Full Length Article

EFFECT OF DIETARY SUPPLEMENTATION OF BORIC ACID ON THE SERUM BIOCHEMICAL, TIBIA MORPHOMETRY AND MINERALIZATION OF JAPANESE QUAIL (*Coturnix coturnix japonica*)

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ABSTRACT

The study was carried out to evaluate the effect of dietary supplementation of boric acid on serum biochemical, tibia morphometry and mineralization of Japanese quail fed diets supplemented with boric acid at different levels of 0, 15, 30, 45 and 60 ppm were prepared in a 5 x 3 design. A total of 150 day-old Japanese quail chicks were individually weighed and allotted randomly into five treatment groups with three replicates containing ten birds each, the birds were fed with experimental diets for a period of 5 weeks. At the end of the 5th week, two birds per replicate an average of six birds per treatment were slaughtered. Blood and tibia were collected accordingly. Serum calcium level significantly (p<0.05) increased but serum phosphorus, serum alkaline phosphatase, tibia morphometry and mineralization were not influenced by dietary boric acid supplementation. Results indicated that dietary boric acid level can be further increased in the quail diet for optimum bone mineralization.

Key words: Boric acid, Japanese quail, serum, tibia.

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INTRODUCTION

The economic production of Japanese quail is usually affected by increased leg problems like spaddled legs, rickets and weak legs and thereby mortalities due to vitamin and mineral deficiencies, which may be reduced by supplementing sufficient dietary minerals and vitamins. The supplementation of Boron (B) to cholecalciferol (vitamin D) deficient chicks results in correction of malformations in the marrow sprouts of bone. Boron plays an important role in the metabolism of macro-minerals like calcium. Boron involves in different mechanisms like compensating

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perturbations in energy substrate utilization induced by vitamin D_3 deficiency, enhancing macro mineral content in normal bone and independently of vitamin D3, B enhances growth cartilage formation.

Boron plays a role in bone mineralisation by involving in parathyroid hormone regulation, and may have implications on bone development and bone strength in broiler chickens (Bozkurt *et al*., 2012) and layer hens (Kucukyılmaz *et al*., 2014).Dietary boron deficiency leads to improper development of bone cartilage, and affects bone ash and bone breaking load, and plasma Ca, P, Mg levels with alkaline phosphatase (ALP) activity. The addition of B and other trace minerals to chick nutrition increased tibial bone ash percentage and reduced the incidence of tibial deformities.

The studies on beneficial effects of dietary boron supplementation of the quails were very limited. Hence this study was conducted with an objective to assess the optimum concentration of boron in the form of boric acid in the diets of Japanese quail for optimum growth and bone strength.

MATERIAL AND METHODS

A total of 150 day old quail chicks were distributed randomly into five treatments with three replicates containing ten birds each and fed with five experimental diets T_1 (Basal diet), T_2 (Basal diet supplemented with 15 ppm boric acid), T_3 (Basal diet supplemented with 30 ppm boric acid), T_4 (Basal diet supplemented with 45 ppm boric acid) and T_5 (Basal diet supplemented with 60 ppm boric acid). Basal diets (Table 1) were formulated with locally available feed ingredients like maize, soybean meal, DORB and fish meal as per the nutrient requirements of poultry (NRC, 1994). The birds were housed in a 5 tier battery cages during the experimental period of 5 weeks and maintained under uniform environment conditions thorough out the experiment.

Table 1. Ingredient composition of Japanese quail experimental diet

Ingredient (Kg)	Basal diet (T_1)
Maize	49.8
Soyabean meal	34.0
DORB	8.9
Fish meal	5.0
DCP	0.2
Shell grit	1.2
Salt	0.25
L-trace minerals*	0.15
L Lysine	0.06
DL methionine	0.10
Vit AB_2D_3	0.10
Choline chloride	0.10
Coccidiostat	0.05
Vit E powder	0.05
Liv 52 powder	0.04
Total	**100**

*Manganese sulphate 8250 mg, Ferrous sulphate 7500 mg, zinc sulphate 7500 mg, cobalt sulphate 75 mg, copper sulphate 450 mg, potassium Iodide 450 mg, sodium selenite 75 mg/Kg.

