Effect of boric acid in rainbow trout (Oncorhynchus mykiss) growth performance

Mustafa Öz, Burak Evren Inanan and Suat Dikel

*Department of Fisheries and Diseases, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey; **Department of Veterinary Science, Eskil Vocational School, Aksaray University, Aksaray, Turkey; †Department of Aquaculture, Faculty of Fisheries, Cukurova University, Adana, Turkey

**ABSTRACT**

The effects of different levels of boron supplementation to feed on the growth parameters of rainbow trout (Oncorhynchus mykiss) were investigated for the first time using four diet variations (0.01%, 0.05%, 0.10%, and 0.20% of boron in feed) in addition to a control diet (without boron). After 120 days of feeding, the final weight (FW), weight gain, condition factor, feed intake, feed conversion rates, specific growth rate, protein efficiency ratio, survival rates, economic conversion rate, and economic profit index were calculated for each treatment and compared with the control as growth parameters. The initial weight was $17.89 \pm 1.12 \text{ g}$ at the end of feeding, the FWs were determined as $89.91 \pm 2.11 \text{ g}$ for the control and $90.56 \pm 1.34 \text{ g}$, $99.21 \pm 3.76 \text{ g}$, $88.14 \pm 2.43 \text{ g}$, and $78.74 \pm 1.47 \text{ g}$ for 0.01%, 0.05%, 0.10%, and 0.20% of boron feed treatments, respectively. The results of this study show that feeding rainbow trout with 0.05% boron-supplemented feed stimulates the growth parameters, but 0.20% of boron supplementation inhibits the growth of rainbow trout.

**Introduction**

As one of the fastest developing industries in the world, aquaculture has increased its production to cover human protein need. This increase induces new requirements for factors such as aquaculture feed. The feed in aquaculture mainly depends on fish meal and fish oil as the main protein sources, and its consumption has been exponentially increasing in the last decades. Accordingly, alternative proteins and some additives, which improve the efficiency of feed intake, have been studied in the feed industry (Tacon and Jackson 1985; Hardy and Tacon 2002). In particular, the limit of protein source encourages the use of additives in feed rations prepared for fish.

Boron as a trace element generally occurs in sedimentary rocks, soils, coal, seawater, etc. in different forms such as borates, boric acid, and boric oxide (Samman et al. 1998). Turkey, the United States, South America, Russian Federation, and China have commercially important deposits of boron-containing minerals (Woods 1994). Serving at cross purposes, boron and China have commercially important deposits of boron-containing minerals (Woods 1994).

Boron as a trace element generally occurs in sedimentary rocks, soils, coal, seawater, etc. in different forms such as borates, boric acid, and boric oxide (Samman et al. 1998). Turkey, the United States, South America, Russian Federation, and China have commercially important deposits of boron-containing minerals (Woods 1994). Serving at cross purposes, boron and China have commercially important deposits of boron-containing minerals (Woods 1994).

Boron as a trace element generally occurs in sedimentary rocks, soils, coal, seawater, etc. in different forms such as borates, boric acid, and boric oxide (Samman et al. 1998). Turkey, the United States, South America, Russian Federation, and China have commercially important deposits of boron-containing minerals (Woods 1994). Serving at cross purposes, boron and China have commercially important deposits of boron-containing minerals (Woods 1994).
the effect of boron-supplemented feed at the rates of 0.00%, 0.01%, 0.05%, 0.10%, and 0.20% on the growth parameters of rainbow trout (*Oncorhynchus mykiss*) and compare it to the control group without boron supplementation.

