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# The Effects of Various Levels of Boric Acid Supplementation to Drinking Water on Body Weight, Some Biochemical and Antioxidant Parameters of Rat

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ABSTRACT

In this study, the effects of different doses of boric acid (BA) added to the drinking water of rats on body weight, serum antioxidant metabolism, and blood parameters were investigated. A total of 24 female Wistar albino rats, aged 21 days, were used in the study. The experiment was organized into three groups, each consisting of 8 animals: Control, BA-100, and BA-200 groups. The Control group received drinking water with 0 mg/L of BA, the BA-100 group received water containing 100 mg/L of BA, and the BA-200 group received water containing 200 mg/L of BA. The duration of the study was 42 days, including 7 days of adaptation period and 35 days of treatment. At the end of the experiment, blood samples were collected under anesthesia following the sacrifice of the animals. It was determined that boric acid had no significant effect on body weight (BW) or average weight gain (AWG). Serum levels of total protein, creatinine, total cholesterol, low-density lipoprotein (LDL) and high density lipoprotein (HDL) were found to be similar across the Control, BA-100, and BA-200 groups. However, glucose levels were significantly higher in the BA-200 group (354.75 mg/dL), and albumin levels were significantly increased in the BA-100 group (3.23 g/dL). Serum triglyceride levels were significantly lower in the BA-100 (56.00 mg/dL) and BA-200 (59.45 mg/dL) groups compared to the Control group (90.05 mg/dL). No statistically significant differences were observed between the groups in terms of antioxidant parameters, including catalase (CAT) and superoxide dismutase (SOD) activities, as well as glutathione (GSH) and malondialdehyde (MDA) levels. In conclusion, boric acid added to the rats' drinking water at the indicated doses had no effect on parameters other than body weight, antioxidant metabolism, and serum glucose, albumin, and triglyceride levels.

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## Introduction

In the development of nutritional strategies, it is well established that not only dietary protein sources (Gümüş et al., 2020; İmik et al., 2024), energy sources (Aydın et al., 2026; Gümüş et al., 2022), and macro-elements, but also plant extracts, volatile fatty acids, phenolic compounds (flavonoids) (Aydın et al., 2026), and trace elements such as boron or boric acid have significant effects (Uysal & Yoruk, 2025). Boron (B) is a critical trace element with growing significance for the health of animals, plants, and humans. This element influences various organs and systems in the body, including the skin, brain, digestive system, skeletal structure, and immune system. In many European countries, B is commonly used as a component of vitamin supplements and mineral support products (Bhasker et al., 2017).

Boric acid (BA) is the most important compound of the element B and is commonly represented by the chemical formula $B(OH)_3$ or $H_3BO_3$ (Woods, 1994). BA is a colorless and odorless substance that can appear as transparent crystals, white granules, or powder. It exhibits high solubility in solvents such as water, alcohol, glycerol, ether, methanol, and liquid ammonia, but shows low solubility in acetone (Yavuz & Denizli 2007; Demirel et al., 2015).

The effects of B on mineral metabolism are also noteworthy. One study found that B supplementation increased HDL levels and decreased LDL levels in the serum of rats (Cakir et al., 2018). Another study demonstrated that B reduced serum alanine aminotransferase (ALT) levels, thereby contributing to liver health (Zafar & Ali, 2013). Additionally, B has been reported to lower serum triglyceride, HDL cholesterol, and ALT levels (Bhasker et al., 2017).

Boron contributes to maintaining the balance between oxidants and antioxidants within cells. It reduces oxidative damage by increasing the levels of GSH and its derivatives or by activating agents that neutralize reactive oxygen species (Hunt & Idso, 1999). Boron compounds are also highly effective in regulating the levels of nicotinamide adenine dinucleotide phosphate (NADPH) (Devirian & Volpe, 2003). Furthermore, B supports the elevation of GSH levels, which play a critical role in protecting the body against toxic oxygen radicals. Experiments conducted on rats have shown that B exposure promotes NADPH production by enhancing glucose-6-phosphate dehydrogenase (G6PD) activity, thereby increasing intracellular GSH levels (Samman et al., 1998; Devirian & Volpe, 2003).

This study aimed to investigate the effects of different doses of BA added to the drinking water of rats (0, 100, and 200 mg/L for the Control, BA-100, and BA-200 groups, respectively) on BW, serum antioxidant metabolism, and some blood parameters.