Feed and water were provided *adlibitum*. At the end of the experiment period of five weeks, two birds per replicate, a total of 30 birds were slaughtered as per standard procedure. Blood samples were collected into anticoagulant free vials and allowed to clot so that the serum got separated. The separated serum was then made clear by centrifuging at 3000 rpm for 10 minutes and stored in a refrigerator for estimation of serum parameters. Serum calcium was estimated calorimetrically by using diagnostic kit (coral clinical systems) by oCPC method. Serum phosphorus was estimated by using diagnostic kit (M/s. ERBA Diagnostics Private Limited) by Ammonium Molyb date method, end point. Serum alkaline phosphatase was estimated by using diagnostic kit (M/s. ERBA Diagnostics Private Limited) by IFCC method, kinetic.

The left tibiae were collected from the 30 birds of five weeks age sacrificed at the end of the experimental period. The bones were identified individually for each treatment separately and their adhered muscles together with connective tissue had been thoroughly removed manually. The bones were dipped in boiling water for 5 minutes to remove the remaining fine and soft tissues. The method described by Shrivastava *et al.* (1996) was slightly modified for the measurement of various bone morphological parameters. The full length of each tibia was measured from its proximal to distal end. The proximal width of each tibia was measured at almost one cm below the proximal end of the dorsal surface (across the flat) and the lateral surface (across the narrow) and their mean was taken as proximal width. The distal width of each tibia was measured at almost one cm above the distal end of the dorsal surface (across the flat) and lateral surface (across the narrow) and their mean were taken as distal width. The mid shaft width of each tibia was measured at middle of the shaft between the proximal and distal ends. The weight of each tibia was determined as such and fat free dried basis. Each tibia was subjected for the estimation of bone ash (percent tibia ash) after drying in hot air oven for overnight and defatting with diethyl ether and petroleum spirit for 8 hours, following the procedure of AOAC (2000), applicable for estimation of calcium and phosphorus. Mortality among the birds during the entire experimental study was recorded and the causes there off were ascertained by detailed autopsy. Statistical analysis of the data was carried out according to the procedures suggested by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Serum biochemical parameters

The Japanese quail fed with diets containing 0, 15, 30, 45 and 60 ppm boric acid showed significant ($p<0.05$) increase in mean serum calcium level among the treatment groups from 1-5 weeks of age in the present study (Table 2). This observation was in consonance with Kucukyilmaz *et al.* (2017) in broiler chicken. In contrary Yesilbag and Eren (2008) in laying hens; Eren*et al.* (2012) and Bozkurt *et al*. (2012) in broiler chicken observed that the boron supplementation had no significant effect on serum calcium.

Serum phosphorus level showed no significant difference among the treatment groups.This observation corroborated with the findings of Yesilbag and Eren (2008) in laying hens; Eren *et al.* (2012) in broiler chicken. In contrary serum phosphorus was significantly ($p<0.05$) increased in the findings of Bozkurt *et al*. (2012) and Kucukyilmaz *et al.* (2017) in broilers. Though the results of this study revealed no significant difference in mean serum phosphorus level among the corresponding dietary groups, the numerically higher mean serum phosphorus level was observed in T_3 when compared with the control group. Serum alkaline phosphatase level showed no significant difference among the treatment groups.This observation was in agreement with the findings of Bozkurt *et al*. (2012), Eren *et al.* (2012) and Kucukyilmaz *et al.* (2017)in broiler chicken.

Tibia morphometry

Mean tibia weight, mean tibia length, mean tibia proximal width, mean tibia distal width and mean tibia mid shaft width (Table 3) of Japanese quail had no significant difference among the treatment groups. Among these observations mean tibia weight results were found to be similar with the findings of Rossi *et al.* (1994) in broilers. In contrary Rossi *et al.* (1993) found significantly ($p<0.05$) higher tibia weights in broilers. Whereas Dumitrescu *et al*.(2012) in broilers and Olgun *et al*. (2013) in laying hens found significantly ($p<0.05$) increased tibia widths.

Tibia mineralization

Tibia ash percentage was not significantly affected among the treatment groups(Table 3). This observation was in consonance with the findings of Yildiz *et al.* (2011), Yildiz *et al*. (2013) and Kucukyilmaz *et al.* (2017) in broilers; Sizmaz and Yildiz (2016) in laying hens. In contrary Bozkurt *et al*. (2012) in broilers and Mizrak *et al*. (2010) in laying hens; observed that boron supplementation resulted in significant ($p<0.05$) increase in tibia ash percentage. Tibia calcium percentage showed no significant difference among the treatment groups.This observation was in agreement with the findings of Kucukyilmaz *et al.* (2017) in broilers. In contrary, Bozkurt

Table2. Mean (±S.E) serum calcium, serum phosphorus and serum alkaline phosphatase of Japanese quail fed with different levels of boric acid from day old to five weeks of age.

