The stimulatory effects of boron on Japanese quail spermatological activity, histopathology, and oxidative stress

Ulku Gulcihan Simsek[1], Burak Karabulut[2], Seyma Ozer Kaya[3], Mine Erisir[4], Yasin Baykalir[5], Sultan Aslan[6], Aydin Cevik[2], Merve Kahramanogullari[4]

[1]Firat University, Faculty of Veterinary Medicine, Department of Animal Science, Elazig, Türkiye
[2]Firat University, Faculty of Veterinary Medicine, Department of Pathology, Elazig, Türkiye
[3]Firat University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Elazig, Türkiye
[4]Firat University, Faculty of Veterinary Medicine, Department of Biochemistry, Elazig, Türkiye
[5]Balikesir University, Faculty of Veterinary Medicine, Department of Biostatistics, Balikesir, Türkiye
[6]Dokuz Eylul University, Faculty of Veterinary Medicine, Department of Animal Science, Izmir, Türkiye

Received February 28, 2023
Accepted June 18, 2024

Abstract

This study looked at how spermatogenesis, lipid peroxidation, antioxidant defense mechanisms, and histological changes in testicular, hepatic, and renal tissues were affected by boric acid (H_3BO_3) added to mixed feed and drinking water at different concentrations for 14 weeks. For this purpose, 60 male Japanese quails (*Coturnix japonica f. domestica*) were used. From the age of 35 days, the birds were given boric acid added to regular soybean meal maize mixed feed as well as drinking water. Experimental groups: Control (no additive), F100: 100 mg/kg boric acid into feed, F300: 300 mg/kg boric acid into feed, W100: 100 mg/l boric acid into drinking water, and W300: 300 mg/l boric acid into drinking water. Both primordial ($P < 0.001$) and mature ($P < 0.05$) sperm counts increased in F300 and W300 groups. Supplemented boric acid in drinking water (300 mg/l) increased the tubule diameters of the testicle ($P < 0.05$). Because of the rising levels of boric acid in the feed and water groups, lipid peroxidation levels increased in testicular ($P < 0.001$), hepatic, and renal tissues ($P < 0.01$). Glutathione (GSH) levels rose in high boric acid groups in testicular and hepatic tissues ($P < 0.001$). Different tissues responded differentially to high amounts of boric acid in terms of antioxidant enzyme activity ($P < 0.001$). As a result, boric acid at high doses showed beneficial effects on spermatological activity; however, continued use caused lipid peroxidation in tissues and some pathological problems in liver tissue.

Boric acid, histopathology, MDA, semen traits, poultry

Boron is in group 3A of the periodic system. The atomic number is 5, atomic mass is 10.811 g/mol, melting point is 2 300 °C. Boron, which is found in low amounts in nature, has rich reserves in Türkiye. The most common boron compounds are boric acid salts, known as acid boric, and the compounds formed by boron with Na, Ca, and Mg. Boric acid is a water-soluble acid that crystallizes as bright, white particles. Dissolution rate in water at different temperatures: 19.5 g/l at 0 °C, 49 g/l at 20 °C, 379 g/l at 100 °C (Kuru and Yarat 2017). Boron is used in many industries; moreover, it is a microelement with important biological effects. It is known to be involved in various biochemical events in human and animal metabolism (Shihab and Khaleel 2023; Yadav 2023). Boron intake with food is effective on lipid metabolism (Avsar Abdik et al. 2019), energy metabolism (Białek et al. 2019), endocrine system (Khaliq et al. 2018), immune system (Kabu et al. 2015), mineral metabolism (Abdelnour et al. 2018), development of bones (Pizzorno 2015; Rondanelli et al. 2020; Simsek et al. 2020), nervous system (Nielsen and Meacham 2011), and cardiovascular systems (Donoiu et al. 2018). Boron also has anti-inflammatory activity and shows this effect by suppressing cytokine production, reducing pro-inflammatory prostaglandin biosynthesis and locotriene synthesis, inhibiting TNF-α,

Address for correspondence:
Professor Dr. Ulku Gulcihan SIMSEK
University of Firat, Faculty of Veterinary Medicine
Department of Animal Science, Elazıg 23119, Türkiye
E-mail: gsimsek@firat.edu.tr
http://actavet.vfu.cz/

