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Boron supplementation provides hepato-protective effect and improves performance in Wistar rats fed calcium deficit diet

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ABSTRACT

This study was conducted to investigate the influence of boron (B) supplementation in rats fed diets with adequate and deficit calcium (Ca). A feeding trial of 90 days duration was conducted in 84 Wistar strain rats divided into 7 groups, viz. Normal-Ca (100%) basal diet (NC) and supplemented with B at 5 (NCB-5), 10 (NCB-10), 20 (NCB-20) and 40 (NCB-40) ppm levels; low-Ca (50%) diet (LC) and supplemented with 40 ppm B (LCB-40). During 80–85 days of feeding, a digestibility trial was conducted to record nutrient digestibility. Rats (8) from each group were sacrificed to collect blood and visceral organs to study biochemical parameters and histopathology. The average daily feed and water intake were similar among the dietary groups. Body weight gain was lower in rats fed LC diet and increased in the LCB-40 group. Nutrient (dry matter, crude protein, crude fat) digestibility was significantly improved with B-supplementation to NC diets. Serum levels of triglycerides, HDL-cholesterol and alanine transaminase were significantly lowered in B-supplemented groups. The levels of glucose, total cholesterol, alkaline phosphatase and aspartate transaminase in serum remained unaltered among the dietary groups. Degenerative changes were observed in the liver of rats fed Ca-deficient diets and were ameliorated with 40 ppm B supplementation. Results indicated that dietary supplementation of B in rats showed improved nutrient digestibility, growth and lowered serum levels of triglycerides, HDL-cholesterol and alanine transaminase. Hepatic tissue alterations due to lower Ca intake were ameliorated with B supplementation.

Key words: Boron, Calcium, Hepato-protection, Histopathology, Nutrient utilization, Wistar rats

Boron (B) is essential for plant growth and its biological importance in animals is not clearly understood in animals (Vijay Bhasker *et al*. 2015). Research indicated a role of B in calcium (Ca) utlisation in rats and chicken (Neilson 2004, Bozkurt *et al*. 2012). Boron supplementation in diet of dairy animals during the periparturient period prevented metabolic disorder (Kabu *et al*. 2013) and alleviated fluoride toxicity (Bharti *et al*. 2008). In tropical conditions, trace minerals play a vital role in health and production of livestock (Prasad and Gowda 2005) and hence there is a need to understand the role of newer elements. Hence, this experiment was conducted in rats to understand the influence of B on nutrient utilization, serum biochemical parameters and tissue changes in visceral organs.

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MATERIALS AND METHODS

The animal experiment protocol was approved by the Institutional Animal Ethics Committee (IAEC). Healthy weaned (3–4 weeks) Wistar strain (*Rattus norvegicus*) albino rats (84) were randomly divided into 7 dietary groups, with 4 replicates of 3 rats in each group. All the rats were housed in polypropylene cages and maintained under similar management conditions. Temperature and humidity were maintained at 23±2ºC and 50 to 70%, respectively, in the proper ventilated animal house. All rats were fed pelleted feed and offered purified drinking water *ad lib*.

All the rats were fed a basal semi-purified diet to meet the nutrient requirement (ICAR 2013) except for the levels of Ca and B, throughout the experiment. Boron was supplemented in the diet as sodium (Table 1). The Ca in the low-Ca diets were reduced to 50% of the control diet by decreasing the quantity of dicalcium phosphate (DCP). The deficit in phosphorus content caused by reduction in DCP was met by adding the calculated amount of sodium orthophosphate dehydrate. Pellets of each experimental diet were prepared by a manual pelletizer, air dried for 24 h, later oven dried at 60°C for 24 h and stored in air tight containers. The rats of different dietary groups were offered

weighed amount of respective experimental diet daily at 9:30 h. They were provided with fresh and clean deionized water *ad lib.* Daily feed intake, refusals and fortnightly body weights were recorded. During 80–85 days of experiment, a digestibility trial of 5 days duration was conducted with conventional total collection method to estimate nutrient digestibility. On 90th day, eight rats from each group were sacrificed with an overdose of ether anaesthesia to collect the vital organs (liver, kidney, spleen and thymus) to study histopathology. Whole blood (3 ml) was drawn by heart puncture into sterile poly propylene tubes and centrifuged at 8,000 g for 20 min to separate serum and preserved at –20°C till analysed.

