B-4493
[1-5]

RESEARCH ARTICLE Indian Journal of Animal Research

Boron Supplementation to Calcium Inadequate Diet Influences Mineral Content of Serum, Tissue and Antioxidant Status in White Leghorn Layers

Adarsh Vijay, N.K.S. Gowda, D.T. Pal, Debpriyo Kumar Dey,
S. Karthik Bhat, A.V. Elangovan

10.18805/IJAR.B-4493

ABSTRACT

Background: Boron (B), a novel micronutrient is known to influence utilization of macrominerals. Present study was conducted in layer birds to study the effect of B supplementation to inadequate calcium (Ca) diet on mineral content in serum, tissue and antioxidant status.

Methods: A total of 80 commrcial White Leghorn layers, 25 weeks old with a uniform body weight were randomly assigned to one of the 4 groups of 20 hens in each dietary group *viz*., Normal calcium (NC), inadequate/low calcium (LC), Normal calcium with 40 ppm B (NCB) and inadequate/low calcium with 40 ppm B (LCB). Dietary level of calcium was maintained at 100 and 90% of the requirement and sodium borate was used as B source. At the end of feeding trial, blood and tissue samples were collected for estimating mineral and biochemical parameters.

Result: Boron supplementation did not significantly alter the serum mineral content, serum alkaline phosphatase and total antioxidant activity but significantly (P<0.025) increased the superoxide dismutase activity in layers fed inadequate Ca diet. Boron supplementation to both diets significantly (P<0.03) increased phosphorus (P) content in bone and muscle, and magnesium (Mg) content in muscle (P<0.019). Boron supplementation to Ca inadequate diet significantly (P<0.006) enhanced the P and Mg content in liver. Content of B was significantly (P<0.01) higher in serum, bone and liver of hens supplemented 40 ppm of B. Implication of this study is amelioration of abiotic stress due to inadequate Ca intake with B supplementation. It is concluded that 40 ppm B supplementation positively influenced the Ca, P and Mg utilization and serum SOD activity.

Key words: Bone, Egg, Enzymes, Liver, Micronutrient, Sodium borate.

INTRODUCTION

Reports suggest a possible role of Boron (B) in various physiological functions, especially in improving the utilization of minerals like calcium (Ca), phosphorus (P) and magnesium (Mg) (Vijay Bhasker *et al*., 2017). Boron's distinctive chemical properties allow it to form complexes with organic molecules to influence cellular activity (Park *et al*., 2005). Reports indicate that dietary B is beneficial in improving Ca level of serum and tibia in broiler chicks fed diets deficient in Vitamin D and Ca (Bozkurt *et al*., 2012). Multiple roles of B in animal biology still needs further investigation, especially during nutrition stress. Laying hens during peak of egg production are more responsive to dietary changes. Hence, this study was undertaken in layers to understand the influence of B supplementation to diet with inadequate Ca level.

MATERIALS AND METHODS

Experimental design, management and diets

The study was conducted at ICAR-National Institute of Animal Nutrition and Physiology (NIANP), Bengaluru, India during the year 2017 (April-July). The animal experimental protocol was approved by Animal Ethics Committee of Institute. Eighty White Leghorn breed of layers of 23 weeks of age were procured. During the experiment, the hens were maintained in a well-ventilated layer house, open sided with windows covered with wire mesh. Hens were reared in individual laying cages (Size: 35cm x 35cm x 40cm) fitted with individual feeder and waterer. They were provided with minimum of 12 h artificial lighting. Birds were fed with *ad libitum* standard layer diet till 24 wk of age and then shifted to experimental diet from 25th wk of age. Birds were randomly assigned to four treatment groups and each treatment group had 20 hens. The experiment was conducted with corn-soybean meal based layer diet (CP: 161g/kg, ME: 11.2 MJ/kg) with a

ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru-560 030, Karnataka, India.

Corresponding Author: N.K.S. Gowda, ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru-560 030, Karnataka, India. Email: nksgowda@rediffmail.com

How to cite this article: Vijay, A., Gowda, N.K.S., Pal, D.T., Dey, D.K., Bhat, S.K. and Elangovan, A.V. (2021): Boron Supplementation to Calcium Inadequate Diet Influences Mineral Content of Serum, Tissue and Antioxidant Status in White Leghorn Layers. Indian Journal of Animal Research. DOi: 10.18805/IJAR.B-4493.