## Materials and Methods

### *Animal, Housing, Diet, Experimental Design*

The study was conducted indoors at the Experimental Animals Application and Research Center of Sivas Cumhuriyet University. A total of 24 female Wistar albino rats, approximately 21 days old, were used in the study. The experiment was organized into three groups, each consisting of 8 animals: Control, BA-100, and BA-200 groups (Table 1). The drinking water of the Control group contained 0 mg/L BA, while the BA-100 and BA-200 groups received 100 mg/L and 200 mg/L BA, respectively. The animals used in the study were fed rat feed with the nutrient and energy content specified in Table 2 throughout the study period. The feeding lasted for a total of 42 days, including a 7-day adaptation period followed by a 35-day experimental period. All groups had *ad libitum* access to both feed and water. The groups consumed equal amounts of water daily.

### *Boric Acid*

Brand: Kimyalab, Product Code: V55901, Molecular Formula: $H_3BO_3$, Purity: 99.9%, Molar Mass: 61.83 g/mol, CAS Number: 10043-35-3, EC Number: 233-139-2, Manufacturer: BeyanLab Laboratory Products Industry and Trade Co. Ltd., Istanbul, Türkiye.

### *Sample Collections*

*Body weight and average weight gain*

Body weight (BW) was measured at the beginning of the trial (d 0) and, every week (d 7, 14, 21 and 28), and at the trial's conclusion (d 35). The difference between two measurements was divided by the number of days elapsed to calculate the average daily gain (ADG). Weight measurements were conducted using a scale with a precision of 1 g (Mettler Toledo, ICS689-B30, Switzerland).

*Collection of blood samples for biochemical analyses*

At the end of the study, blood samples were collected from the hearts of 8 animals per group under anesthesia (Ketamine HCL-Xylazine). Approximately 5 ml of blood was drawn from each animal into anticoagulant-free tubes (Vacutainer SST, Becton Dickinson, USA). The collected blood samples were centrifuged at +4 °C and 4000 rpm for 10 minutes using a refrigerated centrifuge (Hettich 38R, Hettich Zentrifugen, Tuttlingen, Germany). The obtained sera were stored at -80 °C until the day of analysis.

Serum levels of glucose, total protein, albumin, creatinine, triglycerides, total cholesterol, LDL, and HDL were measured using commercial test kits (Archem, Turkey) with an automated analyzer (Mindray BS-200, P.R. China).

Table 1. Experimental groups and the amounts of boric acid added to their drinking water.

| Groups | Boric Acid |
|---|---|
| Control | 0 mg/L |
| BA-100 | 100 mg/L |
| BA-200 | 200 mg/L |

Table 2. Composition and nutrient levels of the basal diet (%).

| Ingredients | Content % |
|---|---|
| Wheat | 25 |
| Corn | 20 |
| Soybean Meal | 15 |
| Milk Powder | 0.6 |
| Wheat Bran | 25 |
| Fish Meal | 10 |
| Vegetable Oil | 3 |
| Salt | 1 |
| Vitamin-Mineral Premix* | 0.4 |
| Calculated Nutritional Values | |
| Metabolizable energy (kcal/kg) | 2737 |
| Crude Protein % | 21 |

* Vitamin-Mineral Premix (per kg): Vitamin A 6,000,000 IU; Vitamin D3 800,000 IU; Vitamin E 8,000 mg; Vitamin K3 2,000 mg; Vitamin B1 (Thiamine) 1,200 mg; Vitamin B2 (Riboflavin) 3,000 mg; Vitamin B6 (Pyridoxine) 2,000 mg; Vitamin B12 8 mg; Niacin 10,000 mg; Folic Acid 400 mg; D-Biotin 20 mg; Choline Chloride 160,000 mg; Manganese 32,000 mg; Iron 16,000 mg; Zinc 24,000 mg; Cobalt 200 mg; Selenium 60 mg; Cal-D-Pan 4,000 mg; Antioxidant 4,000 mg.

Table 3. Effect of boric acid supplementation on body weight of rats.

| Parameters | Groups | | | P value |
|---|---|---|---|---|
| | Control | BA-100 | BA-200 | |
| BW, g | | | | |
| d 0 | 37.5±2.11 | 36.25±1.97 | 36.38±1.32 | 0.869 |
| d 7 | 52.63±3.63 | 54.38±1.72 | 50.63±2.34 | 0.620 |
| d 14 | 67.88±4.15 | 65.50±2.50 | 70.50±2.26 | 0.529 |
| d 21 | 82.13±3.39 | 80.88±3.47 | 88.25±2.04 | 0.209 |
| d 28 | 98.88±4.55 | 89.38±3.66 | 99.75±2.55 | 0.111 |
| d 35 | 116.25±3.50 | 105.00±4.01 | 109.38±1.75 | 0.068 |

All values are presented as mean ± standard error (n = 8). BW: body weight.