The samples of feed offered, residues and faeces were analysed for proximate principles (AOAC 2000). Serum levels of glucose (Henry 1963), triglycerides (McGowan *et al*. 1983), total cholesterol (Herbert *et al*. 1984) and high density lipoprotein (HDL)-cholesterol (Siedel *et al*. 1983), alkaline phosphatase (ALP, EC 3.1.3.1) (Kind and King 1954), alanine transaminase (AST, EC 2.6.1.2), aspartate transaminase (AST, EC 2.6.1.1) (Reitman and Frankel 1957) were determined using diagnostic kits (Span Diagnostics, Surat, India). The acid mineral extracts from feeds and faeces were prepared as per AOAC (2000) and mineral contents were estimated using inductively coupled plasma optical emission spectrophotometer following standard analytical conditions.

The tissues of visceral organs were fixed in 10% formalin and sections stained with haematoxylin and eosin stain (Bancroft and Stevens 1996) and covered with DPX mounting medium and examined under a light microscope to assess the histopathological changes.

The data were analysed using the Statistical Package for Social Sciences (SPSS, version 20.0, Chicago, USA) by one-way ANOVA and comparison of means was tested using Duncan's multiple range tests (Duncan 1995). Contrast analysis was done to distinguish the impact of Ca and B-supplementation. The effects were considered to be significant at $P<0.05$.

RESULTS AND DISCUSSION

The chemical composition of experimental diets was as per the stipulated standards of ICAR (2013) for laboratory animals. The level of Ca (%) in the NC diet was similar, while it was reduced by 50% in the LC diets. The levels of other minerals (P, Mg, Cu, Zn and Mn) were similar in all the diets.

Table 1. Ingredient composition and nutritive value of semi-purified diets

	Dietary groups						
	NC	NCB-5	NCB-10	NCB-20	NCB-40	LC	LCB-40
Composition (g per 1000g)							
Basal diet*	976.96	976.91	976.82	976.65	978.28	977.93	976.96
Dicalcium phosphate	23	23	23	23	23	11.5	11.5
Sodium phosphate	-	-	-	-	-	10.22	10.22
Sodium borate	-	0.044	0.088	0.176	0.352	-	0.352
Calcium	5	5	5	5	5	2.53	2.53
Phosphorus	4.1	4.1	4.1	4.1	4.1	4.1	4.1
Nutrient composition							
Analysed nutritive constituent (% DM)							
DM	96.2	96.6	97.3	96.3	96.3	96.4	95.8
OM	92.7	93.0	93.6	92.6	92.6	93.3	93.0
CP	26.6	25.5	27.4	26.6	25.8	25.3	25.0
CF	4.26	4.49	4.44	4.57	4.05	4.59	4.65
Ash	7.32	6.96	6.37	7.41	7.38	6.74	6.96
Minerals							
Ca (% DM)	0.85	0.89	0.84	0.83	0.83	0.39	0.41
P (% DM)	0.60	0.58	0.58	0.61	0.61	0.58	0.58
Mg (% DM)	0.10	0.10	0.10	0.11	0.10	0.10	0.10
Cu (ppm)	7.85	9.37	8.74	9.69	9.43	9.18	9.36
Zn (ppm)	37.22	39.03	41.21	39.44	42.58	38.21	39.17
Mn (ppm)	86.89	88.50	92.35	93.37	83.97	82.64	79.80
B (ppm)	1.69	5.39	11.2	22.5	38.7	2.85	37.3

*Basal diet (g/1000g): sucrose (450), maida (50), casein (200), corn starch (150), corn oil (50), cellulose (50), mineral mixture‡ (35), vitamin supplement§ (10), dl-methione (3) and choline bitartarate (2). ‡Mineral mixture (g/500g): $Na_3C_6H_5O_7.2H_2O$ (220), NaCl (74), KCl (52), MgO (24), ferric citrate (6.0), $MnSO_4$ (3.5), $ZnCO_3$ (1.6), K, Cr, O‡ (0.55), $CuSO_4$ (0.3), KIO_3 (0.01), Na, SeO*f* (0.01), Sucrose (118.03). §Vitamin supplement (g/200g): Vitamin A (1×10^7 IU), D_3 (2×10^6 IU), K_3 (0.8), E (8.0), B_1 (1.0), B_2 (4.0), B_6 (2.5), B_{12} (9.0), niacin (12), pantothenic acid (6.0), folic acid (0.5) and biotin (10). DM, dry matter; OM, organic matter; CP, crude protein; CF, crude fat; Ca, calcium; P, phosphorus; Mg, magnesium; Cu, copper; Zn, zinc; Mn, manganese; B, boron

