

ARTICLE

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Received.

19.02.2025

Accepted: 23.04.2025

Research Article

The Effect of Distance to Boron on Serum Boron Concentration and Some Biochemical and Hematological Parameters in Sheep

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ABSTRACT

Boron is an essential element in relation to underground resources, with Turkey possessing 73.5 per cent of the global boron reserves. Its prevalence in the field of industry has made it the subject of research in the field of health in recent years. There is a very thin boundary between deficiency and toxicity. Its mechanisms in living organisms are not fully understood, therefore, sufficient and complete and consistent data on human and ecosystem health and its place and dose in these areas have not been reached, and data are needed to maintain and increase its importance and necessity in these areas. The amount and duration of boron exposure may increase in boron fields. This is because boron can be found in air, soil and water. This study investigates the changes in boron levels in living organisms as a function of the distance of boron exposure. Statistically significant differences in serum boron, urea, creatinine and BUN levels were observed between different locations. In parallel, statistically significant differences were also observed between some haemogram parameters. The data obtained are valuable with regard to the possible interaction between boron exposure distance and serum boron levels.

Keywords: Boron, Sheep, Serum

Cite this article as: Celebi, C., Susar, H., Celebi, M., Dinc, P., & Karahan, I. (2025). The effect of distance to boron on serum boron concentration and some biochemical and hematological parameters in sheep. *Manas Journal of Agriculture Veterinary and Life Sciences*, 15(1), 16-23. https://doi.org/10.53518/1642635



INTRODUCTION

Boron (B), a chemical element that has been known to the scientific community for centuries, was first discovered in the year 1808. It is positioned in Group 13 of the periodic table and is represented by the chemical symbol B. Boron is classified as a semi-metal and has an atomic number of 5. It can exist in either a crystalline or amorphous state and is known to exhibit different colours. Boron possesses a distinctive set of chemical properties, leading to its characterisation as one of the most intriguing and complex elements after carbon (Balonos et al., 2004; Kuru and Yarat, 2017). Despite its relative scarcity when compared with hydrogen, carbon, nitrogen or oxygen, boron is ubiquitous in the environment, naturally occurring in rocks, soil and water. Concentrations of boron have been reported in soil ranging from 5-150 mg/kg, in seawater from 0.5-9.6 mg/kg, and in air from 0.5-800 ng/m3. It is the 51st most abundant element in the Earth's crust, with an average concentration of 8 mg/kg (approximately 0.0008%). The combination of its metal and nonmetal properties, in conjunction with its high affinity for oxygen, enables it to form diverse compounds.Boron compounds, of which there are more than 250 types, find primary application in industrial domains such as glass production and fiberglass. Furthermore, some of these compounds have found application in the cleaning sector, for example as antiseptics and in soap. In addition to these uses, they have been employed as herbicides and fertilisers in agriculture, and in recent years they have often been the subject of medical research (Uluisik et al., 2018; Ince et al., 2019).

Its pharmacokinetics have been investigated in both humans and animals. Boron, known to be frequently exposed orally, is absorbed from the gastrointestinal tract. It diffuses rapidly into the body by passive diffusion and has a half-life of approximately 24 hours. Excretion is largely through the kidneys by glomerular filtration. It has been reported to accumulate in certain organs such as the brain, bone, testes and liver until excreted (Oğlakcı-İlhan et al., 2023; Onder et al., 2023). The biochemical mechanism for boron, which is found in low amounts in human and animal tissues, is still unknown. However, it has been reported to play an active role in metabolic and physiological functions, tissue regeneration, anti-inflammatory and antioxidant. In addition, it acts as a co-factor for enzymes in bone mineralization and wound healing (Kuçukkurt et al., 2023; Abdelnour et al., 2018). Furthermore, boron neutron capture therapy (BNCT), which was initially employed in human patients to treat head and neck cancer, is currently being evaluated in animal models of cancer. This is a notable development given the increasing prevalence of cancer, which can be attributed in part to the advancements witnessed in the field of veterinary science (Pizzorno, 2015; Kusaka et al., 2022).

