bioavailability of calcium and phosphorous. Further, boron deficiency caused insufficient growth and abnormal bone development in animals (Naghii 1999). Conversely, excess calcium intake has been shown to reduce growth, feed efficiency in poultry and necessitates higher than the normal levels of the other required minerals in the diet (Shafey 1993). Recommended or excess levels of Ca (Balla *et al.*1984, Nelson and Kirby 1987) and or P (Qian *et al.*1994) in diet are known to reduce the utilization of phosphorous in the chicken gut. A wider Ca: P ratio is also known to disrupt Mn metabolism causing a high incidence and severity of leg problems and associated bone disorders in broiler chicken (Smith and Kabaija 1985). It has also been reported that dietary calcium level is a major factor found to inhibit the absorption of intestinal iron (Cook *et al.* 1991, Hallberg *et al.*1991, 1992; Deehr *et al.*1990) and a dose-response effect of Ca on iron absorption was reported in human (Hallberg *et al.* 1991). In view of these facts and based on our earlier report (Pradhan *et al.* 2020), this experiment was conducted to study the effect of dietary B supplementation on performance and mineral metabolism in broilers fed a diet with optimal or sub-optimal levels of calcium.

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MATERIALS AND METHODS

Housing of birds: A total of 216 one-day old broiler chicks (Vencob) of mixed sex (mean BW 43.79±0.83 g) were used for this experiment. At arrival, the chicks were weighed and randomly allotted to 18 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 conducted in completely randomize design with six treatments, each comprised of three replications. The basal diet was a corn-rice-soya based diet formulated to meet or exceed the nutrient requirement of broiler (NRC 1994) except calcium. The dietary Ca concentration was varied by adjusting the ingredient composition of maize, vegetable oil, corn starch and lime stone powder (Table 1). The birds were offered starter (1 to 21 days) and finisher (22 to 42 days) diet in mash form. The six dietary treatment groups were comprised of B_0C_0 (basal diet with normal calcium (10 g Ca/kg)), B_0C_1 (Basal diet of 20% calcium deficiency (8 g Ca/kg)), B_0C_2 (Basal diet of 40% calcium deficiency (6 g Ca/kg)), B_1C_0 (Basal diet with normal calcium + 25 mg/kg boron), B_1C_1 (Basal diet of 20% calcium deficiency + 25 mg/kg boron) and B_1C_2 (Basal diet of 40% calcium deficiency + 25 mg/kg boron). The formulated diets were analyzed for their nutrient composition (Table 2). Boric acid (Loba Chemie Pvt. Ltd, Mumbai, India) with 17.48 % of elemental boron was used as a source of boron. The calculated amount of boric acid (0.143 g/Kg diet) was mixed with the experimental diets as a premix prior to feeding the birds.

Broiler performances: Chickens were weighed replication wise at weekly intervals to determine the live body weight (BW). Body weight gain (BWG) for different weeks was determined as the difference between the initial and final body weight. Feed intake (FI) was calculated on weekly basis by subtracting the leftover feed at the end of the week from the cumulative feed offered for the whole week. The feed conversion ratio (FCR) was calculated as the ratio of FI to BWG (g feed intake/g gain in body weight). Mortality was recorded daily and dead birds were weighed, and the FCR values was calculated by dividing the total FI by BWG of live plus dead birds.

Metabolism trial: A metabolism trial was conducted during 36 to 40 days of the experimental period. Four birds

Table 1. Ingredients composition of the diets fed to broiler chicken (g/kg)

Ingredient	Starter			Finisher		
	Control	0.8% Ca	0.6% Ca	Control	0.8% Ca	0.6% Ca
Maize	525	528	528	545	549	549
Soya DOC 45%	364	364	364	311	311	311
Rice polish	36.10	36.10	36.10	56.70	56.70	56.00
Vegetable oil	35.25	30.00	26.25	46.30	41.50	37
Corn starch	3.15	9.65	19.25	4.70	9.75	20
Salt	2.00	2.00	2.00	2.00	2.00	2
Sodium bi-carbonate	1.20	1.20	1.20	1.20	1.200	1.2
Dicalcium phosphate	9.00	9.00	9.00	9.00	9.00	9
LSP-Powder	13.50	9.25	3.40	13.50	9.25	3.50
Enzyme Nutrikem XL Pro	0.30	0.30	0.30	0.30	0.30	0.30
DL-Methionine	2.30	2.30	2.30	2.00	2.00	2.00
L-Lysine HCL	1.40	1.40	1.40	1.50	1.50	1.50
L-Threonine	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin premix	2.00	2.00	2.00	2.00	2.00	2.00
Trace mineral mixture	1.00	1.00	1.00	1.00	1.00	1.00
Toxin binder	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride, 60%	0.50	0.50	0.50	0.50	0.50	0.50
Acidifier	1.00	1.00	1.00	1.00	1.00	1.00
Liver tonic hepatocare	1.00	1.00	1.00	1.00	1.00	1.00