Submitted: 22-04-2021 **Accepted:** 24-06-2021 **Online:** 10-09-2021

2x2 factorial design in a completely randomized design consisting of two diets with adequate in calcium (Normal calcium, NC) and inadequate in Ca (Low Ca, LC), and another 2 diets supplemented with 40 mg/kg of B to diet adequate in Ca (NC+B) and inadequate in Ca (LC+B), over and above basal level of B. Calcium level (35g/kg) in the standard layer diet was maintained (NC) as per ICAR recommendations (2013) and Ca level was reduced by 10% for Ca-inadequate diet group(LC). Sodium borate ($Na_2B_4O_7.10H_2O$, Feed grade) was used as B source.

Collection of blood and tissue sample

At the end of the 16 wk of feeding trial, eight birds from each of the four dietary groups were randomly selected and blood samples were collected by cardiac puncture and placed in to blood collection tubes with no anticoagulant. After one hour, the serum was separated by centrifugation at 1800 x g and stored in deep freezer for further analysis. After blood collection, the same birds were sacrificed by cervical dislocation. Tibia bone and soft tissues (breast muscle, liver) collected were excised and all flesh and proximal cartilage was removed and washed with phosphate buffer saline, collected in polythene covers and preserved in deep freezer.

Estimation of serum minerals, biochemicals and total antioxidant activity

Serum samples were digested as per procedure described by Kolmer *et al.* (1951). 1 ml of serum sample with equal volume of concentrated nitric acid was mixed in the digestion tube. The samples were kept overnight at room temperature followed by digestion on low heat (70-80°C) using heating bench, until the volume of samples was reduced to about 1ml. To this 3ml of double acid mixture (3 parts concentrated HNO_3 and 1 part 70% H_2SO_4) was added and digestion continued until the digested samples became watery clear. Final volume of filtrate was made up to 10 ml with Millipore water. Minerals (Ca, P, Mg, B) in digested serum samples were analyzed using inductively coupled plasma optical emission spectrometer (ICP-OES).

The serum alkaline phosphatase (ALP) was estimated with p-nitrophenyl phosphate (p-NPP) substrate method (Linhardt and Walter, 1965), Superoxide dismutase (SOD) activity in serum was determined by the method on the ability of the enzyme to inhibit the auto-oxidation of pyrogallol (Marklund and Marklund, 1974).Total antioxidant activity (TAA) in serum was measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1999). For estimating total antioxidant activity in liver, frozen samples were thawed at room temperature and around 1.0g of liver sample was minced in homogenizer and prepared 10% tissue homogenate in PBS buffer and then centrifuged at 1800 x g for 15 minutes. The supernatant was collected and used for the analysis of TAA.

Estimation of minerals in bone and soft tissue

The samples (tibia, breast muscle and liver) were thawed at room temperature. The soft tissues (liver and breast muscle) were taken in pre- weighed silica crucible and oven dried at 80°C for 24h, later subjected to de-carbonization followed by ashing at 550-600°C for 4h. The residue left in the crucible was taken as total ash which was digested with 5N HCl on a hot plate for 15 minutes and then cooled. The digested sample was then filtered through Whatman filter paper No: 41 in to a 100 ml volumetric flask followed by washing with deionized water and volume was made up to the mark.

The tibia bones of experimental birds were boiled in de-ionized water at 80°C for 30 min and cooled. Later, muscle tissues attached to bone were manually removed and air dried for 24h. Bone samples were solvent extracted in petroleum ether at 60°C for 8h. Mineral extract from bone was prepared as mentioned above. The minerals (Ca, P, Mg and B) were estimated using ICP-OES.

Statistical analysis

The data of the present study was analyzed using Statistical Package for Social Sciences with two-factorial ANOVA using the GLM procedure found in SPSS (2009) software.