Boron is a trace element that is present in the diet of many organisms. The recommended daily intake of boron for humans is approximately 0.5-1 mg. However, as with all elements, boron can have adverse effects on living organisms. The nature of these effects can vary depending on the concentration, duration and variety of exposure. It is therefore important to understand the risk/benefit balance associated with boron use and exposure, and to assess the potential health effects of boron intake from diet and drinking water. This has reportedly become a critical component of risk assessment in recent years. Toxicity has been reported to occur at elevated boron levels. For this reason, the World Health Organization (WHO) published in 2011 that drinking water should contain 2.4 ppm boron. The amount of boron that should be taken daily because of nutrition has been reported as 2 mg (Ince et al., 2016; Wang et al., 2018; Nielsen and Eckhert, 2020). Moreover, boric acid is categorised by the European Union as a "Category 1B" compound. This designation signifies that the substance belongs to a group of compounds that have the potential to compromise fertility (Kan and Kucukkurt, 2023; Bolt et al., 2020). Excess boron has been shown to be hazardous when administered in excess. The possible adverse effects include nausea, vomiting, diarrhoea and abdominal pain. Furthermore, symptoms such as seizures, irritability, and oedema have been documented. It is noteworthy that the lethality of a single dose is very low, since the excess is excreted in the urine (Devirian and Volpe, 2003). The known side effects of boron are few and far between. Nevertheless, the findings of recent research have served to underscore the significance of this issue.

Urea is a product of protein metabolism and is considered to be one of the quality indicators of the filtration process of the kidney, with BUN (blood urea nitrogen) being another such indicator. Creatine, a by-product of muscle metabolism, has been shown to be related to muscle mass and kidney function in living organisms. Hematologic parameters are of great importance in the detection of disease and metabolic disorders, and they



are also used to monitor the side effects of drugs and drug-like substances. Routinely measured parameters are also useful in explaining changes in mechanisms involved in physiological events.

In the light of all the information given in the literature, in this study, it was thought that blood boron levels, some biochemical and hematological parameters would differ in sheep raised in regions where boron exposure is thought to be at different levels. For this hypothesis, 2 different regions were determined as close and far from the boron mine site. It was aimed to measure blood boron levels, hematological parameters, urea, creatine, BUN by taking blood from sheep living in these regions.

MATERIALS AND METHODS

Animals and Sample Collection

Two locations were determined for the hypothesis. The first study location was the Bigadiç district, where a boron mine is located. The second location was the centre of Balıkesir province. Blood was taken from a total of 20 sheep. The sheep were checked for pregnancy and general health status. The neck area was shaved and two tubes were taken from the vena jugularis. One tube was used to obtain whole blood (for haemogram) and the other tube was used to obtain serum (for urea, creatine, BUN and boron concentration).

Biochemical and Hematological Analysis

The collected whole blood samples were kept in cold chain and analyzed within 2 hours and without clotting/disintegration. Blood tubes were carefully placed in the VETMAC VH30 Hemogram Device to obtain information on WBC, LYM#, MID#, GRAN#, LYM%, MID%, GRAN%, NLR, PLR, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, PDW-CV, PDW-SD, PCT, P-LCC, P-LCR parameters.

After the other blood tubes were collected from the animals, they were transported in cold chain and serum was separated by centrifugation at 4000 rpm for 5 minutes (ISOLAB). Serum samples were collected in double-redundant 2 ml ependorfs and stored at -18°C until analysis. Serum samples for urea, creatine and BUN analysis were delivered to BALLAB without breaking the cold chain. These analyzes were performed as a service procurement and the results were obtained (ERBA, XL 2000).

Boron Analysis

Serum samples for determination of boron concentration were thawed at +4. Then the sample volume was determined and taken into the container and nitric acid and hydrogen peroxide were added according to the method. It was left to heat under a fume hood, taking care not to boil. The samples purified from organic substances were then taken into a balloon jug and the volume was completed to 10 ml by adding ultrapure water. The prepared samples were analyzed in ICP-OES device at Balıkesir University Science and Technology Application and Research Center (Perkin-Elmer OPTIMA7300) (Tokay and Bagdat, 2022a ve 2022b).