[a]Provides per kg of diet: trans-retinol 12000 IU; cholecalciferol 1500 IU; α-tocopherol acetate 75 mg; Vitamin K_3 5 mg; Vitamin B1 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.

Table 2. Chemical composition of experimental feeds

	Starter			Finisher		
Analyzed nutrients (%)	Control	0.8% Ca	0.6% Ca	Control	0.8% Ca	0.6% Ca
Dry matter	90.22	89.34	89.4	90.3	90.11	90.18
Organic matter	93.75	93.92	94.11	94.23	94.41	94.57
Crude protein	21.86	21.94	22.03	19.94	19.98	20.06
Ether extract	5.87	5.40	5.08	7.89	7.54	7.21
Crude fibre	4.51	4.51	4.513	4.37	4.41	4.46
Total ash	6.25	6.08	5.89	5.77	5.59	5.43
Nitrogen free extract	61.51	62.06	62.48	62.03	62.48	62.84
Mineral composition						
Ca (%)	0.98	0.81	0.62	0.97	0.79	0.61
Available P (%)	0.46	0.45	0.461	0.42	0.43	0.43
Mg (%)	0.17	0.17	0.16	0.18	0.17	0.16
Mn (mg/kg)	50.56	53.12	56.26	52.87	54.23	51.61
Cu (mg/kg)	12.10	13.26	15.14	11.26	11.64	12.57
Zn (mg/kg)	82.59	78.59	84.65	80.43	82.21	80.59
Fe (mg/kg)	205.14	199.54	207.56	201.58	207.26	205.84
Boron (mg/kg)	2.02	1.97	1.68	1.96	1.98	2.03

per replica close to the mean body weight were selected and transferred to the metabolic cages, and kept for 5 days with 2 days adaptation and 3 days collection period. During the metabolism trial, the quantitative measurement of the feed offered and that of the residues left were recorded replica wise. The excreta were removed at every 2 h interval and put in self-zipped polyethylene sachets and the total amount of excreta obtained in a 24 hours period was weighed, manually mixed and a subsample ($1/20^{th}$) was kept daily in a hot air oven at 80°C for 18 h to determine the dry matter and total ash (AOAC 2000). Excretion and apparent absorption of calcium, phosphorus, magnesium, copper, iron, manganese, zinc and boron during the metabolism trial were determined. Approximately 2 g of oven dried feed and excreta sample was ignited in quartz crucible at 550°C for 4 h. The cooled sample was treated with 3N nitric acid and boiled for 10 min under cover. The treated sample was filtered into a 50 mL volumetric flask and diluted up to the mark with deionized water (obtained from a Milipore Water Purification System, Elix 3, Mosheim, France). The concentration of said minerals in feed and excreta were determined using the Microwave Plasma Atomic Emission Spectrometer (MP-AES) (MP-AES, Agilent, Santa Clara, California, USA) with operating conditions as suggested by the manufacturer. Apparent retention coefficient was calculated as

$$\frac{[\text{Element} \times \text{feed intake} - \text{trace element} \times \text{excreta}]}{\text{Element} \times \text{feed intake}} \times 100$$

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

Lower than the recommended level of calcium significantly increased ($P<0.05$) the BW, feed intake, and FCR in commercial broilers at six weeks of age (Table 3). The effects of high calcium can be largely explained by its effects on intestinal pH and reducing the soluble fraction of minerals, and the proportion of minerals in small complexes leads to their reduced bioavailability (Shafey 1993). The growth depression of chickens fed on diets high in calcium may be partly caused by the decreased availability of other minerals needed for growth.