**Materials and methods**

**Experimental fish and handling**

This study was approved by the Animal Experiments Local Committee in Adana, Turkey (no. 3-2/2016). The feeding activities were performed for 120 days in a commercial rainbow trout fish (*O. mykiss*) farm in Pozanti-Adana, Turkey. In total, 1575 rainbow trout were used in the study. Each treatment and control consisted of three equal replicates (105 fish per replicate); the fish in each replicate were randomly assigned to 15 net cages of 1 m³ each (10 mm mesh size). At the beginning of the experiment, the average live weight of all experimental fish was 17.89 ± 1.12 g. The water temperature and oxygen content of the pools in the experiment were checked twice daily using an OxyGuard oxygen meter (OxyGuard, Birkerød, Denmark). The water temperature was 8°C, and the oxygen contents were 8.9–13.2 mg/L during the experimental period.

**Diet preparation and experimental design**

During the experiment and for 2 weeks prior to the beginning of the experiment, the fish were fed a commercial trout feed produced by Skretting (Stavanger, Norway), of which nutritional values are listed in Table 1. The concentration of boron in the initial feed and the water was determined by the curcumin method spectrophotometrically (Dyrssen et al. 1972). A 5 g feed was crushed and extracted with 10 mL of distilled water boiling for 15 minutes under reflux and was filtered as quickly as possible (Porter et al. 1981). The absorbance of the boric acid in the obtained solutions and the water samples with three replicates based on complexing with curcumin was measured at 540 nm. The minimum detection limits for boron were 0.02 mg/g in the feed and 0.1 mg/L in the water (Grinstead and Snider 1967).

Four different boron level diets (0.01%, 0.05%, 0.10%, and 0.20% of boron in feed) were prepared by supplementing boron to the feed, and a boron-free diet was used as the control. The boron levels in feed were adjusted with boric acid (Sigma–Aldrich, Steinheim, Germany). The feed treatments were prepared in 5-kg-weight batches. First, the powdery boric acid was diluted with 500 mL of water and impregnated with the feeds by spraying and impregnation. Then, it was lubricated to prevent boric acid in the feed from passing to the water by washing. The feed batches were dried in the shade and stored in buckets with covers. Similarly, water lubrication was applied to the control group. Hand-feeding to visual satiation was applied to the triplicates by feeding the fish twice daily (08:30 h and 16:30 h), where satiation was defined as the point when the fish did not approach the surface when food was offered. The satiation feeding was applied at the precise time by the same person throughout the feeding experiment to avoid the possibility of ‘inter-worker’ error.

**Results**

The boron concentrations of the water and initial feed used in this study were found lower than the detection limit. In addition to the initial fish weights, the growth parameters of rainbow trout calculated after 120 days of feeding are shown in Table 2. The FW of the feed treatment that contained 0.05% of boron was significantly the highest value (99.21 ± 3.76 g) compared to the other feed treatments (*P* < .05). However, the highest boron treatment, i.e., 0.20% of boron in feed treatment, has the significantly lowest FW value (78.74 ± 1.47 g). Similarly, the highest weight gain (WG) was found in the 0.05% boron treatment as 81.32 ± 3.76 g (*P* < .05). There were significant differences in WG between the 0.05% boron treatment and the other groups (*P* > .05). The feed intakes (FIs) of the 0.01% and 0.05% boron feed treatments (80.88 ± 0.86 g and 80.45 ± 1.42 g, respectively) were lower than the FIs of the control and the 0.10% and 0.20% boron treatments. The 0.05% boron treatment had the lowest FCR score (0.99 ± 0.02), whereas the scores were 1.15 ± 0.03 in the control and 1.30 ± 0.05 in the 0.20% boron treatment. The 0.05% boron feed treatment also had a higher SGR than the other treatments.