The analysed values of B (ppm) in diets NC, NCB-5, NCB-10, NCB-20, NCB-40, LC and LCB-40 were 1.69, 5.39, 11.2, 22.5, 38.7, 2.85 and 37.3 ppm against the desired values of 0, 5, 10, 20, 40, 0 and 40 ppm, respectively (Table 1). Dietary B-supplementation did not alter the feed intake in rats, which was agreement with earlier reports (Hegsted *et al*. 1991, Gorustovich *et al*. 2008). Significant (P<0.05) decline in average daily gain (14.6%) was observed in rats fed Ca restricted diet, which was in agreement with the findings of Persson *et al*. (1993). However, weight gain improved (P< 0.05) with 40 ppm B supplementation (Table 2). Dietary B (30 and 60 ppm) improves weight gain in broiler chicks fed Ca and P deficient diets (Bozkurt *et al*. 2012).

It was observed that the gut absorption of Ca improved (P<0.05) with B-supplementation (5 to 20 ppm) in rats fed on both Ca-adequate and Ca-deficient diets. The increased luminal concentration of Ca is known to induce gastric acid secretion either indirectly through gastrin release or directly through parietal cell activation (Kopic and Geibel 2013). This might have enhanced the digestive process to facilitate higher digestibilities of nutrients in B-supplemented rats (Table 3). The crude fibre digestibility in LC group was higher (P<0.05) than control, due to the fact that low Ca intake could lower faecal fat, as Ca in diet has negative

Table 2. Performance of rats of different dietary groups

Attribute	Normal-Ca diets					Low-Ca diets		SEM	P-value	Contrasts‡			
	NC (A)	NCB-5 (B)	NCB-10 (C)	NCB-20 (D)	NCB-40 (E)	LC (F)	LCB-40 (G)			B	A *v.* F	A *v.* G	F *v.* G
Feed intake (g/rat/day)	14.0	14.9	14.0	14.7	13.7	13.9	13.7	0.19	NS	NS	NS	NS	NS
Water intake (ml/rat/day)	76.0	75.0	84.9	83.5	83.4	75.1	79.9	3.94	NS	NS	NS	NS	NS
Body weight													
Initial BW (g)	59.5	59.3	59.6	58.8	58.8	60.0	58.4	1.14	NS	NS	NS	NS	NS
Final BW (g)	294	290	279	267	268	260	286	5.03	NS	NS	*	NS	*
BW gain (g)	235	230	219	208	210	200	228	4.49	NS	NS	*	NS	*
ADG (g)	2.60	2.56	2.44	2.32	2.33	2.22	2.53	0.05	NS	NS	*	NS	*

Each mean value is an average of four replicates. ‡Significant effects of contrast analysis. BW, Body weight; ADG, average daily gain; SEM, standard error of the mean; NS, nonsignificant. *P<0.05.

Table 3. Digestibility of nutrients and serum biochemical profile in rats of different dietary groups

Attribute	Normal-Ca diets					Low-Ca diets		SEM	P-value	Contrasts‡			
	NC (A)	NCB-5 (B)	NCB-10 (C)	NCB-20 (D)	NCB-40 (E)	LC (F)	LCB-40 (G)			B	A *v.* F	A *v.* G	F *v.* G
[1]*Nutrients digestibility (%)*													
DM	89.7^{b}	91.9^{ab}	92.2^{a}	92.7^{a}	91.4^{ab}	91.0^{ab}	91.5^{ab}	0.28	NS	**	NS	*	NS
OM	91.6^{c}	93.3^{ab}	93.8^{ab}	94.5^{a}	93.0^{abc}	93.0^{abc}	92.3^{bc}	0.22	*	**	*	NS	NS
CP	93.6^{b}	95.2^{a}	94.6^{ab}	95.0^{ab}	93.8^{ab}	94.5^{ab}	93.8^{ab}	0.19	NS	*	NS	NS	NS
CF	93.5^{b}	96.0^{a}	94.5^{ab}	95.6^{ab}	95.7^{ab}	95.9^{ab}	94.7^{ab}	0.29	NS	*	*	NS	NS
[2]*Biochemical parameters*													
Glucose (mg/dl)	89.8	82.9	69.4	72.7	80.2	92.3	88.2	3.3	NS	NS	NS	NS	NS
Triglycerides (mg/dl)	123^{a}	104^{ab}	113^{ab}	102^{ab}	87^{b}	110^{ab}	106^{ab}	3.14	NS	**	NS	NS	NS
Cholesterol (mg/dl)	75.0	70.8	77.2	63.3	74.2	80.2	65.8	3.18	NS	NS	NS	NS	NS
HDL-Cholesterol (mg/dl)	53.0^{a}	48.4^{ab}	45.4^{ab}	41.2^{b}	45.7^{ab}	54.1^{a}	48.6^{ab}	1.05	*	**	NS	*	*
ALP (KA units)	38.8	30.8	29.9	36.8	38.3	43.0	38.9	1.82	NS	NS	NS	NS	NS
ALT (IU/l)	69.8^{ab}	46.7^{bc}	37.4^{c}	55.3^{abc}	59.0^{abc}	62.6^{abc}	77.8^{a}	3.44	NS	**	NS	NS	NS
AST (IU/l)	139	124	131	130	151	153	151	5.02	NS	NS	NS	NS	NS