Table 4. Effect of boric acid supplementation on average daily gain of rats.

| Parameters | Groups | | | P value |
|---|---|---|---|---|
| | Control | BA-100 | BA-200 | |
| ADG, g/d | | | | |
| d 0-7 | 2.16±0.48 | 2.59±0.31 | 2.04±0.32 | 0.559 |
| d 7-14 | 2.18±0.27 | 1.59±0.51 | 2.84±0.51 | 0.158 |
| d 14-21 | 2.04±0.57 | 2.20±0.79 | 2.54±0.25 | 0.825 |
| d 21-28 | 2.39±0.39$^{a}$ | 1.21±0.09$^{b}$ | 1.64±0.24$^{ab}$ | 0.018 |
| d 28-35 | 2.48±0.60 | 2.23±0.61 | 1.38±0.29 | 0.311 |
| d 0-35 | 2.25±0.11 | 1.96±0.10 | 2.09±0.05 | 0.121 |

All values are presented as mean ± standard error of the mean. (n = 8). ADG: average daily gain., $^{a, b}$: Means within a row with different superscript letters are statistically significant ($P < 0.05$).

Table 5. Effect of boric acid supplementation on blood parameters of rats.

| Parameters | Groups | | | P value |
|---|---|---|---|---|
| | Control | BA-100 | BA-200 | |
| Glucose, mg/dL | 119.38±28.02$^{b}$ | 92.88±9.31$^{b}$ | 354.75±54.58$^{a}$ | 0.00 |
| Total Protein, g/dL | 5.38±0.09 | 5.65±0.12 | 5.33±0.12 | 0.107 |
| Albumin, g/dL | 3.04±0.31$^{b}$ | 3.23±0.72$^{a}$ | 3.01±0.63$^{b}$ | 0.031 |
| Creatinine, mg/dL | 0.39±0.14 | 0.38±0.20 | 0.43±0.19 | 0.115 |
| Triglyceride, mg/dL | 90.05±14.94$^{a}$ | 56±3.13$^{b}$ | 59.45±8.85$^{b}$ | 0.043 |
| Total Cholesterol, mg/dL | 67.41±3.23 | 73.16±3.68 | 65.60±5.12 | 0.409 |
| LDL, mg/dL | 48.25±3.92 | 50.63±1.71 | 41.75±2.47 | 0.150 |
| HDL, mg/dL | 39.66±1.80 | 43.70±2.36 | 35.85±3.62 | 0.146 |

All values are presented as mean ± standard error of the mean. (n = 8). ADG: average daily gain., $^{a, b}$: Means within a row with different superscript letters are statistically significant ($P<0.05$)., LDL: Low-density lipoprotein, HDL: High density lipoprotein.

***Determination of Antioxidant Parameters***

Serum levels of GSH, SOD, CAT and MDA were determined using specific commercial kits from Reed Biotech (China) following the manufacturer's instructions. Measurements were conducted with an ELISA Reader (Thermo Scientific Multiskan FC, USA). A calibration curve was generated for the calculation of absorbance values, and the concentrations of GSH, CAT, SOD, and MDA in the samples were calculated in ng/ml based on their absorbance.

***Statistical Analyses***

The data obtained in the study were analyzed using SPSS statistical software (SPSS, 2011). One-way ANOVA was used to determine statistical differences among the parameters obtained from the groups, and Duncan's multiple range test was applied to identify which groups differed from each other ($P<0.05$).

## Results

In the study, data related to BW are presented in Table 3. The mean initial (d 0) BW of the Control, BA-100, and BA-200 groups were 37.50 g, 36.25 g, and 36.38 g, respectively, while the final (d 35) BW at the end of the experiment were measured as 115.00 g, 104.38 g, and 103.75 g, respectively. Throughout the study, no statistically significant differences were observed in the average body weights among the groups at the weekly weigh-ins ($P>0.05$).

At the end of the study, the ADG was calculated as 2.25 g, 1.96 g, and 2.09 g for the Control, BA-100, and BA-200 groups, respectively. Between days 0-35 of the study, the ADG of the BA-100 group was found to be significantly lower than that of the Control and BA-200 groups ($P<0.05$). In other periods, no statistically significant differences were observed among the groups ($P>0.05$) (Table 4).

The results of the biochemical analyses performed on the serum samples obtained from the animals at the end of the study are presented in Table 5. No statistically significant differences were found among the Control, BA-100, and BA-200 groups in terms of total protein, creatinine, total cholesterol, LDL, and HDL levels ($P>0.05$). Serum glucose levels were statistically similar in the Control (119.38 mg/dL) and BA-100 (92.88 mg/dL) groups ($P>0.05$), but significantly higher in the BA-200 group (354.75 mg/dL) ($P<0.05$).