Tamim and Angel (2003) conducted an experiment with 1, 4 and 9 g of calcium/ kg diet and reported the regulatory effect of high dietary calcium on intestinal pH affecting the digestibility of nutrients in broilers. Similar findings were also reported by Tamim *et al.* (2004) who experimented with Ca levels equivalent to 0, 0.1, 0.2, 0.4, 0.7, or 0.9% of the diet. It has been reported that high dietary calcium regulates the bird's appetite and feed intake by regulating the blood ionic calcium level (Lobaugh *et al.*1981). Rama Rao *et al*. (2006) reported that increasing dietary calcium concentration from 6 to 9 g/kg between 22 to 42 days affect the growth in broilers. In another study, Rama Rao *et al*. (2003) predicted the requirements of calcium for maximum weight gain in broiler chicken was 7.56 g/kg diet. The results of our study on performance indices agree with Letourneau-Montminy *et al.* (2008) which suggested an enhanced feed intake and growth rate in broilers fed a diet with 5.3 g Ca/kg as compared to 8.3 g Ca/kg diet. Similar results were reported by Lim *et al*. (2003) in layers fed a diet with two levels (3% and 4%) calcium. Wilkinson *et al*. (2014) also reported a decreasing trend for BW gain in broilers fed a diet with 5 and 10 g Ca/kg diet. The negative effects of excess dietary calcium on growth and feed efficiency (Shafey 1993) were also reported in growing chickens.

Further, supplementation of boron at 25 mg/kg improved the performances of commercial broilers irrespective

Table 3. Effects of boron supplementation on performances of broiler chickens fed with calcium deficient diets

Parameter	Treatment							
	B_0C_0	B_0C_1	B_0C_2	B_1C_0	B_1C_1	B_1C_2	SEM	P value
Body weight (g)	1902[d]	1964[bc]	1953[c]	1959[bc]	2008[a]	1992[ab]	10.68	0.001
Gain in body weight (g)	1858[d]	1920[bc]	1909[c]	1915[bc]	1964[a]	1948[ab]	8.69	0.001
Feed intake (g)	3415[c]	3504[ab]	3488[b]	3485[b]	3560[a]	3537[ab]	17.83	0.002
FCR	1.838[a]	1.825[bc]	1.827[b]	1.820b[cd]	1.812[d]	1.816[cd]	0.003	0.001

[abcd]Mean with different superscript in a row differ significantly.

of dietary calcium level (Table 3). Supplemental boron promote intestinal mucosal immunity, intestinal cell proliferation and inhibit apoptosis (Sun *et al.* 2016); an indication of better gut health leads to better digestion and absorption of nutrients (Van der Aar *et al.* 2017) and could be the reason behind increased feed intake in the present study. Supplementation of boron up to 40 (Rossi *et al.*1993) and 120 ppm (Fassani *et al.*2004) into the broiler basal diet improved the BW at 21 and 42 days of age, respectively. Bozkurt *et al*. (2012) also reported that up to 60 ppm boron supplementation tend to increase feed intake in broiler chicken during 42 days of rearing. In the present study, significantly better performances of broilers supplemented with boron into calcium deficient diets over a calcium adequate diet might be explained by the synergistic and/or additive effect of boron and dietary calcium concentration.

Calcium and phosphorous retention were significantly higher (P<0.05) with decreased dietary calcium concentration. Dietary level of calcium influenced its retention and excreta concentration was reported in several studies (Rama Rao *et al.* 2006, Dawson-Hughes *et al.* 1988). Wider Ca: P ratio has been reported to negatively influence the utilization of both Ca and P and a positive impact on retention of either could be achieved through narrower ratios (Mohammed *et al.* 1991, Qian *et al.* 1997, Tamim *et al.* 2004, Santos *et al.* 2008). The inter-relationship that exists between dietary Ca and P concentrations in poultry nutrition and metabolism has been reported (Suttle 2010) and the negative effects of high dietary Ca on P absorption were well documented (Young *et al.* 1966, Kaup *et al.* 1990, Liu *et al.* 2000). In the present study, the Ca: non phytate P ratio of 1.3:1 and 1.7:1 imparted a higher P absorption than the recommended level of 2.2:1 (NRC 1994). Boron supplementation enhanced the retention of calcium and phosphorous with subsequent reduction in their excreta concentration. It has been reported that boron increased the serum vitamin D_3 level by suppressing the activity of microsomal enzyme 24-hydroxylase, primarily responsible for vitamin D_3 catabolism (Hunt 1994, Miljkovic *et al.* 2009). Bozkurt *et al*. (2012) reported a decrease in fecal excretion of calcium and phosphorus in broiler chicks fed a Ca-P deficient diet supplemented with 30 mg/kg boron. Rama Rao *et al*. (2007) reported that supplementation of vitamin D_3 significantly decreased (P<0.01) the fecal calcium excretion in broiler fed a calcium deficient diet. In the present study, we did not measure the serum vitamin D level but assumed that the beneficial effect of boron on calcium absorption may be due to its regulatory effect on vitamin D. Supplementation of boron at 25 mg/kg diet significantly increased (P<0.05) the retention of P than the non-supplemented Ca adequate diet indicates that boron positively interacted with absorption of P which might be due to up-regulatory impact of boron on function of vitamin D (Miljkovic *et al.* 2004).