Statistical Analysis

In the study, Kolmogorov-Smirnov test was applied for normality assumptions. Independent t-test, one of the parametric tests, was applied to determine whether there was a significant difference between the groups. The data obtained were analysed using SPSS 25.0 (IBM Corp., Armonk, NY, USA). In case of a significant difference between the groups, post-Hoc test was used to determine between which groups there was a significant difference. p<0.05 was considered statistically significant.

RESULTS

Blood samples taken from sheep in the Centre group and Bigadiç group selected from the region close to the boron mine site were analyzed. The proximity to the mine site was determined by the high boron levels in the Bigadiç group. The statistically significant difference between the groups is shown (*Figure 1*).



Figure 1. Serum boron levels (mg/L) of the animals in the Centre and Bigadiç groups.

Urea, creatine and BUN were measured in the sera obtained from the blood collected from the Bigadiç group and the Centre group. Significant differences in these parameters were determined statistically and are shown below (Figure 2).



Figure 2. Serum urea, BUN and creatinine levels (mg/L) of the animals in the Centre and Bigadiç groups.

Whole blood samples taken from the sheep were analyzed considering the time period. There were no differences between the groups in most of the parameters, but GRAN%, NLR, RDW-CV, PLT and MPV values showed statistically significant differences. All parameters are shown in the table (Table 1).



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	Bigadiç		Centre		
	Mean±SD	Min-Max	Mean±SD	Min-Max	Р
WBC (10^9/L)	32.90±4.64	15.69-58.88	28.96±4.32	9.46-49.32	0.542
LYM# (10^9/L)	21.01±2.41	12.00-36.18	17.12±2.53	5.90-30.09	0.281
MID# (10^9/L)	6.36±1.23	1.29-12.33	4.41 ± 0.84	1.22-9.22	0.209
GRAN# (10^9/L)	6.98±1.17	2.01-14.19	7.43 ± 1.08	2.34-13.26	0.784
LYM% (%)	0.64 ± 0.02	0.56-0.78	0.59 ± 0.01	0.54-0.66	0.076
MID% (%)	0.14 ± 0.01	0.08-0.21	0.14 ± 0.01	0.11-0.18	0.970
GRAN% (%)	0.20 ± 0.01	0.08-0.26	0.26 ± 0.01	0.18-0.31	0.014*
NLR	0.32±0.03	0.11-0.45	0.44±0.03	0.28-0.58	0.022*
PLR	28.84 ± 4.21	16.13-52.08	32.93±6.42	14.97-67.80	0.602
RBC (10^12/L)	10.72±0.34	9.60-12.85	9.75±0.38	8.17-11.37	0.079
HGB (g/L)	104.6±3.58	92-128	97.40±3.81	80-114	0.185
НСТ	0.36 ± 0.01	0.31-0.43	0.33 ± 0.01	0.28-0.38	0.141
MCV (fL)	33.95±0.34	32.50-35.80	34.74±0.38	32.90-36.70	0.143
MCH (pg)	9.83±0.12	9.30-10.40	10±0.15	9.50-11	0.402
MCHC (g/L)	291±0.96	283-294	287.5±2.36	274-299	0.187
RDW-CV (%)	0.20 ± 0.001	0.20-0.21	0.19 ± 0.002	0.18-0.20	0.002*
RDW-SD (fL)	25.71±0.39	23.40-27.60	25.34±0.27	23.70-26.90	0.426
PLT (10^9/L)	568.2 ± 46.87	331-757	436.4±27.58	296-592	0.026*
MPV (fL)	4.32±0.03	4.10-4.50	4.57±0.10	4.10-5.00	0.033*
PDW-CV (%)	0.14 ± 0.08	0.11-0.18	0.15 ± 0.01	0.11-0.20	0.519
PDW-SD (fL)	9.60±1.96	4.70-26.20	8.21±0.97	4.40-12.40	0.533
PCT (%)	2.27±0.23	1.19-3.20	1.98 ± 0.10	1.40-2.40	0.275
P-LCC (10^9/L)	171.7±11.92	107-218	147.8±6.20	121-187	0.092
P-LCR (%)	0.30 ± 0.08	0.27-0.35	0.34±0.02	0.21-0.41	0.127

Table 1. Blood parameters of the animals in the Centre and Bigadic groups.