Table 6. Effect of boric acid supplementation on antioxidant parameters in serum tissue of rats.

| Parameters | Groups | | | P value |
|---|---|---|---|---|
| | Control | BA-100 | BA-200 | |
| CAT, ng/ml | 0.057±0.004 | 0.055±0.003 | 0.055±0.003 | 0.833 |
| SOD, ng/ml | 0.357±0.003 | 0.356±0.003 | 0.364±0.002 | 0.103 |
| GSH, ng/ml | 0.711±0.000 | 0.711±0.000 | 0.710±0.000 | 0,190 |
| MDA, ng/ml | 2.529±0.002 | 2.530±0.003 | 2.495±0.038 | 0.450 |

All values are presented as mean ± standard error of the mean. (n = 8). CAT: Catalase, SOD: Superoxide dismutase, GSH: Gutathione, MDA: Malondialdehyde.

Serum albumin levels were statistically similar in the Control (3.04 g/dL) and BA-200 (3.01 g/dL) groups ($P$>0.05), while the BA-100 group (3.23 g/dL) showed a significantly higher level ($P$<0.05). In addition, the serum triglyceride levels of the BA-100 (56.00 mg/dL) and BA-200 (59.45 mg/dL) groups were significantly lower than that of the Control group (90.05 mg/dL) ($P$<0.05).

Data on antioxidant parameters measured in the serum obtained from the rats are presented in Table 6. According to the analysis results, no statistically significant differences were observed among the groups in terms of CAT and SOD activities, as well as GSH and MDA levels ($P$>0.05).

## Discussion

Boron compounds, which are frequently mentioned across various industries today, hold economic significance in the livestock sector. In recent years, numerous studies have been conducted on the use of B compounds in animal nutrition (Sızmaz et al., 2014; Uysal & Yoruk, 2025; Başeğmez & Doğan, 2024).

It is believed that BA, used as a feed additive, may positively influence the digestive system and thereby improve performance parameters. In one study, the inclusion of BA in rainbow trout diets at levels of 0.010%, 0.025%, and 0.050% was reported to increase feed conversion ratio (FCR) and weight gain, while reducing feed intake and improving feed efficiency (Öz, 2025). Conversely, another study reported that the addition of 175 ppm BA to broiler diets had no effect on FCR, feed intake, or feed efficiency (Sızmaz et al., 2014). Similar to these findings, the current study also observed that the inclusion of BA in the drinking water of rats at doses of 100 and 200 mg/L did not affect FCR or weight gain. These results may be associated with the dosage of BA used and the duration of its application.

Although the exact biochemical mechanism of B in human and animal tissues is not fully understood, it is believed to react with compounds containing cis-hydroxyl groups, such as sugars and polysaccharides, thereby potentially playing a role in cell membrane function and stability. It has also been suggested that B may influence the transmission of impulses between hormone receptors and cell membranes (Yeşilbağ, 2008). Furthermore, B is reported to play significant roles in bone and mineral metabolism, as well as in lipid and energy metabolism, the immune and endocrine systems, and brain functions, contributing positively to overall performance. Additionally, it has been proposed that B may be effective in the prevention of conditions such as osteoporosis, osteoarthritis, and arthritis (Bhasker et al., 2017; Yeşilbağ, 2008).

Serum parameters are considered indicators of various metabolic processes. Among extracellular antioxidant defense components, albumin is regarded as the most potent, alongside transferrin, haptoglobin, and ceruloplasmin (Sitar et al., 2013). Although albumin has little or no enzymatic activity, it can bind to several molecules such as copper (Cu), zinc (Zn), magnesium (Mg) and calcium (Ca), thereby forming biologically important compounds. Plasma albumin concentration may vary under conditions that affect the liver, such as malnutrition, impaired absorption of certain molecules, or drug use (Levit & Levit, 2016). Serum albumin is recognized as a biomarker in numerous clinical situations, including assessments of nutritional status (Chien et al., 2017; Smith, 2017).

Looking at studies investigating the effects of B on glucose, the body's primary energy source, several experiments have been conducted on different animal species. These studies indicate that dietary B supplementation can lead to decreased glucose levels. This effect is thought to result from B forming complexes with hydroxyl groups in the glucose molecule (Hunt, 1989). In rats with cholecalciferol deficiency, B deficiency was found to increase plasma insulin levels (Hunt, 1998). In a study on broilers, high levels of dietary B significantly reduced glucose concentrations (Eren et al., 2012). Similarly, in another study conducted on chickens, increasing doses of boric acid were shown to decrease glucose levels (Eren & Uyanık, 2007). In contrast, a study on chicks fed a B-deficient diet reported that peak insulin levels were 75% lower than in chicks receiving B supplementation (Nielsen, 2000).