ILT1, ILT6, and nitric oxide release (Hunter et al. 2019). Moreover, the antioxidant defense system that protects against oxidative stress brought on by malathion is made more active by boron, which also has a protective effect against lipid peroxidation (Karabag Coban et al. 2015). It has been discovered that boron promotes sexual activity by increasing the synthesis of steroid hormones like oestrogen and testosterone (Khaliq et al. 2018). In their study, Cortés et al. (2017) added boron to drinking water for humans at doses of 3 mg/l (low), 3–7 mg/l (moderate), and 7 mg/l (high). They discovered that while the addition of medium-dose boron improved the quality of the semen, a high dose had a negative impact on sperm concentration, morphology, motility, and viability. Similarly, Korkmaz et al. (2011) found taking 6.5 mg of boron daily with drinking water had no negative effects on sperm quality in men. According to Krishnan et al. (2019), dietary boron supplementation (40 ppm) increased sperm output, sperm motility, and immunological and antioxidant defense capacity in male goats. Notwithstanding these results, it is still debatable whether the inorganic boron compounds have advantageous or detrimental effects and what the sufficient dose for reproduction is (Estevez-Fregoso et al. 2023). While the World Health Organization (WHO) determines the daily amount of boron that can be taken safely for adults as 1–13 mg/day, the European Food Safety Authority (EFSA) considers the maximum dose that can be taken 10 mg/day (Rondanelli et al. 2020). On the other hand, Scialli et al. (2010) stated that the effect of boron on reproductive traits in males differed among species, especially since the sensitivity was higher in some species such as rats, mice, and dogs.

The aforementioned information explains that the objective was to investigate the effects of boric acid (H_3BO_3) added to mixed feed and drinking water for a prolonged period of time (14 weeks) at various doses on spermatogenesis, lipid peroxidation, and antioxidant defense mechanisms of testicles, liver, and kidney tissues. Moreover, this research was also conducted to show the effects of boron on the histopathological structure of tissues in Japanese quails.

Materials and Methods

This study was conducted with the ethical approval of the Elazig Veterinary Control Institute Animal Experiments Local Ethics Committee (Decision no: 2018/05). Sixty male Japanese quails (*Coturnix japonica f. domestica*), aged 30 days were used in the study. After the quails were acclimated for five days to the coop environment, the sex of all quails was confirmed by checking the cloaca. After taking the live weights of the quails on day 35, they were divided into experimental groups, considering their live weights were similar among the groups (Fig. 1). Experimental groups included: Control - no additive; F100: 100 mg/kg boric acid into feed; F300: 300 mg/kg boric acid into feed; W100: 100 mg/l boric acid into drinking water; and W300: 300 mg/l boric acid into drinking water. Boric acid (TekKim, Chemical Industry, Türkiye, CAS no: 10043-35-3) used in the research had > 99.5 purity, > 56.0 boron trioxide (B_2O_3), < 0.02 sulphate (SO_4), < 0.001 chloride (Cl), < 0.0007 iron (Fe). Each experimental group was arranged into four replicates with three quails in each replicate. Each group included 12 quails. Quails were fed *ad libitum* with boric acid supplemented standard soybean meal and maize mixed feed and drinking water in the groups between 35 and 140 days of the rearing period. Boric acid was dissolved well in fresh drinking water daily. Composition of the feed and nutritional value are presented in Table 1. The birds were reared in plastic cages of 30 cm × 45 cm × 25 cm, width × length × height/3 birds. The lighting program was implemented at 16 h light/8 h dark (3 watts/m^2). The rooms were at a temperature of 19–21 °C and a humidity of 55–60%. Natural ventilation was done in the rooms. The quails were slaughtered (Turkish standard; Poultry - Rules for slaughtering and carcass preparing, TS 5925) at the age of 140 days; blood, testis, liver, and kidney samples were taken. The testicles were weighed with a digital scale with 0.001 precision. Testosterone level was measured in serum by Olympus AU 2700 auto analyzer (Beckman-Coulter, Inc., Fullerton, CA). Spermatological characteristics were examined by biochemical and histopathological analyses in the testes of each bird.

The haemocytometric method was used for the cell count. Cleared out right testis samples, of which tunica albuginea was expelled, were minced and homogenized in 10 ml of 0.9% NaCl solution containing 0.5% TritonX 100. One hundred µl were taken from this homogenate and diluted with the same solution once more at the ratio of 1:9. Then, 10 µl of the final homogenate were placed to the counting chamber of a haemocytometer (Improved Neubauer, depth 1/10 mm, field size 0.0025 mm^2; LABART, Munich, Germany) and the spermatogenic cells were counted in the light magnifying instrument at × 200 magnification. Counts of spermatogonium, spermatocyte, spermatid, sperm were calculated and expressed as million per testis (Grote et al. 2008).