[1]Each mean value is an average of four replicates. [2]Each mean value is an average of eight replicates. DM, dry matter; OM, organic matter; CP, crude protein; CF, crude fat; HDL-Cholesterol, High density lipoprotein-cholesterol; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; SEM, standard error of the mean; NS, nonsignificant, ‡Significant effects of contrast analysis. ^{abcd}Means with different superscripts in a row differ significantly. *P<0.05 and **P<0.01.

effect on fat digestibility (Buchowski *et al*. 2009).

The serum glucose level between the dietary groups did not vary. However, it was reported that dietary B decreases the peak pancreatic *in situ* insulin release in chicks to maintain plasma glucose (Bakken and Hunt 2003). The lowest serum level of triglycerides observed in the NCB-40 group was in agreement with the earlier reports in rats (Hall *et al*. 1989) and pigs (Armstrong and Spears 2001). The present findings indicate a plausible role of B in energy substrate utilization, which was also previously reported (Hunt *et al*. 1994). The decreased serum levels of both total cholesterol and HDL-cholesterol in the NCB-20 group (Table 3) was also previously reported in rats (Naghii and Samman 1997). The unaltered serum levels of ALP and AST among the dietary groups had been previously reported in rats (Zafar and Ali 2003). However, elevated ALP activity observed in the LC group may be possibly attributed to bone depletion caused by dietary Ca restriction (Table 3). Lower serum level of ALT in B-supplemented groups (5 to 40 ppm) was reported in rats (Zafar and Ali 2003), thus suggesting the role of B in promoting hepatic health. The elevated serum levels of ALT and AST in rats fed Ca restricted diets could be due to hepatic tissue changes and abiotic stress induced by lower dietary Ca intake. Dietary