Contrary to the above findings, the present study found that administration of 200 mg/L BA in drinking water significantly increased serum glucose levels, a result that could not be correlated with existing literature. Oral administration of BA at a dose of 1330 mg/kg in rats was reported to increase serum Ca and Mg levels while decreasing phosphorus levels (Ismail, 2022). In a study conducted on broilers, supplementation of 175 ppm BA in the diet had no effect on levels of total protein, cholesterol, triglycerides, aspartate aminotransferase (AST), or ALT (Sızmaz et al., 2014). Another study reported that dietary inclusion of BA at levels of 0.025% and 0.050% in rainbow trout increased serum levels of alkaline phosphatase (ALP), albumin, globulin, and total protein, while decreasing glucose and cholesterol concentrations (Öz, 2025). In the current study, supplementation of BA at doses of 100 and 200 mg/L in drinking water did not affect serum levels of total protein, creatinine, total cholesterol, LDL and HDL. However, 200 mg/L BA increased glucose levels, 100 mg/L BA increased albumin concentration, and both 100 and 200 mg/L BA significantly reduced triglyceride levels.

There are studies demonstrating the importance of B compounds in antioxidant activity by neutralizing reactive oxygen species (ROS) (Türkez et al., 2007; Karabağ et al., 2023). Oral administration of BA has been reported to increase the levels of antioxidant enzymes such as GSH and CAT, while preventing oxidative damage by inhibiting ROS (Cengiz, 2018). The activities of antioxidant enzymes SOD, CAT, and glutathione peroxidase (GSH-Px) play a crucial role in cellular defense mechanisms (Tapiero et al., 2004). Superoxide dismutase plays a central role in protection against oxidative damage by catalyzing the conversion of superoxide radicals into hydrogen peroxide and oxygen (Kakarla et al., 2005). Catalase activity protects the lipids and proteins in erythrocyte membranes against peroxidative radicals. Glutathione peroxidase activity is associated with increased GSH levels. GSH is a critical tripeptide for erythrocytes and plays a key role in antioxidant enzyme functions (Türkez et al., 2007). Oxidative stress occurs when antioxidant levels decrease; therefore, the activities of antioxidant enzymes (SOD, CAT, GSH) are essential for cellular defense (Scandalios, 2005).

In one study, oral administration of BA at doses of 20 and 40 mg/kg in rats was found to increase CAT and SOD activities and GSH levels in liver and kidney tissues, while reducing MDA levels (Başeğmez & Doğan, 2024). Similarly, another study indicated that B compounds exert protective effects against oxidative damage through their influence on SOD, CAT, and GSH activity (Hunt et al., 1996). In a study conducted on broilers, the group receiving 175 ppm BA in their diet exhibited plasma MDA levels significantly lower than the control group, although liver MDA levels remained similar (Sızmaz et al., 2014). In another experiment, dietary supplementation with 100 mg/kg BA in rats reduced MDA levels, which are considered indicators of lipid peroxidation, and increased GSH levels, but had no effect on CAT and SOD activities (Ince et al., 2010). Likewise, another study reported that BA had no effect on liver SOD activity in rats (Mohora et al., 2002). In the present study, the inclusion of BA in drinking water at doses of 100 and 200 mg/L was found to have no significant effect on SOD and CAT enzyme activities, or on GSH and MDA levels. These findings may be associated with the dosage and duration of administration.

## Conclusions

The finding that boric acid supplementation at varying levels in rat diets did not significantly affect live body weight or serum antioxidant parameters, while it did influence serum glucose, albumin, and triglyceride levels, may provide important implications for the formulation of nutritional strategies. However, further studies involving different doses and longer durations of administration are needed to determine the optimal dosage and application frequency of substances such as BA, as well as to comprehensively evaluate their effects on animals.

## Declarations

### *Ethical Approval Certificate*

All animal procedures in this study were approved by the Local Ethics Committee for Animal Experiments of (Approval No: 578/2023).

### *Author Contribution Statement*

*Taha Can Taş:* investigation, data curation, methodology, writing–review and editing. *Recep Gümüş:* conceptualization, validation, visualization, methodology, data curation, data analysis, resources, investigation, data interpretation, original draft preparation, writing–review and editing, supervision.

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### *Conflict of Interest*

The authors declare no conflict of interest.

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