**Growth performance calculations and statistical analysis**

The specific growth rate (SGR, % day⁻¹) is 100 × (ln w₁ − ln w₀)/t, where w₁ and w₀ are the wet weights at times t₁ and t₀. The economic conversion ratio (ECR, US$ kg⁻¹) is [feed cost (US$ kg⁻¹) + boron cost (US$ kg⁻¹)]/feed conversion ratio (kg diet kg⁻¹ fish). The feed conversion ratio (FCR) is (Wfinal − Winitial)/consumed feed, where Wfinal and Winitial are the live weights (g) of the fish on the initial (t) and final (T) days, respectively. The economic profit index (EPI (US$ fish⁻¹) is: final weight (FW, kg fish⁻¹) × fish sale price (US$ kg⁻¹) − ECR (US$ kg⁻¹) × weight increase (kg)), which was developed by Martinez-Llorens et al. (2007). The protein efficiency ratio (PER) is Weight gain (g)/protein intake (g) (Skalli et al. 2004). The condition factor (CF) is calculated as CF = (Body weight (g)/[Fish size (cm)]³ × 100) (Martinez and Vázquez 2001).

The mean values and standard deviations of each growth parameter were calculated from the data of the three replicates for each treatment. The growth parameters were statistically tested by ANOVA using SPSS 15.0 for Windows. When only two groups were compared, the differences between the groups were tested using *post hoc* Tukey’s HSD tests. The effects with *P* < .05 were considered significant.

### Table 1. Nutritional values of the trout feed used in the study.

| Diet composition          | Average of total feed (%) |
|---------------------------|---------------------------|
| Crude protein             | 45                        |
| Lipid                     | 20                        |
| Crude cellulose           | 2.0                       |
| Crude ash                 | 10                        |
| Macro elements            |                           |
| Phosphorus                | 1.10                      |
| Calcium                   | 1.90                      |
| Sodium                    | 0.30                      |

*Boron was found lower than the detection limit (0.02 mg/g) in the feed.*
and the control. The 0.20% boron treatment had the lowest SGR value (1.23 ± 0.01). The average PERS of all treatments were between 1.80 ± 0.07 and 2.35 ± 0.06, the 0.20% boron feed treatment had the minimum average value, and the 0.05% boron feed treatment had the maximum average value. All treatments including the control had 100% survival rate (SUR) values. The maximum and minimum ECR were calculated for the 0.20% and 0.01% boron (1.84 ± 0.07 and 1.41 ± 0.04), respectively. There was no significant difference in EPI of the control, 0.01%, and 0.10% boron feed treatments. However, the 0.05% boron treatment had the highest EPI of 0.21 ± 0.01.

Discussion

The growth experiment was performed to reveal the effects of boron in the diets at 0.01%, 0.05%, 0.10%, and 0.20% in feed and control (without B). The difference between the averages which is remarked in the same line with different letters (a–e) is significant (P < .05). IFW: Initial fish weight.

| Growth parameters | Control | 0.01% | 0.05% | 0.10% | 0.20% |
|-------------------|---------|-------|-------|-------|-------|
| IFW (g)           | 17.75 ± 1.01a | 17.89 ± 1.19a | 18.03 ± 1.26a | 17.73 ± 1.09a | 18.04 ± 1.06a |
| FW (g)            | 89.91 ± 2.11b | 90.56 ± 1.34b | 99.21 ± 3.76b | 88.14 ± 2.43b | 78.74 ± 1.47b |
| WG (g)            | 72.02 ± 2.11b | 72.67 ± 1.34b | 81.32 ± 3.76b | 69.26 ± 2.43b | 60.85 ± 1.46b |
| CF                | 1.47 ± 0.02b | 1.32 ± 0.01c | 1.24 ± 0.02c | 1.39 ± 0.01b | 1.32 ± 0.02c |
| FI                | 82.97 ± 1.51a | 80.88 ± 0.86b | 80.45 ± 1.42b | 79.31 ± 1.44b | 78.82 ± 2.33b |
| FCR               | 1.15 ± 0.03b | 1.11 ± 0.03b | 0.99 ± 0.02 | 1.15 ± 0.05b | 1.30 ± 0.05b |
| SGR               | 1.35 ± 0.19b | 1.35 ± 0.01b | 1.43 ± 0.03b | 1.32 ± 0.02b | 1.23 ± 0.01c |
| PER               | 2.02 ± 0.05b | 2.09 ± 0.05b | 2.35 ± 0.06b | 2.03 ± 0.08b | 1.80 ± 0.07b |
| SUR (%)           | 100      | 100    | 100    | 100    | 100    |
| ECR (5/kg)        | 1.64 ± 0.04b | 1.58 ± 0.04b | 1.41 ± 0.04c | 1.63 ± 0.07b | 1.84 ± 0.077 |
| EPI               | 0.18 ± 0.01b | 0.18 ± 0.01b | 0.21 ± 0.01a | 0.17 ± 0.01b | 0.15 ± 0.01c |