¹Analysis results were considered significant for p values less than 0.05.

²Standart deviation, Min: Minumum, Max: Maximum, WBC: White blood cell, LYM: Lenfosite, MID: Monosite, GRAN: Granulosite, NLR: Neutrophil lymphocyte ratio, PLR: Platelet-lymphocyte ratio RBC: Red blood cell, HGB: Haemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin concentration, RDW-CV: Red blood cell distribution width, PLT: Platelet count, MPV: Mean platelet volume, PDW: Platelet distribution width, PCT: Procalcitonin, P-LCR: Platelet large cell ratio, P-LCC: Platelet large cell coefficient

DISCUSSION

Boron is an important element whose metabolism is not yet fully understood, especially in oral exposure. Although there are limited studies on its distribution in tissues, it has been reported that it is almost completely absorbed in the gastrointestinal system and frequently excreted in the urine (Kuru and Yarat, 2017). Its effects in living metabolism include osteoporosis and arthritis treatment, antioxidant and memory effects and serious diseases such as cancer. However, toxicity research on boron compounds is ongoing. Therefore, boron exposure is important for all living things today (Pizzorno, 2015). Alterations in hematologic and biochemical parameters are of paramount importance in the diagnosis, severity determination and treatment follow-up of diseases. Furthermore, metabolic events within the body offer researchers invaluable insights into organ function (Etim et al., 2014). The present study investigates the effects of varying distances to the boron mine site on serum boron levels, along with selected biochemical and haematological parameters.

In a study, leukocytes decreased due to gentamicin application and decreased as the dose of boron application increased. This showed that boron, which is claimed to have antioxidant properties, was not sufficient to protect leukocytes. In the same study, it was observed that any dose of boron application had no effect on leukocyte percentages. It was reported that erythrocyte count and haemoglobin levels were not affected by boron concentrations, but the decrease in haematocrit value with gentamicin was prevented by boron. In addition, erythrocyte volume (MCV), mean erythrocyte haemoglobin (MCH), mean erythrocyte haemoglobin level (MCHC) and platelet count were found within normal limits compared to rats (Durmus et al., 2018). In a study investigating the protective effect of boron on blood parameters in experimental cadmium (Cd) toxicity, 15 mg/kg lithium borate (LTB) was given orally for 5 days. In the study, WBC, % neutrophil increased in Cd and



Cd+LTB groups. Lymphocyte and monocyte levels decreased significantly. It was reported that LTB did not cause any change in RBC, haematocrit and haemoglobin levels (Yildirim et al., 2018). In another study, cyclophosphamide (CP) toxicity was induced and the effects of boron on changes in some blood parameters were investigated. In another study, cyclophosphamide (CP) toxicity was induced and the effects of boron on changes in some blood parameters were investigated. In particular, erythrocyte and hemoglobin counts, which decreased with CP treatment, approached the control group with B treatment. While CP alone suppressed leukocytes by 96%, boron significantly reduced the suppression of the affected bone marrow and decreased the leukocyte count by 50% compared to control. A similar result was obtained in platelet count (Cengiz, 2018). In a study investigating the effect of boron application on haematological parameters in dairy cattle in the periparturient period, there was no change in MCHC levels in the prenatal and calving period, but it caused a decrease in the postpartum period. WBC levels did not change for control and boron groups in the periparturient period. No significant change was reported for RBC, MCV, HCT, HB, PLT, MPV, Lymphocyte, Monocyte values (Kabu et al., 2014). In our study, some results were obtained which were in parallel with most of the studies. Although WBC, LYM, MID, LYM%, RBC, HGB, HCT, MCHC, P-LCC, PCT, PDW-SD values decreased between the groups, this difference was not statistically significant. GRAN%, NLR, RDW-CV, PLT, MPV values showed a significant difference between the groups. The increase in some of these values may be due to infection especially in sheep. Therefore, it is recommended to conduct more comprehensive research on whether the difference is due to boron exposure. Expanding the scope of the study in terms of both the number of animals and the parameters examined will greatly facilitate the evaluation of possible results. In addition, the significant difference in urea, creatinine and BUN results in our study was in parallel with the results of some studies. It is thought that this may be due to the boron intake from the water and feed material consumed and the air inhaled due to the small distance to boron sources. The increase in the amount of boron entering the body also affected the parameters by making changes in excretion.