RESULTS AND DISCUSSION

Chemical composition of diets

The basal diet contained 11.26 MJ/kg ME and 161 g/kg CP in all the groups and is as per the recommended level for layers. The estimated mineral content in all 4 diets were: Diet 1, Normal calcium diet (NC): Ca 34.6 g/kg, P 6.00 g/kg, Mg 4.60g/kg, and B 19.7 ppm; Diet 2, Low calcium diet (LC): Ca 31.3 g/kg, P 6.50 g/kg, Mg 5.10 g/kg, and B 18.8 ppm; Diet 3, Normal calcium diet with 40 mg/kg B supplementation (NC+B): Ca 34.8 g/kg, P 6.10 g/kg, Mg 4.80 g/kg, and B 57.8 ppm; Diet 4, Low calcium diet with 40 mg/kg B supplementation (LC+B): Ca 31.6 g/kg, P 6.30 g/kg, Mg 5.00 g/kg, and B 57.9 ppm, respectively.

Mineral content in serum, bone, muscle and liver

The data on mineral content in serum of layer birds is presented in Table 1. In serum, macro minerals such as Ca, P and Mg concentrations did not differ significantly among different dietary groups, while the B content was significantly ($P<0.01$) higher in groups supplemented with 40 ppm B irrespective of level of Ca. Statistically, similar level of serum Ca, in spite of varied dietary Ca intake in the dietary groups might be due to the homeostatic mechanism involving bone and blood. Kurtoglu *et al*. (2002) reported that B supplementation (50, 100, 150, 200 and 250 mg/kg) in laying hens increased serum levels of Ca and P. Similarly, Eren *et al*. (2004) reported that the serum Ca, inorganic P and Mg levels were elevated in the laying hens fed on diets supplemented with boric acid for 8 weeks. Olgun *et al*. (2012) observed that the addition of boron (60, 120 and 240 mg/kg) to layer ration significantly increased plasma B concentration. Amina *et al*. (2017) reported that supplementing B at 100, 200 and 300 ppm significantly increased plasma Ca, P and B

concentrations in laying hens. The increase of minerals in plasma was attributed to the interaction of B with macro-elements (Ca, P,Mg), thereby modifying their concentration in the plasma of chickens (Kurtoglu *et al*., 2001, 2005; Bozkurt *et al*., 2012). Moreover, Kucukyilmaz *et al*. (2017) confirmed that the addition of B to the diet resulted in significant increase in B concentration of serum. In contrast, Kaya and Macit (2018) reported that B supplementation did not change serum P and Mg level, while decreased serum Ca content in laying hens. Kabu *et al*. (2013) reported that dietary sodium borate @ 30 g/day in Holstein cattle resulted in higher serum concentrations of Ca and Mg at the time of calving and prevented metabolic disorders. This indicates that B has sufficient influence on Ca,P and Mg utilization.

Boron supplementation to diets increased the P content in bone (P<0.03). The B content in bone was significantly (P<0.01) higher in both the groups supplemented with 40 ppm B with significant (P<0.02) interaction with Ca levels, indicating that bone is a major storage site of B similar to Ca (Table 2). During the active phase of egg laying, much of the Ca in blood is diverted for egg shell formation and this is the probable reason for no significant change in Ca content of bone. The data on mineral content in breast muscle of layer birds is presented in Table 4. Boron supplementation did not significantly influence the Ca and B content of muscle. However, it significantly increased P (P<0.001) and Mg (P<0.019) content of muscle, indicating better gut absorption of P and Mg due to B supplementation (Table 3).

Boron supplementation to Ca inadequate diet increased the Ca content in liver (Table 4). Feeding diet with inadequate Ca reduced the Ca content in liver and was restored to control value upon B supplementation with significant Ca x B interaction (P<0.04). Feeding diet with inadequate Ca resulted in higher P (P<0.006) and Mg (P<0.001) content in liver, indicating better P and Mg utilization when Ca intake is less. The P (P<0.003) and Mg (P<0.021) content in liver was further increased with B supplementation, confirming that B promotes P and Mg deposition in liver. The interaction effect for Ca (P<0.004) and B (P<0.001) was significant, indicating that B and Ca has a close relationship.