Notes: Each value indicates the average ± standard deviation. The averages expressed using different letters in each row are significantly different (P < .05). IFW: Initial fish weight.

Table 2. Effect of boron (B) in diet on growth parameters of rainbow trout after 120 days of feeding (n = 105 for each replicate) and the initial fish weight (n = 1575).

B Levels in Feed

The results of our study show that <0.05% of boron in the feed caused an increase in WG values and a decrease in FCR values, but >0.05% of boron in the feed reduced the growth parameters. Two main reasons are assumed to cause the positive effects of boron supplementation on growth. First, boron has a role in the transportation of cell membranes (Takano et al. 2008). NaB1C1, which is a boron transporter in cell membrane, serves as an electrogenic Na+-coupled borate transporter, which is essential for cell growth and proliferation (Park et al. 2004). This transporter is related to the integrity and healthiness of the cell membrane (Park et al. 2005). The cell membrane has a cell division, which is mainly responsible for the growth of an organism and can be the main reason for the growth stimulation effect of boron. Second, boron attends to the pathways of numerous enzymatic reactions in animal metabolism (Hoye 1998; Adhikari and Mohanty 2012). The boron compounds in animal metabolism protect against the development and decrease in triglyceride, notably low-density lipoprotein, total cholesterol, insulin, and non-esterified fatty acids in serum (Basoglu et al. 2002; Kabu and Civelek 2012). Moreover, boron plays a part in the oxidative stress reducing mechanisms such as the glucose–alanine cycle and methionine metabolism, and boron positively affects the lipid profile (Basoglu et al. 2011). Additionally, boron has many effects related to the bone metabolism in animals. The boron supplementation increases the bone abnormalities and magnesium concentrations in bones and enhances the absorption and retention of calcium and phosphorus (Hegsted et al. 1991; Bai and Hunt 1996). The relationship between boron and these cations induces the development of chickens (Hunt et al. 1994). Boron that was added to the diet of chickens at a level of 3 mg/kg promotes the growth (Hunt and Nielsen 1981). Nevertheless, boron can be toxic when its concentrations are more than the requirements of the animal species, although it is an essential element for organisms (Goldbach et al. 2007). The current study has shown that 0.20% boron in feed inhibits the growth of rainbow trout. This inhibition could have resulted from histopathological damage in fish tissues. Especially, the high concentrations of boron caused interstitial oedema, degenerative and atrophic changes in the myofibrils of muscle tissues of rainbow trout (Topal et al. 2016).
Conclusion
The present study indicates that adding 0.05% boron to the feed improves the growth of rainbow trout at unit time and lower cost. Higher boron concentrations than this level cannot enhance the growth parameters of rainbow trout. We suggest that using boron as an additive in the feed is beneficial for growth in rainbow trout. Moreover, further studies are required to examine the effects of the boron-supplemented feed on the fish meat quality, gamete quality, blood parameters, and resistance to diseases. The results of the current study demonstrate the utility of boron mineral for the feed industry as a new field.

Disclosure statement
No potential conflict of interest was reported by the author(s).

Funding
This study has been financially supported by Aksaray University Scientific Research Projects Unit Foundation (Project No: 2016-027).

