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Harmful effects of dietary supplementation of boron on blood parameters of Rainbow Trout (*Oncorhynchus mykiss*)

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ABSTRACT: Even though boron (B), as a trace micronutrient, occurs in natural waters and organisms, its high concentration could cause harmful and even toxic effects for organisms. Lately, studies about positive and negative effects of B on aquatic organisms have been increased with the growing scientific awareness. The aim of the present study was to determine the effects of B-containing feed (0.01%, 0.05%, 0.10%, and 0.20% of B in feed) on hematological and serum biochemical parameters of Rainbow Trout (*Oncorhynchus mykiss*) compared to the control feed without B. Among the most remarkable results, red blood cell, hemoglobin, and hematocrit values were dramatically decreased in the 0.20 % of B group compared to the control ($P<0.05$). Also, activities of liver enzymes increased with the increasing B level in the feed. Consequently, B supplementation ($> 0.01\%$) to feed induced negative changes in blood parameters of rainbow trout.

Keywords: Boron, fish feed, hematological parameters, serum biochemical parameters, rainbow trout

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INTRODUCTION

Blood is a major bio-material in assessing the health and disease status of all fish species, just as in all other animals (Coles, 1986; Bush, 1991). Hematological and biochemical parameters of blood in fish are general metabolic, physiological, biological, pathological and biochemical health-disease indicators. These parameters are also important indicators of the effects of environmental stress sources and water eco-system conditions. Fish, which are cold-blooded organisms, are very easily affected by various factors such as environmental and nutritional factors (Lusková, 1997; Gabriel et al., 2011). It is known that factors such as nutrition, stress, seasonal changes, disease, age, gender, species and race affect the physiological values of blood parameters (Fazio et al., 2013; Gabriel et al., 2004).

Rainbow trout (*Oncorhynchus mykiss*), a member of the Salmonidae family, is of high commercial importance and is widely produced and consumed in the world. This species is extensively cultured in various European countries, owing to its rapid growth and high nutrient content (Öz, 2018a; Öz, 2018b; Öz et al., 2017a; Öz, 2016). Boron (B) is considered an essential element for plant growth and development (Ahmad et al., 2009). It is not yet certain whether it is essential for humans and animals. However, after the 1980s it has been examined various studies on human and animal metabolism as a nourishing micro element (Devirian and Volpe, 2003; Yıldız et al., 2009; EFSA, 2013). Recently, it has been defined as a trace element that can affect the metabolism of macromolecules, triglycerides, glucose, amino acids, proteins and estrogenic compounds (Nielsen, 1997). It can have a role in minerals (Kurtoglu et al., 2001), lipids (Eren et al., 2006), energy metabolism (Hunt and Herbel, 1991) and in enzyme and steroid hormone activities (Hunt, 1994; Naghii and Mofid, 2008). Moreover, it is thought that the mechanism of boron mineral, which is thought to be essential for humans and animals in trace amounts, has important functions in lipid metabolism and energy metabolism, immune and endocrine system and brain, positively affects performance, and may be effective in preventing osteoporosis, osteoarthritis and arthritis (Nielsen, 1997). A part of dietary boron intake from nutrition is digested through the gastrointestinal system and accumulated in tissues and organs in various concentrations (Sayli, 2000). Boron concentrations in tissues are increased with the consumption of boron (Rossi et al., 1993).

In recent years, studies on the evaluation of the ecological and nutritional effects of water borne and food borne boron in different fish species have increased in number (Öz et al., 2017; Acar et al., 2018; Öz et al., 2018; Alak et al., 2018). These studies have reported that there were some positive effects of boron at certain levels, but higher concentrations of boron caused toxic effects in fish. For this reason, the aim of the current study was to investigate the effects of dietary boron on some hematological and serum parameters of Rainbow Trout (*Oncorhynchus mykiss*).