Low dietary calcium increased (P<0.05) the retention of magnesium, iron and manganese. Supplementation of boron significantly increased the excreta concentration of iron and manganese. However, the antagonistic effect of boron on iron absorption could be restored by low dietary Ca concentration. Manganese retention was significantly increased (P<0.01) at 8 g of Ca /kg diet but not at 6 g of Ca /kg diet. Supplementation of boron decreased the absorption of Mn. However, the absolute and relative retention of Mn was unaffected when boron was supplemented into a low calcium diet (Table 4). Adverse effect of high dietary calcium on the metabolism of magnesium was confirmed by several researchers (Nicar and Pak 1982, Whiting and Wood 1997, Kronqvist *et al.* 2011). It has also been proposed that a dietary calcium: magnesium molar ratio greater than 3.5 may pose a risk for inducing magnesium deficiency (Seelig 1994). Dietary calcium concentration has been found to inhibit the absorption of both heme and non-heme iron (Cook *et al.* 1991, Hallberg *et al.* 1991, 1992; Deehr *et al.* 1990) and there is a dose-response effect of Ca on iron absorption in which adding as little as 300 mg calcium can exert maximal inhibition of iron absorption (Hallberg *et al.* 1991). Lim *et al*. (2003) reported a decreased retention of iron in layer fed a diet with 4% calcium. Similarly, Rama Rao *et al*. (2006) reported an increased fecal excretion of iron by increasing the dietary calcium concentration from 6 to 9g/kg in broilers. In agreement to the above studies, significantly increased (P<0.01) retention and decreased (P<0.01) excreta concentration of iron was observed with reduced level of dietary Ca concentration (10g/kg to 6g/kg diet) observed in this experiment. Supplementation of boron decreased the absorption of iron in the present study agrees with Kucukyilmaz *et al.* (2017), who reported that supplementing 20 mg/kg boron in the diet increased the excreta iron concentration. However, the absolute and relative retention of iron in the boron supplemented Ca deficient diets groups were comparable with normal diet without boron supplementation indicating the antagonistic effect of boron on iron might be restored by low dietary Ca concentration.

Table 4. Effects of boron supplementation on mineral composition of excreta in broiler chickens fed with calcium deficient diets

Mineral	Treatment							
	B_0C_0	B_0C_1	B_0C_2	B_1C_0	B_1C_1	B_1C_2	SEM	P value
Calcium								
% Retention	36.05^{c}±0.95	40.82^{b}±1015	42.53^{ab}±0.77	43.00^{ab}±0.68	45.10^{a}±1.92	45.12^{a}±0.69	1.11	0.001
% in excreta	2.43^{a}±0.06	1.91^{c}±0.02	1.40^{d}±0.01	2.18^{b}±0.02	1.82^{c}±0.08	1.36^{d}±0.03	0.04	0.001
Phosphorus								
% Retention	27.77^{c}±1.37	33.65^{b}±1.66	35.18^{ab}±1.17	35.59^{ab}±0.92	38.48^{a}±2.12	38.18^{ab}±0.91	1.42	0.002
% in excreta	1.19^{a}±0.01	1.03^{b}±0.02	1.04^{b}±0.03	1.06^{b}±0.02	1.09^{b}±0.01	1.09^{b}±0.01	0.02	0.001
Magnesium								
% Retention	53.99^{c}±2.56	58.29^{ab}±0.61	60.52^{a}±0.86	54.47^{bc}±0.59	59.28^{a}±1.21	58.63^{ab}±0.87	1.301	0.019
% in excreta	0.32^{a}±0.02	0.29^{ab}±0.003	0.26^{b}±0.01	0.32^{a}±0.003	0.29^{ab}±0.02	0.28^{b}±0.01	0.010	0.008
Manganese								
% Retention	38.59^{ab}±4.01	41.54^{a}±2.10	34.97^{abc}±1.59	28.72^{c}±1.98	33.57^{bc}±2.23	29.88^{c}±1.13	2.347	0.016
Excreta (µg/g)	0.127^{cd}±0.007	0.125^{d}±0.002	0.137^{bc}±0.003	0.148^{ab}±0.003	0.146^{ab}±0.002	0.150^{a}±0.002	0.004	0.001
Copper								
% Retention	45.56±3.14	43.86±1.52	47.10±1.72	47.32±4.67	47.67±4.00	46.12±3.69	3.33	0.967
Excreta (µg/g)	0.024±0.002	0.027±0.001	0.027±0.001	0.023±0.002	0.025±0.002	0.027±0.002	0.002	0.477
Zinc								
% Retention	46.76±1.60	48.91±1.19	47.88±2.24	46.53±1.08	52.64±3.50	49.07±1.12	2.02	0.358
Excreta (µg/g)	0.17±0.003	0.17±0.005	0.17±0.007	0.17±0.004	0.16±0.006	0.17±0.004	0.005	0.823
Iron								
% Retention	35.66^{b}±0.96	43.97$^{a±}$0.85	43.50^{a}±1.33	28.23^{c}±1.32	32.55^{b}c±1.45	31.31^{bc}±1.34	1.11	0.001
Excreta (µg/g)	0.51^{b}±0.01	0.47^{c}±0.01	0.46^{c}±0.01	0.57^{a}±0.01	0.58^{a}±0.01	0.57^{a}±0.01	0.01	0.001
Boron								
% Retention	49.92^{a}±1.78	47.55^{a}±1.72	47.85^{a}±1.59	33.69^{b}±1.96	35.11^{b}±4.30	34.74^{b}±2.45	2.47	0.001
Excreta (µg/g)	0.0038^{b}±0.00	0.0042^{b}±0.00	0.0042^{b}±0.00	0.0683^{a}±0.002	0.0706^{a}±0.006	0.0692^{a}±0.002	0.0026	0.001