Some blood parameters and blood boron levels were investigated in rabbits following boron administration at different doses. In the study, no statistically significant difference was found in urea and creatinine levels at any level. However, serum B concentrations increased in parallel with the boron doses administered. This was reported as an indication that the boron taken into the body was absorbed and passed into the blood (Yigit et al., 2013). Thirteen healthy women were added boron-containing foods (more than 10 mg) to their diet for 1 month. Increased boron concentrations were observed in blood, urine and saliva samples taken before and after the diet. However, decreases in body weight, body fat weight and body mass index were reported. There is a need to investigate the effects of boron-rich diet on animal health with more detailed studies (Kuru et al., 2019). In a study, pregnant rats were given 0-20 mg B/kg/day (boric acid) by gavage from day 6 to day 21 and pups from day 1 to day 28. It was reported that postnatal weight gain was significantly reduced at 20 mg B/kg and plasma boron concentrations in the offspring increased in proportion to the dose. These findings confirmed that growth was affected by boron exposure (Watson et al., 2020). In a study on boron, which is reported to be risky in terms of developmental toxicity, mothers and babies in a settlement with boron in drinking water between 0.38 and 16.1 mg B/l were examined. Boron concentrations in breast milk and serum showed a strong correlation, while urinary boron concentrations in infants showed an inverse relationship with body weight, head circumference and length (Hjelm et al., 2019). In our study results, a statistically significant elevation was determined between serum boron levels. The elevation in the samples taken from Bigadiç may be due to the small distance to the mine site. Boron concentrations are also high in water, air and soil materials in the living areas near the mine site.

CONCLUSION

In conclusion, the present literature review and study have determined that the distance of exposure to boron is an important factor in the concentration of boron in the blood. Furthermore, although significant differences were found in hemogram and some biochemical parameters in the present study, it should be determined whether this situation is boron-induced with a more comprehensive study. The toxic effects of boron in environmental contamination or its possible beneficial effects for living organisms are very open areas for study. Further research is required to address the existing lacunae in the extant literature pertaining to boron.

NOTE

*This study was presented as an oral presentation at the "7th International Eurasian Conference on Biological and Chemical Sciences", October 02-04, 2024.



ETHICAL APPROVAL

During the writing process of the study titled 'The Effect of Distance to Boron on Serum Boron Concentration and Some Biochemical and Haematological Parameters in Sheep', scientific, ethical and citation rules were followed; no falsification was made on the collected data and this study was not sent to another academic medium for evaluation. Approval for this study was granted by the Balıkesir University Animal Experiments Local Ethics Committee (Approval no. 2024/4-8). Significant efforts were made to minimise animal suffering, with strict adherence to the '3Rs' rule in accordance with established ethical principles.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHOR CONTRIBUTION

All authors contributed equally.

FINANCIAL SUPPORT

This research was supported within the scope of TÜBİTAK-2209 University Students Research Projects Support Programme with Project number 1919B012325412.

DATA AVAILABILITY

The original data collected during the study are included in the article. Further inquiries can be directed to the corresponding author.

APPROVAL FOR PUBLICATION

All authors declare that they have seen and approved the final version of the submitted manuscript.

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