As per the reports, B is a regulator of mineral metabolism and it is beneficial for Ca metabolism. Hunt *et al*. (1994) reported that chicks fed on diets with 15.60 ppb (adequate) Vitamin D_3 supplemented with B exhibited enhanced content of macro minerals in bone compared to those fed on diet having inadequate Vitamin D_3. Kurtoglu *et al*. (2007) reported that B supplementation in laying hen diets at 50, 100, 150, 200 and 250 mg/kg decreased the tibial Ca level, while gradually increasing the B concentration up to seven-fold without impacting the bone ash. Bozkurt *et al*. (2012) concluded that B supplementation @ 30 ppm was effective in increasing the Ca, P and ash content of tibia bone in total rearing period of 1-42 days of broiler birds fed on diets deficient in Ca and P.

Table 1: Effect of boron supplementation on serum mineral content

Treatment	Ca (mg/dL)	P (mg/dL)	Mg (mg/dL)	B (ppm)
NC	24.2	5.47	4.14	0.41
LC	20.8	5.16	3.64	0.24
NC+B	24.1	5.10	4.07	1.46
LC+B	24.9	5.13	3.94	1.57
SEM	0.77	0.15	0.10	0.13
Main effects				
Ca level				
Adequate	24.2	5.28	4.10	0.93
Inadequate	23.1	5.14	3.81	1.00
B level (mg/kg)				
0	22.7	5.34	3.92	0.34[a]
40	24.5	5.11	4.01	1.51[b]
P-value				
Ca	0.401	0.649	0.115	0.856
B	0.197	0.522	0.551	0.001
Ca x B	0.173	0.585	0.349	0.374

NC:Normal calcium (35.0 g/kg); LC: Low calcium (31.5 g/kg); NC+B: Normal calcium plus 40ppm boron; LC+B:Low calcium plus 40ppm boron

[a,b]Means bearing different superscripts in same column differ significantly

Table 2: Effect of boron supplementation on bone mineral content.

Treatment	Ca (%)	P (%)	Mg (%)	B (ppm)
NC	25.1	10.5	0.37	5.22
LC	23.5	9.4	0.36	4.80
NC+B	24.1	11.3	0.34	21.5
LC+B	24.2	11.1	0.37	23.7
SEM	0.55	0.30	0.01	1.63
Main effects				
Ca level				
Adequate	24.6	10.9	0.36	13.3
Inadequate	23.7	10.3	0.37	14.9
B level (mg/kg)				
0	24.3	9.99[a]	0.37	5.02[a]
40	24.1	11.2[b]	0.36	22.6[b]
P-value				
Ca	0.44	0.27	0.70	0.09
B	0.93	0.03	0.45	0.001
Ca x B	0.39	0.52	0.30	0.02

[a,b]Means bearing different superscripts in same column differ significantly.

Serum biochemicals and total antioxidant activity

Boron supplementation to diets did not significantly alter the TAA in serum and liver, as well as ALP activity in serum (Table 5). However, the reduced SOD activity due to feeding inadequate Ca diet was ameliorated with B supplementation and was restored to control level. Elkomy *et al*. (2015)

reported that supplementing B as boric acid to rabbit significantly increased total antioxidant capacity. El-Saadany *et al*. (2016) reported that supplementing B @ 100 mg/kg in male chicks improved total antioxidant capacity. Turkez *et al*. (2007) reported that SOD activity was significantly higher in erythrocyte of human peripheral blood culture exposed to 15 ppm borax. However, few studies indicated that there was no significant difference in SOD activity of erythrocytes (Ince *et al*., 2010) and liver tissues (Zafar and Ali, 2013) with borax supplementation. Vijay Bhasker *et al*. (2016) reported that boron supplementation improved SOD activity in rats by upregulating SOD genes. Elkomy *et al*. (2015) reported that supplementing boron as boric acid at 100 mg/kg feed in rabbit bucks increased SOD activity.

Table 3: Effect of boron supplementation on muscle mineral content.