*Basal diet (B_0C_0); Basal diet with 20% calcium deficiency (B_0C_1); Basal diet with 40% calcium deficiency (B_0C_2); Basal diet with supplemental boron @ 25 mg/kg (B_1C_0); Basal diet with 20% calcium deficiency with supplemental boron @ 25 mg/kg (B_1C_1); Basal diet with 40% calcium deficiency with supplemental boron @ 25 mg/kg (B_1C_2). ^{abcd}Mean with different superscript in a row differ significantly; SEM, standard error of mean.

In the present study, a significant increase (P<0.01) in retention of Mn was observed in the dietary group with 8 mg of Ca/kg diet but not with 6 mg of Ca/kg diet. Schoulten *et al*. (2002) reported that increasing the dietary Ca level decreases absorption of manganese. On the other hand, Sebastian *et al*. (1996) found no effect on the relative retention of Mn in 1-21week-old broiler chickens when dietary Ca was increased from 6 to 12.5 g/kg. The contradictory results may be due to the source and form of manganese (Li *et al.* 2005). They suggested that organic source of Mn with moderate or strong chelating strength could partially or completely resist the antagonistic effect of increased dietary calcium. The reduced retention of Mn at 0.6 % dietary Ca may be due to decrease in Ca: P ratio in the diet. Scott *et al*. (1976) reported that with narrower calcium and phosphorous ratio, P forms a flocculent precipitate of calcium phosphate in the alkaline duodenum and this precipitate may form an un-absorbable complex with manganese, creating an apparent manganese deficiency. Supplementation of boron decreased the absorption of Mn in the present study is in line with the findings of Bhasker *et al.* (2016) who reported a negative interaction of boron with manganese and observed a significantly (P<0.01) lowered level of manganese in the serum of rat fed a diet supplemented with graded levels of boron at 5-40 mg/kg diet. However, the absolute and relative retention of Mn in the boron supplemented Ca deficient dietary treatment groups were comparable with that of group with normal diet without boron supplementation indicating the negative effect of boron can be compensated by low dietary Ca concentration. Dietary calcium concentration has no effect on the excreta concentration of Zn which is in corroboration with Rama Rao *et al*. (2006) who reported that the Zn concentration in excreta was not affected by the variation in dietary Ca level. About 85-90% of ingested boric acid is rapidly absorbed across the gastrointestinal epithelia and excreted mostly in urine shortly after ingestion in humans and animals (Hunt *et al.* 1997, Sutherland *et al.* 1998). Supplementation of boron enhanced the excreta boron concentration suggesting that the boron is under homeostatic control (Vaziri *et al.* 2001).