BA supplemen-tation	Serum Calcium (mg/dl)	Serum Phosphorus (mg/dl)	Serum alkaline phosphatase (IU/L)	Tibia Ash %	Tibia Calcium %	Tibia Phosphorus%
T_1(0 ppm BA)	12.28^{b} ±0.83	9.53±0.87	495.67±39.78	56.96 ±0.51	18.63 ±0.42	14.77 ±0.35
T_2(15 ppm BA)	12.80^{b} ±0.64	11.02±0.29	507.88±25.82	57.26 ±0.58	18.78 ±0.38	15.94 ±0.52
T_3(30 ppm BA)	13.75^{ab} ±0.75	11.42±0.95	530.91±77.50	57.53 ±1.31	19.32 ±0.51	16.24 ±0.18
T_4(45 ppm BA)	14.10^{ab} ±0.93	9.08±0.90	532.99±43.08	57.95 ±1.31	19.85 ±0.81	16.72 ±1.72
T_5(60 ppm BA)	15.92^{a} ±0.95	10.52±0.57	526.31±88.81	60.19 ±2.20	20.74 ±0.68	17.15 ±0.26
SEM	0.41	0.35	25.06	0.59	0.28	0.37
p value	0.045	0.186	0.99	0.399	0.103	0.343
n	6	6	6	6	6	6

[a,b]Means within a column with no common superscripts differ significantly ($p<0.05$)

*Significant ($P<0.05$); [NS] Not significant ($p>0.05$)

Table 3. Mean (±S.E) tibia proximal width, tibia distal width, tibia mid shaft width, tibia length (mm) and tibia weight (g) of Japanese quail fed with different levels of boric acid from day old to five weeks of age.

	Tibia Morphometry				
BA supplementation	**Proximal Width (mm)**	**Distal Width (mm)**	**Mid Shaft Width (mm)**	**Length (mm)**	**Weight (g)**
T_1 (0 ppm BA)	3.87 ±0.09	3.22 ±0.06	2.74 ±0.13	52.97 ±0.82	0.42 ±0.01
T_2 (15 ppm BA)	3.88 ±0.11	3.19 ±0.13	2.76 ±0.08	54.83 ±0.71	0.44±0.01
T_3 (30 ppm BA)	4.01 ±0.12	3.06 ±0.08	2.82 ±0.07	53.83 ±0.51	0.45 ±0.01
T_4 (45 ppm BA)	4.19 ±0.10	3.24 ±0.02	2.98 ±0.06	54.43 ±1.14	0.48 ±0.01
T_5 (60 ppm BA)	3.87 ±0.15	3.08 ±0.05	2.73 ±0.05	51.29 ±1.08	0.49 ±0.03
SEM	0.05	0.03	0.04	0.43	0.01
p value	0.289	0.376	0.268	0.068	0.175
n	6	6	6	6	6

et al. (2012) and Yildiz *et al*. (2013) in broilers;Sizmaz and Yildiz (2016) and Olgun *et al*. (2013) in laying hens reported that boron supplementation in the diet resulted in significant ($p<0.05$) increase of tibia calcium percentage. Tibia phosphorus level showed no significant difference among the treatment groups.This observation was similar with the findings of Mizrak *et al*. (2010), Kucukyilmaz *et al*. (2017) in broilers and Sizmaz and Yildiz (2016) in laying hens. In contrary Bozkurt *et al*. (2012) in broilers and Olgun *et al*. (2013) in laying hens found significant ($p<0.05$) increase in tibia phosphorus percentage by supplementation of boron in the broilers diet from 0 to 60 ppm. The results in this study also revealed a numerically increasing trend in tibia ash, calcium phosphorus percentages was noticed from T_1 (0 ppm) to T_5 (60 ppm).

CONCLUSION

From the results it was observed that boric acid supplementation had no significant difference in the serum phosphorus, serum alkaline phosphatase, tibia morphometry and tibia mineralization, but significant ($p<0.05$) increase in serum calcium level was noticed. Further research work may be carried out with various higher levels of boric acid supplementation to Japanese quail rations to improve bone mineralization.

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