Treatment	Ca (ppm)	P (ppm)	Mg (ppm)	B (ppm)
NC	191	1412	270	0.53
LC	116	968	284	0.66
NC+B	251	2053	263	2.31
LC+B	468	2067	250	2.20
SEM	56.9	135	4.44	0.52
Main Effects				
Ca level				
Adequate	221	1733	267	1.42
Inadequate	292	1518	267	1.43
B level (mg/kg)				
0	154	1190^{a}	277^{b}	0.59
40	360	2060^{b}	257^{a}	2.25
P-value				
Ca	0.521	0.349	0.962	0.997
B	0.069	0.001	0.019	0.122
Ca x B	0.190	0.318	0.111	0.911

^{a,b}Means bearing different superscripts in same column differ significantly.

Table 4: Effect of boron supplementation on liver mineral content.

Treatment	Ca (ppm)	P (ppm)	Mg (ppm)	B (ppm)
NC	131^{b}	2289^{a}	186^{a}	1.90^{b}
LC	79.7^{a}	3325^{b}	255^{b}	1.82^{b}
NC+B	91.5^{ab}	3400^{bc}	235^{b}	2.16^{a}
LC+B	123^{b}	4330^{c}	268^{b}	2.52^{c}
SEM	7.45	202	8.26	0.11
Main Effects				
Ca level				
Adequate	111	2845^{a}	210^{a}	1.63^{a}
Inadequate	101	3828^{b}	262^{b}	2.17^{b}
B level (mg/kg)				
0	105	2807^{a}	220^{a}	1.86
40	107	3865^{b}	252^{b}	2.32
P-value				
Ca	0.453	0.006	0.001	0.004
B	0.905	0.003	0.021	0.070
Ca x B	0.004	0.873	0.173	0.001

^{a,b,c}Means bearing different superscripts in same column differ significantly.

Table 5: Effect of boron supplementation on TAA and serum enzyme activity.

Treatment	TAA (µm/ml) (serum)	TAA (µm/gm) (liver)	ALP (µmoles/ml) (serum)	SOD (U/ml) (serum)
NC	52.8	4.13	123	46.6^{b}
LC	33.4	2.50	139	37.6^{a}
NC+B	46.2	4.16	187	43.2^{ab}
LC+B	43.1	3.37	131	45.7^{b}
SEM	3.15	0.31	14.7	1.31
Main effects				
Ca level (%)				
Adequate	49.5	4.15	147	44.9
Inadequate	38.9	2.95	135	41.7
B level (mg/kg)				
0	43.1	3.33	129	42.1
40	44.4	3.77	159	44.5
P-value				
Ca	0.083	0.066	0.533	0.192
B	0.793	0.462	0.383	0.336
Ca x B	0.195	0.486	0.268	0.025

^{a,b}Means bearing different superscripts in same column differ significantly

CONCLUSION

From the results, it is concluded that 40 ppm B supplementation positively influenced the tissue deposition of Ca, P, Mg and serum SOD activity and the response was enhanced when B was supplemented to Ca inadequate diet.

ACKNOWLDEGEMENT

The authors thankfully acknowledge the support of Director, ICAR-NIANP, Bengaluru in conducting this work.

REFERENCES

Amina, S. El-Saadany., Effat, Y. Shreif and Amal, M. EL-Barbary. (2017). The influence of dietary boron supplementation on performance and some physiological parameters in Bandarah chickens 2- laying period. Egyptian Poultry Science Journal. 37: 105-122.

Benzie, I.F. and Strain, J.J. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurements of total antioxidant power and ascorbic acid concentration. Methods in Enzymology. 299: 15-27.

Bozkurt, M., Kuçukyılmaz, K., Catli, A.U., Cinar, M., Cabuk, M. and Bintaþ, 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 Science. 25: 248-255.

Elkomy, E.A., Abd El-hady, A.M. and Elghalid, O.A. (2015). Dietary boron supplementation and its impact on semen characteristics and physiological status of adult male rabbits. Asian Journal of Poultry Science. 9: 85-96.

El-Saadany, S., Amina., Amal, M., El-Barbary. and Effat, Y. Shreif. (2016). The influence of dietary boron supplementation on performance and some physiological parameters in Bandarah chikens 1-Growing period. Poultry Science. 36: 1131-1146

Eren, M., Uyanik, F. and Kucukersan, S. (2004). The influence of dietary boron supplementation on egg quality and serum calcium, inorganic phosphorus, magnesium levels and alkaline phosphatase activity in laying hens. Research in Veterinary Science. 76: 203-210.