The outstanding overall performances of broilers fed on a diet with either 20 or 40 % Ca deficiency as compared to adequate Ca suggest that the level of Ca in the broiler diet should likely be much lower than current recommendation. Boron has an adverse effect on the retention of iron and manganese but diet with lower level of Ca increase the retention of iron and manganese indicating the negative

impact of boron on these minerals can be overcome by a low Ca diet. Therefore, it can be concluded that diet with 40% Ca deficiency with 25 mg/kg boron supplementation impart a better performance in broiler without much affecting the metabolism which suggest that 0.6 g Ca/kg diet with supplemental boron at 25 mg/kg is optimum in broilers for better performances.

REFERENCES

AOAC. 2000. Officials Methods of Analysis of the Association of Analytical Chemists. 17th edn. Association of Official Analytical Chemists, Washington, DC.

Armstrong T A and Spears J W. 2001. Effect of dietary boron on growth performance calcium and phosphorus metabolism and bone mechanical properties in growing barrows. *Journal of Animal Science* **7912**: 3120–27.

Armstrong T A, Spears J W, Crenshaw T D and Nielsen F H. 2000. Boron supplementation of a semipurified diet for weanling pigs improves feed efficiency and bone strength characteristics and alters plasma lipid metabolites. *Journal of Nutrition* **130**(10): 2575–81.

Balla G C, Nelson T S and Kirby L K. 1984. Effect of different dietary levels of calicum and phosphorus on phytate hydrolysis by chicks. *Nutrition Reports International* **32**: 909–13.

Bhasker T V, Gowda N K S, Mondal S, Krishnamoorthy P, Pal D T, Mor A and Pattanaik A K. 2016. Boron influences immune and antioxidant responses by modulating hepatic superoxide dismutase activity under calcium deficit abiotic stress in Wistar rats. *Journal of Trace Elements in Medicine and Biology* **36**: 73–79.

Bozkurt M, Kucukyilmaz K, Catli A U, Cinar M, Cabuk M and Bintas E. 2012. Effects 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 Sciences* **252**: 248–55.

Cook J D, Dassenko S A and Whittaker P. 1991. Calcium supplementation: effect on iron absorption. *American Journal of Clinical Nutrition* **53**(1): 106–111.

Cufadar Y, Olgun O and Bahtiyarca Y I L M A Z. 2011. Effects of different sources of boron supplementation to diet on egg shell quality and bone characteristics in laying hens. *Archivos de Zootecnia* **14**: 37–42.

Dawson-Hughes B E S S, Stern D T, Shipp C C and Rasmussen H M. 1988. Effect of lowering dietary calcium intake on fractional wholebody calcium retention. *Journal of Clinical Endocrinology and Metabolism* **671**: 62–68.

Deehr M S, Dallal G E, Smith K T, Taulbee J D and Dawson-Hughes B. 1990. Effects of different calcium sources on iron absorption in postmenopausal women. *American Journal of Clinical Nutrition* **511**: 95–99.

Devirian T A. and Volpe S L. 2003. The physiological effects of dietary boron. *Critical Reviews in Food Science and Nutrition* **43**: 219–31.

Duncan D B. 1955. Multiple range and multiple F tests. *Biometrics* **11**: 1–42

Fassani E J, Bertechini A G, Brito J A G, Kato R K, Fialho E T and Geraldo A. 2004. Boron supplementation in broiler diets. *Brazilian Journal of Poultry Science* **4**: 213–17.

Hakki S S, Dundar N, Kayis S A, Hakki E E, Hamurcu M, Kerimoglu U and Nielsen F H. 2013. Boron enhances strength and alters mineral composition of bone in rabbits fed a high energy diet. *Journal of Trace Elements in Medicine and Biology* **272**: 148–53.

Hallberg L, Brune M, Erlandsson M, Sandberg A S and Rossander-Hulten L. 1991. Calcium: effect of different amounts on nonheme-and heme-iron absorption in humans. *American Journal of Clinical Nutrition* **531**: 112–19.

Hallberg L, Rossander-Hulten L, Brune M and Gleerup A. 1992. Calcium and iron absorption: mechanism of action and nutritional importance. *European Journal of Clinical Nutrition* **465**: 317–27.

Hunt C D. 1994. The biochemical effects of physiologic amounts of dietary boron in animal nutrition models. *Environmental Health Perspectives* **1027**: 35–42.

Hunt C D. 1997. Measurement of boron in rodent diets and tissue. *Trace Elements in Laboratory Rodents.* (Ed.) R R Watson. CRC Press, Boca Raton, FL. pp. 255–267.