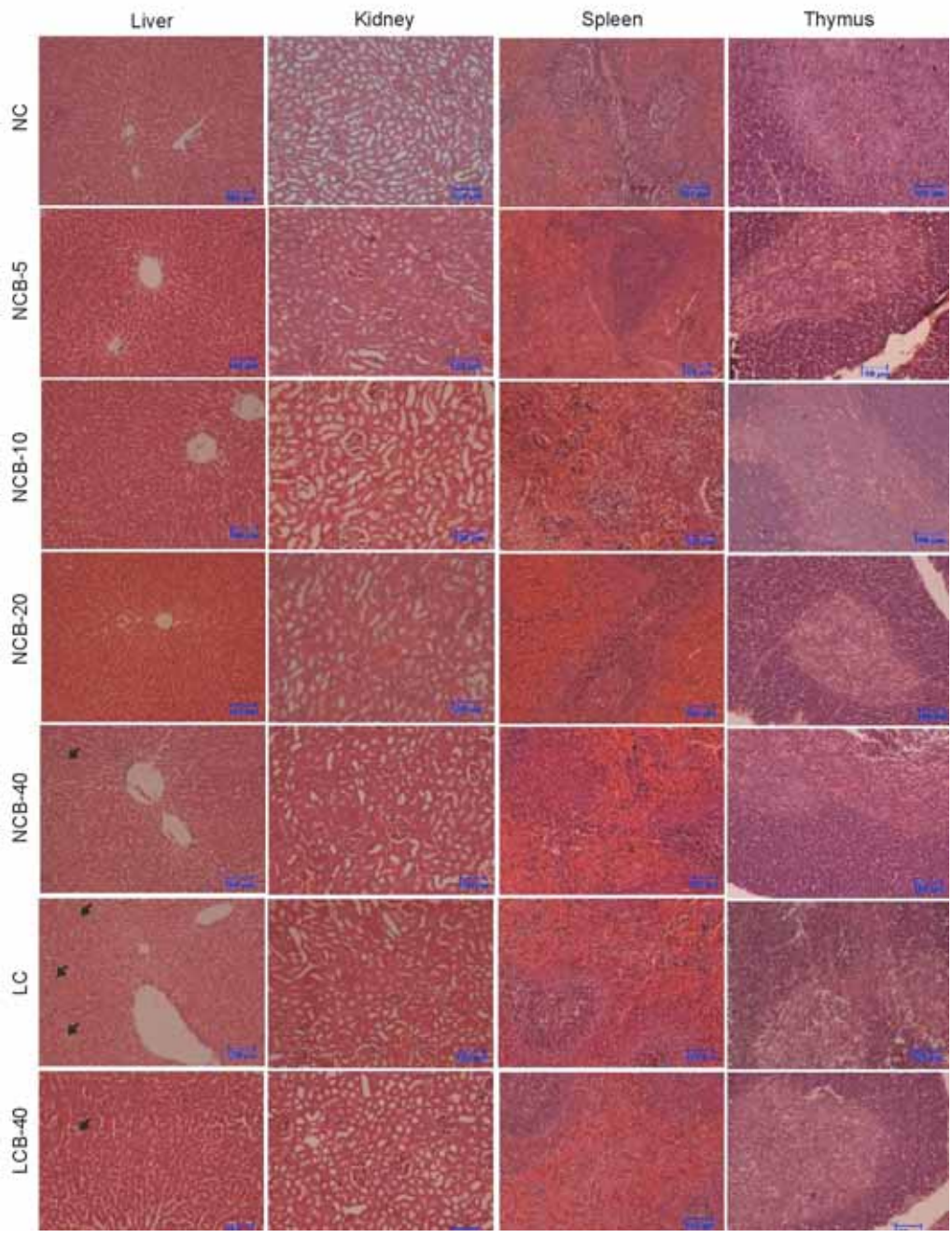

Fig. 1. Photomicrograph showing tissue architecture of liver, kidney, spleen and thymus of rats in different dietary groups. Hematoxylin and Eosin staining. Scale bars = 100 µm. Arrows indicate vacuolar degeneration and nuclear condensation in liver of dietary groups (NCB-40, LC, and LCB-40).

B-supplementation is reported to modulate the gene expression of superoxide dismutase enzyme in rats (Vijay Bhasker *et al*. 2016). Boron supplementation to dairy cows showed lipotrophic effect and improved energy status in postparturient period (Bosoglu 2017).

The histopathological investigation of liver tissue revealed that B-supplementation did not induce any pathological changes in liver (Fig. 1). Similarly, oral administration of borax @ 1000 mg/kg/d did not cause any pathological changes in vital organs of cows (Kabu and Uyarlar 2015). Hence the mild degenerative changes in liver observed in the NCB-40 group might be caused by altered Ca homeostasis rather than the B element itself. In the LC group, severe degenerative changes were observed in hepatocytes with loss of cellular architecture, distortion of hepatic cords and dilatation of the central vein. This might be due to the fact that reversible cell injury occurs when cytosolic Ca is increased with the enhanced release of Ca from mitochondria and endoplasmic reticulum under Ca deficit conditions. The severity of degenerative changes observed in the LC group was reduced in LCB-40 to milder forms similar to that observed in the NCB-40 group. This suggests that 40 ppm B-supplementation has an ameliorative effect on pathology induced by dietary Ca restriction. Boron (30 ppm) supplementation prevented degenerative changes in liver and kidney of rabbits fed high level of protein and energy (Baspinar *et al*. 2015). The histological sections of the kidney did not reveal any pathological changes (Fig. 1). However, earlier reports have indicated pathological effects in kidney of rats induced by fluoride toxicity (Krishnamoorthy *et al*. 2015) and Malathion toxicity (Coban *et al*. 2015) were ameliorated with B-supplementation at the level of 50 ppm and 20 ppm, respectively. Also, the B-supplementation (100 mg/l) in drinking water showed a positive effect on the development and microstructure of immune organs (spleen and thymus) in broilers (Jin *et al*. 2014) and 40 mg/l improved spleen tissue structure in rats (Hu *et al*. 2014). However, in the present study, the anatomical architecture of the spleen and thymus remained unaltered among the dietary groups (Fig. 1).

Hence it can be concluded that B-supplementation in diets of Wistar rats showed positive influence on digestibility of dry matter, crude protein, crude fat and growth performance. Further B-supplementation lowered the levels of serum triglycerides, HDL-cholesterol, ALT and ameliorated the hepatic tissue alterations induced by the lower Ca intake, thus exhibiting hepato-protective effect.

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