References
Adhikari S, Mohanty M. 2012. Effect of waterborne boron and molybdenum on survival, growth and feed intake of Indian major carp, Cirrhinus mrigala (Hamilton). Chem Ecol. 28(2):113–121.
Ardó L, Yin G, Xu P, Váradi L, Szigeti G, Jeney Z, Jeney G. 2008. Chinese herbs (Astragalus membranaceus and Lonicera japonica) and boron enhance the non-specific immune response of Nile tilapia (Oreochromis niloticus) and resistance against Aeromonas hydrophila. Aquaculture. 275:26–33.
Bai YS, Hunt CD. 1996. Dietary boron enhances efficacy of cholecalciferol in broiler chicks. J Trace Elem Exp Med. 9(3):117–132.
Basoglu A, Baspinar N, Ozturk AS, Akalin PP. 2011. Effects of long-term boron administrations on high-energy diet-induced obesity in rabbits: NMR-based metabolicome evaluation. J Anim Vet Adv. 10(12):1512–1515.
Basoglu A, Seving M, Birdane FM, Boydak M. 2002. Efficacy of sodium of borax in the prevention of fatty liver in dairy cows. J Vet Intern Med. 16:732–735.
Bustos-Obregón E, Olivares C. 2012. Boron as testicular toxicant in mice (Mus domestica). Int J Morphol. 30(3):1106–1114.
Durmuş M, Polat A, Öz M, Ozogul Y, Ucak I. 2014. The effects of seasonal dynamics on sensory, chemical and microbiological quality parameters of vacuum-packed sardine (Sardina pilchardus). J Food Nutr Res. 53(4):344–352.
Dyrrsen DW, Novikov YP, Uppström LR. 1972. Studies on the chemistry of the determination of boron with curcumin. Anal Chim Acta. 60(1):139–151.
Emiroğlu Ö, Çiçek A, Arslan M, Aksan S, Rüzgar M. 2010. Boron concentration in water, sediment and different organisms around large borate deposits of Turkey. Bull Environ Contam Toxicol. 84:427–431.
Fort DJ, Stover EL, Strong PL, Murray FJ. 1999. Effect of boron deprivation on reproductive parameters in Xenopus laevis. J Trace Elem Exp Med. 12:187–204.
Goldbach HE, Huang L, Wimmer MA. 2007. Boron functions in plants and animals: recent advances in boron research and open questions. In: Xu F, Goldbach H, Brown PH, Bell RW, Fijiwara T, Hunt CD, Goldberg S, Shi LF, editors. Advances in plant and animal boron nutrition. Dordrecht: Springer; p. 3–25.
Grinstead RR, Snider S. 1967. Modification of the curcumin method for low level boron determination. Analyst. 92:532–533.
Hardy RW, Tacon AGJ. 2002. Fish meal: historical uses, production trends and future outlook for sustainable supplies. In: Stickney RR, McVey JP, editors. Responsible marine aquaculture. London: CABI Publishing; p. 311–325.
Hegsted M, Keenan MJ, Siver F, Wozniak P. 1991. Effect of boron on vitamin D deficient rats. Biol Trace Elem Res. 28:243–255.
Howe PD. 1998. A review of boron effects in the environment. Biol Trace Elem Res. 66:153–166.
Hunt CD. 1994. The biochemical effects of physiologic amounts of dietary boron in animal nutrition models, Environ Health Perspect. 102(7):35–43.
Hunt CD, Herbel JL, Idso JP. 1994. Dietary boron modifies the effects of vitamin D3 nutrition on indices of energy substrate utilization and mineral metabolism in the chick. J Bone Min Res. 9:171–182.
Hunt CD, Nielsen F. 1981. Interaction between boron and cholecalciferol in the chick. In: Gawthorne J, White C, editor. Trace element metabolism in man and animals. Canberra: Australian Academy of Science; p. 597–600.
Kabu M, Akosman MS. 2013. Biological effects of boron. Rev Environ Contam Toxicol. 225:57–75.