Kaup S M, Behling A R, Choquette L and Greger J L. 1990. Calcium and magnesium utilization in rats: Effect of dietary butterfat and calcium and of age. *Journal of Nutrition* **1203**: 266–73.

Kronqvist C, Emanuelson U, Sporndly R and Holtenius K. 2011. Effects of prepartum dietary calcium level on calcium and magnesium metabolism in periparturient dairy cows. *Journal of Dairy Science* **943**: 1365–73.

Kucukyilmaz K, Bozkurt M, Cinar M and Tuzun A E. 2017. Evaluation of the boron and phytase alone or in combination in broiler diets. *Journal of Poultry Science* **541**: 26–33.

Letourneau-Montminy M P, Lescoat P, Narcy A, Sauvant D, Bernier J F, Magnin M and Jondreville C. 2008. Effects of reduced dietary calcium and phytase supplementation on calcium and phosphorus utilisation in broilers with modified mineral status. *British Poultry Science* **496**: 705–15.

Li S F, Luo X G, Lu L, Crenshaw T D, Bu Y Q, Liu B and Yu S X. 2005. Bioavailability of organic manganese sources in broilers fed high dietary calcium. *Animal Feed Science and Technology* **123**: 703–15.

Lim H S, Namkung H and Paik I K. 2003. Effects of phytase supplementation on the performance egg quality and phosphorous excretion of laying hens fed different levels of dietary calcium and nonphytate phosphorous. *Poultry Science* **821**: 92–99.

Liu J, Bollinger D W, Ledoux D R and Venum T L. 2000. Effects of dietary calcium: phosphorus ratios on apparent absorption of calcium and phosphorus in the small intestine cecum and colon of pigs. *Journal of Animal Science* **781**: 106–09.

Lobaugh B, Joshua I G and Mueller W J. 1981. Regulation of calcium appetite in broiler chickens. *Journal of Nutrition* **1112**: 298-306.

Miljkovic D, Miljkovic N and McCarty M F. 2004. Up-regulatory impact of boron on vitamin D function–does it reflect inhibition of 24-hydroxylase?. *Medical Hypotheses* **636**: 1054–56.

Miljkovic D, Scorei R I, Cimpoiaşu V M and Scorei I D. 2009. Calcium fructoborate: plant-based dietary boron for human nutrition. *Journal of Dietary Supplements* **63**: 211–26.

Mızrak C, Yenice E, Can M, Yildirim U and Atik Z. 2010. Effects of dietary boron on performance egg production egg quality and some bone parameters in layer hens. *South African Journal of Animal Science* **403**: 257–64.

Mohammed A, Gibney M J and Taylor T G. 1991. The effects of dietary levels of inorganic phosphorus calcium and cholecalciferol on the digestibility of phytate-P by the chick. *British Journal of Nutrition* **662**: 251–59.

Naghii M R, Torkaman G and Mofid M. 2006. Effects of boron and calcium supplementation on mechanical properties of

bone in rats. *Biofactors* **283-4**: 195–201.

Naghii M R. 1999. The significance of dietary boron with particular reference to athletes. *Nutrition and Health* **131**: 31–37.

Nelson T S and Kirby L K. 1987. The calcium binding properties of natural phytate in chick diets. *Nutrition Reports International* **35**: 949–956.

Nicar M J and Pak C Y. 1982. Oral magnesium load test for the assessment of intestinal magnesium absorption. Application in control subjects absorptive hypercalciuria primary hyperparathyroidism and hypoparathyroidism. *Mineral and Electrolyte Metabolism* **81**: 44-51.

Nielsen F H. 2004. Dietary fat composition modifies the effect of boron on bone characteristics and plasma lipids in rats. *Biofactors* **203**: 161-71.

NRC 1994. National Research Council. *Nutrients Requirements of Poultry*. 9th ed. National Academic Press, Washington, DC, pp 155.

Pradhan S K, Kumar B, Banakara K B, Patel V R, Pandya H R and Singh R R. 2020. Effect of boron supplementation on the performance and metabolism of minerals in broiler chicken. *Animal Nutrition and Feed Technology* **20**: 39-49.

Qian H, Kornegay E T and Denbow D M. 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase cholecalciferol and the calcium: total phosphorus ratio in broiler diets. *Poultry Science* **761**: 37–46.

Qian H, Kornegay E T, Veit H P, Denbow D M and Ravindran V. 1994. Effect of supplemental Natuphos® phytase on tibial traits of turkeys fed soybean meal-based semi-purified diets. *Poultry Science* **73**: 89–95.