MATERIALS AND METHODS

Experimental Design and Diet composition

This study was approved by the Animal Experiments Local Committee (no: 4, 24.04.2017). The feeding experiment was carried out in Pozantı, Adana for 132 days. A total of 750 fish (of 20.14 ± 1.21 g body weight) were randomly assigned to 15 cages ($1 \times 1 \times 1$ m; 10 mm mesh size, 50 individuals per cage) for 5 treatments including a control group. A commercial trout feed produced by Skretting (Stavanger, Norway) was used as the control diet and the base feed for the preparation of the other experimental diets. In a previous study, 0.05% boron in feed has been reported to increase the immune response of Nile tilapia (*Oreochromis niloticus*) (Ardó et al. 2008). Another study in rainbow trout reported that boron supplemented to fish feed enhanced growth (Oz et al. 2018). The dose recommended in these two studies was taken into consideration while determining the boron rates used in our study. Thus, four different diets containing 0.01%, 0.05%, 0.10%, and 0.20% of boron were prepared by boric acid (Sigma-Aldrich, Steinheim, Germany). As described in our previous study (Oz et al., 2018), the powdery boric acid was diluted with 500 mL of water, impregnated by spraying to the feed and the oiled. 5 kg feed batches were dried in the shade and stored in buckets with covers. The fish were fed with the experimental and the control diets two times a day at 08:30 and 16:30, according to visual satiation determined as the fish did not approach the surface when the feeds was offered.

Determination of blood parameters

The fish were anaesthetized by 0.30 mL/L 2-phenoxyethanol (Velíšek and Svobodová, 2004), and the blood samples were collected from the caudal vein by vacuum tubes with anticoagulant for hematological parameters and without anticoagulant for biochemical analyses of serums. Serums were separated with cen-

trifugation at 3000 rpm for 10 min (Coles, 1986). The serums were stored in a -20 °C freezer until biochemical analyzes were performed. The hematological parameters were carried out with the commercial test kits (Mindray V-28 Reagent Kit, China) of Mindray BC-2800-Vet (China) Auto-hematology analyzer. White blood cells (WBC, 10^9 / L), hemoglobin (Hgb, g / dL), Hemotocrit (Hct, %), red blood cells (RBC, 10^{12} / L), mean red blood volume (MCV, fL), mean red blood cell hemoglobin (MCH, pg) and the mean red blood cell hemoglobin concentration (MCHC, g / dL) values were measured. The serum biochemical analyses were determined by colorimetric estimation using semi auto-analyzer (Humalyzer 3000 Semianalyzer, Germany) with commercial test kits (Assel, Italy). The measured biochemical parameters were total protein (TP, g/dL), glucose (Glu, mg/dL), albumin (Alb, g/dL), globulin (Glu, g/dL), urea (Ure, mg/dL), aspartate amino transferase (AST, U/L), alanine aminotransferase (ALT, U/L), alkaline phosphatase (ALP, U/L). Also, albumin and globulin ratios (A/G) were calculated for the samples.

Statistical analysis

The data are expressed as means \pm standard deviation (SD). Significance differences in the treatments

were determined by one-way analysis of variance (ANOVA), followed by a Tukey's pair-wise multiple comparison test using SPSS 15.0 (SPSS, Inc., Chicago, USA) software. Statistical significance was established at $P < 0.05$.

RESULTS

The effects of dietary boron on the hematological and serum biochemical parameters are presented in Table 1 and 2, respectively. Significant differences were found in all treatments regarding all blood parameters. WBC values were found similar in all treatments. RBC, Hgb, and Hct values were dramatically decreased ($P < 0.05$) while the boron concentrations were increased in the feed. The highest values were found in the control group, while the lowest values were observed in the 20% boron treated group. MCV, MCH, and MCHC values were increased in the 0.20 % of boron group. Decrease in the serum TP, Alb, Glu, and Ure values were determined compared to the control ($P < 0.05$). Also, A/G values were found significantly lower in the 0.20 % of boron group when compared to others ($p < 0.05$). Glu values and the activities of liver enzymes the 0.20 % of boron group were higher than the control and the other boron groups.

Table 1. Hematological parameters of rainbow trout (*Oncorhynchus mykiss*) fed diets containing boron for 132 days.