Hunt, C.D., Herbel, J.L. and Idso, J.P. (1994). Dietary boron modifies the effects of vitamin D_3 nutrition on indices of energy substrate utilization and mineral metabolism in the chicks. Journal of Bone Mineral Research. 9: 171-182.

Ince, S., Kucukkurt, I., Cigerci, I.H., Fidan, A.F. and Eryavuz, A. (2010). The effects of dietary boric acid and borax supplementation on lipid peroxidation, antioxidant activity, and DNA damage in rats. Journal of Trace Elements in Medicine and Biology. 24: 161-164.

Kabu, M., Birdane, F.M., Civelek, T. and Uyarlar, C. (2013). Affects of boron administration on serum Ca, Mg and P for peripartum cows. Archieves in Animal Breeding. 56: 733-741.

Kaya, H.A. and Macit, M. (2018). The effects of boron (orthoboric acid) supplementation into diets of laying hens on egg shell quality and tibia biomechanic parameters and serum, shell and tibia mineral concentrations during late laying period. Ataturk Universitesi Veteriner Bilimleri Dergisi. 13: 42-53.

Kolmer, J.A., Spanbling, E.H. and Robinson, H.W. (1951). Approved laboratory technique. Appleton Century Crofts, New York.

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. 54: 26-33.

Kurtoglu, F., Kurtoglu, V., Celik, I., Kececi, T. and Nizamlioglu, M. (2005). Effects of dietary boron supplementation on some biochemical parameters, peripheral blood lymphocytes, splenic plasma cells and bone characteristics of broiler chicks given diets with adequate or inadequate cholecalciferol (vitamin D_3) content. British Poultry Science. 46: 87-96.

Kurtoglu, V., Firuze kurtoglu., Sur. E., Bulut. Z. and Onder, F. (2007). Effect of boron supplementation to the diet on tibia mineral concentration, pheripheral blood leucocytes percentage and some selected variables of layers. Archiv fur Geflugelkunde. 71: 13-18.

Kurtoglu, V., Kurtoglu, F. and Coskun, B. (2001). Effects of boron supplementation of adequate and inadequate vitamin D_3-containing diet on performance and serum biochemical characters of broiler chickens. Research in Veterinary Science. 71: 183-187.

Kurtoglu, V., Kurtoglu, F., Coskun, B., Seker, E., Balevi, T. and Cetingul, I.S. (2002). Effects of boron supplementation on performance and some biochemical parameters in laying hens. Rev de Medecine Veterinaire. 53: 823-828.

Linhardt and Walter, K. (1965). Phosphomonoesterases. Methods of Enzyme Analysis. Bergmeyer H U. New York, Academic press. 779-89.

Marklund, S. and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry. 47: 469-74.

Olgun, O., Yazgan, O. and Cufadar, Y. (2012). Effect of supplementation of different boron and copper levels to layer diets on performance, egg yolk and plasma cholesterol. Journal of Trace Elements in Medicine and Biology. 27: 132-136.

Park, M., Li, Q., Shcheynikov, N., Muallem, S. and Zeng, W. (2005). Borate transport and cell growth and proliferation. Cell Cycle. 4: 24-26.

S.P.S.S. (2009). PASW Statistics for Windows, Version 18.0. Chicago, SPSS Inc.

Turkez, H., Geyikoglu, F. and Tatar, A. (2007). Effects of some boron compounds on peripheral human blood. Zeitschrift für Naturforschung C. 62: 889-896.

Vijay Bhasker, T, Gowda, N.K.S., Pal, D.T., Karthik Bhat, S., Krishnamoorthy, P., Mondal, S., Pattanaik, A.K. and Verma, A.K. (2017). Influence of boron supplementation on performance, immunity and antioxidant status of lambs fed diets with or without adequate level of calcium. PLoS ONE. 12(11): doi.10.1371/journal.pone.0187203.

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

Zafar, H., and Ali, S. (2013). Boron inhibits the proliferating cell nuclear antigen index, molybdenum containing proteins and ameliorates oxidative stress in hepatocellular carcinoma. Archives of Biochemistry and Biophysics. 529: 66-74.