Kabu M, Civelek T. 2012. Effects of propylene glycol, methionine and sodium borate on metabolic profile in dairy cattle during periparturient period. Rev Med Vet. 163(8–9):419–430.
Loewengart G. 2001. Toxicity of boron to rainbow trout: A weight-of-the-evidence assessment. Environ Toxicol Chem. 20(4):796–803.
Martínez AM, Vázquez BPC. 2001. Reproductive activity and condition index of Holocentrus passer (Teleostei:Pomacentridae) in the Gulf of California, Mexico. Rev Biol Trop. 49(3-4):939–943.
Martínez-Llorens S, Moñino AV, Tomás A, Pla M, Jover M. 2007. Soybean meal as a protein source in gilt-head sea bream (Sparus aurata L.) diets: effects on growth and nutrient utilization. Aquac Res. 38:82–90.
Nielsen FH. 1997. Boron in human and animal nutrition. Plant Soil. 193:199–208.
Oz M. 2016. Nutrition and gender effect on body composition of rainbow trout (Oncorhynchus mykiss). J Adv VetBio Sci Tech. 1(1):20–25.
Ozogul Y, Aydin M, Durmus M, Karadurmus U, Öz M, Yuvka I, Kosger A. 2013. The effects of season and gender on the proximate and fatty acid profile of male and female warty crab (Eriphia verrucosa) from Black Sea. Rapport Commission International Mer Mediterranee. 40:634.
Park M, Li Q, Shchechinov N, Mualem S, Zeng W. 2005. Borate transport and cell growth and proliferation: Not only in plants. Cell Cycle. 4(1):24–26.
Park M, Li Q, Shchechinov N, Zeng WZ, Mualem S. 2004. NaBC1 is a ubiquitous electrogenic Na+-coupled borate transporter essential for cellular boron homeostasis and cell growth and proliferation. Mol Cell. 16(3):331–341.
Porter SR, Spindler SC, Widdowson AE. 1981. An improved automated colormetric method for the determination of boron in extracts of soils, soilless peat-based composts, plant materials and hydroponic solutions with Azomethine-H. Commun Soil Sci Plant Anal. 12(5):461–473.
Rowe RI, Eckhart CD. 1999. Boron is required for zebrafish embryogenesis. J Exp Biol. 202:1649–1654.
Samsan S, Naghii MR, Lyons WM, Verus AP. 1998. The nutritional and metabolic effects of boron in humans and animals. Biol Trace Elem Res. 66:227–235.
Scialli AR, Bonde JP, Brüskes-Hohlfeld I, Culver BD, Li Y, Sullivan FM. 2010. An overview of male reproductive studies of boron with an emphasis on studies of highly exposed Chinese workers. Reprod Toxicol. 29:10–24.
Skalli A, Robin J. H. 2004. Requirement of n-3 long chain polyunsaturated fatty acids for European sea bass (Dicentrarchus labrax) juveniles: growth and fatty acid composition. Aquaculture. 240:399–415.
Tacon AGJ, Jackson AJ. 1985. Utilization of conventional and unconventional protein sources in practical fish feeds. In: Cowey CB, Mackie AM, Bell JG, editors. Nutrition and feeding of fish. London: Academic Press; p. 119–145.
Takano J, Miwa K, Fujiwara T. 2008. Boron transport mechanisms: collaboration of channels and transporters. Trends Plant Sci. 13(8):451–457.
Taşbozan O, Gökte MA, Erbaça Ç. 2016. The effect of different growing conditions to proximate composition and fatty acid profiles of rainbow trout (Oncorhynchus mykiss). J Appl Anim Res. 44(1):442–445.
Topal A, Oruç E, Altun S, Ceyhun SB, Ateamanalp M. 2016. The effects of acute boric acid treatment on gill, kidney and muscle tissues in juvenile rainbow trout. J Appl Anim Res. 44(1):297–302.
Woods WG. 1994. An introduction to boron: history, sources, uses and chemistry. Environ Health Perspect. 102(7):5–11.