Parameters	Control	0.01% B	0.05% B	0.10% B	0.20% B
WBC (10^9 /L)	26.40 \pm 0.07 ^a	25.26 \pm 0.07 ^d	24.99 \pm 0.06 ^e	25.75 \pm 0.03 ^c	26.00 \pm 0.04 ^b
RBC (10^{12} /L)	1.64 \pm 0.01 ^a	1.09 \pm 0.02 ^b	0.94 \pm 0.00 ^c	0.92 \pm 0.02 ^d	0.82 \pm 0.00 ^e
Hgb (g/dL)	11.03 \pm 0.13 ^a	9.05 \pm 0.01 ^b	8.86 \pm 0.02 ^c	8.52 \pm 0.01 ^d	8.37 \pm 0.00 ^e
Hct (%)	30.44 \pm 0.01 ^a	28.32 \pm 0.01 ^b	27.48 \pm 0.18 ^c	25.11 \pm 0.02 ^d	22.64 \pm 0.03 ^e
MCV (fL)	186.12 \pm 0.81 ^d	260.19 \pm 4.10 ^c	291.86 \pm 1.83 ^a	273.52 \pm 6.42 ^b	276.18 \pm 0.90 ^b
MCH (pg)	67.40 \pm 0.84 ^c	83.18 \pm 1.30 ^d	94.12 \pm 0.44 ^b	92.81 \pm 2.20 ^c	102.18 \pm 0.25 ^a
MCHC (g/dL)	36.21 \pm 0.43 ^b	31.97 \pm 0.01 ^c	32.25 \pm 0.23 ^d	33.93 \pm 0.04 ^c	36.99 \pm 0.01 ^a

WBC (white blood cell), RBC (red blood cell), Hgb (hemoglobin), Hct; (hematocrit), MCV (mean red blood cell volume), MCH (mean red blood cell hemoglobin), MCHC (mean red blood cell hemoglobin concentration)

Table 2. Serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*) fed diets containing boron for 132 days.

Parameters	Control	0.01% B	0.05% B	0.10% B	0.20% B
TP (g/dL)	3.56 \pm 0.15 ^a	3.19 \pm 0.05 ^b	2.93 \pm 0.08 ^c	2.68 \pm 0.04 ^d	2.08 \pm 0.11 ^e
Alb (g/dL)	1.58 \pm 0.02 ^a	1.41 \pm 0.01 ^b	1.28 \pm 0.02 ^c	1.17 \pm 0.02 ^d	1.04 \pm 0.07 ^e
Glo (g/dL)	1.98 \pm 0.15 ^a	1.78 \pm 0.06 ^b	1.65 \pm 0.09 ^c	1.51 \pm 0.04 ^d	1.40 \pm 0.07 ^e
A/G	0.80 \pm 0.05 ^a	0.79 \pm 0.03 ^a	0.78 \pm 0.04 ^a	0.77 \pm 0.03 ^a	0.74 \pm 0.07 ^a
Glu (mg/dL)	70.63 \pm 1.26 ^e	82.36 \pm 1.55 ^d	92.04 \pm 1.55 ^c	100.56 \pm 1.54 ^b	108.09 \pm 2.17 ^a
Ure (mg/dL)	4.51 \pm 0.08 ^a	4.16 \pm 0.07 ^b	3.84 \pm 0.05 ^c	3.20 \pm 0.06 ^d	2.97 \pm 0.11 ^e
ALP (U/L)	81.84 \pm 84.67 ^a	102.16 \pm 0.83 ^b	123.95 \pm 5.13 ^c	149.21 \pm 3.89 ^d	193.87 \pm 11.19 ^e
ALT (U/L)	12.71 \pm 0.68 ^c	16.59 \pm 0.53 ^d	18.33 \pm 0.40 ^c	21.23 \pm 0.33 ^b	27.87 \pm 0.85 ^a
AST (U/L)	325.57 \pm 4.94 ^a	355.61 \pm 3.53 ^b	375.88 \pm 11.82 ^c	421.87 \pm 8.52 ^d	474.82 \pm 12.15 ^e

Total protein (TP), glucose (Glu), albumin (Alb), globulin (Glo), urea (Ure), aspartate amino transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP). Also, albumin and globulin ratios (A/G)

DISCUSSION

The increase in WBC indicates that fish increase their defense capacity against microbial or bacterial infection. In the present study, WBC values were found higher than those in previous studies in rainbow trout (Atamanalp et al., 2008; Talas and Gülhan 2009; Zorriehzahra et al., 2010; Bianchi et al., 2014; Khodadadi et al., 2018). Similar results were found regarding WBC values obtained from fish exposure to cobalt chlorite (Atamanalp et al., 2011). RBC values in the current study were found higher than in some studies (Atamanalp et al., 2008, Atamanalp et al., 2011) and lower than in some studies (Vazquez and Guerrero, 2007; Bianchi et al., 2014; Qadir et al., 2014). RBC values were found to have similar values in some studies (Talas and Gülhan, 2009; Zorriehzahra et al., 2010; Kankaya and Kaptaner, 2016). The number of erythrocytes decreases as the amount of boron in feed increases. It should be taken into consideration that the addition of boron minerals to the feed in small amounts and in short intervals stimulates the body defense system in fish while minimizing the decrease in erythrocyte count. The Hgb values obtained in the study showed a statistically significant decrease as the amount of boron mineral. Despite this decrease, the Hgb value obtained from all groups was higher than some studies (Vazquez and Guerrero, 2007; Atamanalp et al., 2008; Talas and Gülhan, 2009; Atamanalp et al., 2011; Kankaya and Kaptaner, 2016; Bianchi et al., 2014). The values found in the control group were higher than the values reported by Zorriehzahra et al., (2010), Fazio et al., (2013) and Cakici and Aydin (2006).