Rama Rao S R, Panda A K, Raju M V L N, Sunder G S and Praharaj N K. 2003. Requirement of calcium for commercial broilers and white leghorn layers at low dietary phosphorus levels. *Animal Feed Science and Technology* **1061-4**: 199–208.

Rama Rao S R, Raju M V L N, Reddy M R and Pavani P. 2006. Interaction between dietary calcium and non-phytate phosphorus levels on growth bone mineralization and mineral excretion in commercial broilers. *Animal Feed Science and Technology* **1311-2**: 135–50.

Rama Rao S R, Raju M V L N and Reddy M R. 2007. Performance of broiler chicks fed high levels of cholecalciferol in diets containing sub-optimal levels of calcium and non-phytate phosphorus. *Animal Feed Science and Technology* **1341-2**: 77–88.

Rossi A F, Miles R D, Damron B L and Flunker L K. 1993. Effects of dietary boron supplementation on broilers. *Poultry Science* **7211**: 2124–30.

Santos F R, Hruby M, Pierson E E M, Remus J C and Sakomura N K. 2008. Effect of phytase supplementation in diets on nutrient digestibility and performance in broiler chicks. *Journal of Applied Poultry Research* **172**: 191–201.

Schoulten N A, Teixeira A S, Silva H O, Conte A J, Bertechini A G and Fialho E T. 2002. Effect of calcium levels in phytase supplemented diets on the absortion of minerals in broiler chickens from 22 to 42 days. *Ciência Animal Brasileira* **4**: 31–37.

Scott M L, Nesheim M C and Young R J. 1976. *Nutrition of the Chicken*. eds. M. L. Scott and Associates, Ithaca, New York. pp. 7–54.

Sebastian S, Touchburn S P, Chavez E R and Lague P C. 1996. Efficacy of supplemental microbial phytase at different dietary calcium levels on growth performance and mineral utilization of broiler chickens. *Poultry Science* **7512**: 1516–23.

Seelig M S. 1994. Consequences of magnesium deficiency on the enhancement of stress reactions; preventive and therapeutic implications a review. *Journal of the American College of Nutrition* **135**: 429–46.

Shafey T M. 1993. Calcium tolerance of growing chickens: Effect of ratio of dietary calcium to available phosphorus. *World's Poultry Science Journal* **491**: 5–18.

Smith O B and Kabaija E. 1985. Effect of high dietary calcium and wide calcium-phosphorus ratios in broiler diets. *Poultry Science* **649**: 1713–20.

Sun P, Luo Y, Wu X T, Ansari A R, Wang J, Yang K, Xiao K and Peng K. 2016. Effects of supplemental boron on intestinal proliferation and apoptosis in African ostrich chicks. *International Journal of Morphology* **343**: 830–35.

Sutherland B, Strong P and King J C. 1998. Determining human dietary requirements for boron. *Biological Trace Element Research* **661**: 193–204.

Suttle N F. 2010. *Mineral Nutrition of Livestock*. 4th ed. CABI Publishing, CAB International, Wallingford.

Tamim N M and Angel R. 2003. Phytate phosphorus hydrolysis as influenced by dietary calcium and micro-mineral source in broiler diets. *Journal of Agricultural and Food Chemistry* **5116**: 4687–93.

Tamim N M, Angel R and Christman M. 2004. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. *Poultry Science* **838**: 1358–67.

Van der Aar P J, Molist F V and Van Der Klis J D. 2017. The central role of intestinal health on the effect of feed additives on feed intake in swine and poultry. *Animal Feed Science and Technology* **233**: 64–75.

Vaziri N D, Oveisi F, Culver B D, Pahl M V, Andersen M E, Strong P L and Murray F J. 2001. The effect of pregnancy on renal clearance of boron in rats given boric acid orally. *Toxicological Sciences* **602**: 257–63.

Whiting S J and Wood R J. 1997. Adverse effects of high-calcium diets in humans. *Nutrition Reviews* **551**: 1–9.

Wilkinson S J, Bradbury E J, Bedford M R and Cowieson A J. 2014. Effect of dietary nonphytate phosphorus and calcium concentration on calcium appetite of broiler chicks. *Poultry Science* **937**: 1695–1703.

Young V R, Richards W P C, Lofgreen G P and Luick J R. 1966. Phosphorus depletion in sheep and the ratio of calcium to phosphorus in the diet with reference to calcium and phosphorus absorption. *British Journal of Nutrition* **204**: 783–94.