The reduction in MCV suggests that boron mineral may interfere with normal physiology of RBC (Latif et al., 2015). The main constant values of erythrocytes, MCV, MCH and MCHC are important parameters for the assessment of adaptation of respiratory function to practical oxygenation conditions in water (Radu et al., 2009). In terms of MCV, MCH and MCHC, there are values given in literature (Çelik et al., 2006). The increase of MCV, MCH and MCHC indicate that there is a relevant reaction. MCV, MCH and MCHC values were obtained by investigating and while their values were found to be high (Latif et al., 2015, Bianchi et al., 2014, Charoo et al., 2014), the MCV and MCH values are found lower by researching (Altun and Diler, 1999). Some researchers reported that in *Channa marulius* MCV and MCH values were different in different seasons (Latif et al. 2015). In the literature, very different reports cause difficulties in the evalu-

ation of hematologic values in fish physiology. It is necessary to prevent this confusion with studies conducted with a large number of animals.

In a review published in 2007, the mean values of some biochemical parameters commonly used in different healthy fish species were provided (Celik and Bilgin, 2007). According to this review, the published values were: total protein $3,49 \pm 1,007$ (0,10-7,50) g / dL, albumin $1,23 \pm 0,639$ (0,10-3,20) g / dL, globulin, $2,38 \pm 0,664$ (0,40 -4,37 g / dL, urea $5,33 \pm 4,056$ (0,00-18,00) mg / dL. Based on this information, the amount of serum TP 3.56 g / dL measured in the control group is within normal physiological limits; the amount of Alb (1.58 g / dL) is higher than normal and globulin (1.98 g / dL) (Table 2) is observed as lower than normal values. When the literature was reviewed, it was observed that *O. mykiss* TP values differed even in the same type:

The lowest serum TP amount mentioned in literature was 0.88 g / dL (Handy et al., 1999), while it was reported as 4.14 g / dL. in the study of Shimma et al. (1984). The results of TP (Giles, 1984) reported as 3.60 g / dL in the control group in literature are consistent with the results in the control group of our study. Furthermore, low serum protein may result from increased proteolytic activity in order to compensate for increased energy demand for coping with molecular stress which may be caused by high doses of boron.

Albumin, while increasing in the rate of dehydration, is quite reduced in cases of liver, kidney and circulatory system diseases or in cases of malnutrition and intoxication. Some of globulins are connected to hemoglobin, and globulins are responsible for the transport of metals such as iron in blood and help with the anti-inflammatory system (Kaneko et al., 2008). The albumin / globulin ratio is used to identify the causes of changes in total serum protein. However, it is not a specific indicator in the diagnosis due to not showing which particular proteins are changed. The specific A / G ratio in mammals is between 0.8-2.0. In all living organisms, this ratio increases in cases where albumin increases, while the ratio decreases in the table where globulin increases (Kaneko et al., 2008).

In parallel to the total protein decrease, Alb and Glo values decreased in 4 experimental groups in comparison to the control group (table 2). This is quite natural if the total protein content is considered to be a

total of Alb + Glo + fibrinogen. Protein catabolism for the homeostatic balance of protein metabolism and energy cycle may be reflected in blood parameters in the forms of intense enzymatic activities, protein degradation in response to stress, possibly liver damage due to chemical materials and the decrease in serum protein.

Average reported urea values in rainbow trout grown in three different environments were: 4-10 mg / dL in *Oncorhynchus mykiss* raised in natural environment, 5-11 mg / dL for ones in cages and 5-8 mg / dL for ones raised in pools (Ural et al, 2013). The urea value reported here in the control group is in accordance with the reference values. The amount of urea measured in the control group of our study was 4.51 mg / dL, which was slightly less than the value 5.33 mg / dL which was reported in a review on the mean biochemical parameters in all fish species by Çelik and Bilgin (2007).

From this point of view, it can easily be said that the low urea level found in our study primarily indicates gill and kidney dysfunction, especially if we keep in mind that blood urea nitrogen and urea levels are important metabolic products of protein catabolism in fish.

Enzymes are proteins that convert substrates into products. It is clinically important to know the serum levels of various enzymes. The presence of these enzymes in serum (low and high amounts) indicates that damage to cells occurs and causes intracellular components to be released into the blood. It is important to measure the enzyme levels in serum or plasma to detect, diagnose and monitor diseases, monitor status and control of treatment, and to detect tissue response to toxic substances exposed. In the bone, alkaline phosphatase (ALP) allows the progression of mineralization by destroying the pyrophosphate, a potent inhibitor of mineralization in bone tissue. It is also involved in the detoxification with the *in vivo* dephosphorylation of bacterial endotoxin, especially in the liver and intestines. They are varying values according to species, subspecies, regional changes, age and gender differences. They are reliable indicators for liver damage (Kaneko et al., 2008). Transaminases (AST and ALT) are critical and important enzymes in biological processes. ALT and AST enzymes are frequently used in the diagnosis of damages caused by pollutants (liver, kidney, intestine, placenta) in different tissues of fish (Sastri and Subhadra, 1985; Kaneko et al., 2008).

In the present study, aminotransferases (AST and ALT) and alkali phosphatase (ALP) values have also been researched as liver enzymes. In the control group ALP values have been found as 81.84 U/ L, AST values as 325.57 U/L and ALT values as 12.71 U/L. A increase has been observed in the values of ALP, ALT and AST due to increasing boron concentrations in the experimental groups. In the study, published in 2016, which researched the effects of permethrin insecticide on fish it has been reported that *O. mykiss* control group had AST values of 125,6 and ALT values of 14.5 U/L (Mozhdeganloo et al, 2016).

As is known, metals can alter the enzyme activity by binding enzymes to functional groups such as sulfhydryl, carboxyl, or inhibit the activity of the enzyme by replacing the metal in the active site of the enzyme (in metal-containing enzymes such as ALP). Increased levels of heavy metals in living organisms cause the formation of ROS (Reactive Oxygen Species) which causes lipid peroxidation, inhibition of enzymes and DNA damage. Especially serum levels of liver enzymes are also used in clinical biochemistry as a stress indicator (Kaneko et al., 2008). Enzymes used as biomarkers of aquatic contamination are frequently used to determine the effects of environmental pollutants on the organism due to their sensitivity to the effects of very low pollutants and their immediate response (Sastri and Subhadra, 1985). Significant changes in serum activities of these enzymes express the target tissue damage caused by stress.

The concentration-physiological response curve of boron mineral on living things can vary for many animal species. While negative effects can be observed in very high and very low concentrations, there is no negative effect at the intermediate concentrations and it has been determined that there are positive effects in physiological doses. The most sensitive tests report that the acute effects on the fish are in the range of 10-20 mg-B / L. Toxic effects of boron compounds on aquatic organisms such as fish, insects, molluscs etc. have also been reported (Loewengart, 2001). Although these studies provide toxicological assessments of the boron, there is still no sufficient information about the metabolic systematic and toxic effects of boron. Our study demonstrated that boron mineral can have a nutritional compound effect until certain dosages while it can also show toxic effects above a certain dose.

This type of descriptive and determinant biochemical research in animals in different countries, or even

in different regions of the same country, provides useful information for academic perspectives as well as for clinical studies (Kaneko et al., 2008).

CONCLUSION

When all parameters were taken into consideration, it was observed that differences in region, race, sex, age, season and nutrition sources affect hematological and biochemical values and cause changes. The detection and monitoring of hemo-biochemical parameter values reflecting the metabolic profile may indicate whether homeostatic mechanisms can maintain blood composition under different conditions (different races, different regions & areas, different feed-nutrition regimes, different age and gender characteristics) in physiological limits.

It is very important to include the physiological, biochemical and hematological values among the investigations that complement and support the clinical findings in a way to shed light on academic, clinical and economic scientific studies. At this point, it will be the most useful approach to determine, calculate and use hemo-biochemical reference ranges of species with racial and regional differences.

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CONFLICT OF INTEREST

None declared.

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