The malondialdehyde (MDA) assay was carried out based on the MDA forming a pink complex with thiobarbituric acid, and the absorbance was read in 532 nm. The MDA values were expressed as nmol/g tissue (Turker et al. 2016). Reduced glutathione (GSH) was assayed by the method of Chavan et al. (2005) and expressed as µmol/g tissue. Sulphhydryl groups in the tissue reacted with Ellman's reagent and formed a yellow color with maximum absorbance at 412 nm. The Góth method was used to measure catalase (CAT) activity (Góth 1991). The yellow complex of molybdate and hydrogen peroxide was measured at 405 nm. The CAT activity was expressed as k/g protein. Glutathione peroxidase (GSH-Px) activity was assayed by the method of Matkovics et al. (1988) and determined by cumene hydroperoxide. Reduced GSH as cosubstrates and the loss of GSH following enzymatic reaction at 37 °C was measured spectrophotometrically with Ellman's reagent at 412 nm. The activity was expressed as U/g protein. The superoxide dismutase (SOD) activity was determined by the method of Sun et al. (1988) on a spectrophotometer at 560 nm and expressed as U/g protein. The SOD activity was measured based on the degradation of nitroblue tetrazolium by the superoxide radical, which was produced by the xanthine-xanthine oxidase system. The blue-colored formazan obtained at the end of the reactions was maximally absorbed in 560 nm.

Left testis, liver, and kidney samples were fixed in 10% neutral formalin solution. The tissues were left in fixation solution for approximately 48 h, then paraffin blocks were prepared by routine tissue follow-up. The 3–5 µm thick sections were taken from the prepared blocks using a microtome, stained with haematoxylin-eosin and Masson trichrome staining methods, and examined under a light microscope. Microscopic evaluations were made according to Johnsen's scoring system which is shown in Table 2, and the seminiferous tubular diameter

Table 1. Composition of mixed feed and nutritional values.

Feedstuffs	%	Nutrients	%
Corn	51.40	Dry matter	90.40
Wheat bran	9.00	Crude protein	18.00
Soybean meal (44% HP)	22.00	Crude cellulose	4.40
Corn germ meal	2.00	Raw oil	5.35
Sunflower meal (45% HP)	4.30	Ash	10.19
Vegetable oil	3.50	Calcium[3]	2.50
Calcium phosphate	0.88	Phosphorus[3]	0.35
Calcium carbonate	4.50	Sodium[3]	0.18
Limestone	1.43	Lysine[3]	1.00
L-lysine hydrochloride	0.16	Methionine+Cystine[3]	0.59
L-threonine	0.12	Threonine[3]	0.76
Sodium bicarbonate	0.16	Tryptophan[3]	0.25
Salt	0.20	ME, kcal/kg[3]	2800
Vitamin-mineral mix[2]	0.35		

[1] In the experimental groups, 100 and 300 ppm boric acid was added to the basal ration
[2] Vitamin-mineral mix (per 1 kg): Vitamin A 15,500 IU; Vitamin D3 3.500 IU, manganese 120 mg; iron 40 mg; zinc 100 mg; copper 16 mg; cobalt 200 mg; iodine 1.25 mg; selenium 0.30 mg
[3] Determined by calculation

Table 2. Johnsen's histological scoring criteria.

Score	Histological criteria
10	Full spermatogenesis, perfect tubules, many spermatozoa
9	Almost a full spermatogenesis, a disorganized epithelium, a large number of mature spermatids.
8	The number of spermatozoa per tubule lumen is less than five, and the number of mature spermatids is decreased.
7	No spermatozoa, no mature spermatids, but early spermatids present.
6	No spermatozoa, no mature spermatids, few early spermatids.
5	No spermatozoa, no spermatids, many spermatocytes present.
4	No spermatozoa, no spermatids, few spermatocytes.
3	Only spermatogonium are present
2	No germ cells, only Sertoli cells present
1	No cells in the tubule, no seminiferous epithelium.

measurement was evaluated by measuring 15 tubules in each testicular section, taking into account the transverse sections. In sections stained with Masson's trichrome, testicular tunica albuginea thicknesses were evaluated qualitatively between groups. The liver and kidneys were subjected to general histological evaluation. Selected sections were photographed microscopically (Foot 1933).

Distribution of all data was checked by performing normality analysis test (Shapiro Wilk). Analysis of variance (ANOVA) was used to compare the spermatological characteristics, testosterone, and tubule diameters of the groups; Tukey HSD post-hoc test was used for further analysis. The data of Johnsen's scoring system were compared with Kruskal-Wallis analysis of variance. Data are presented as mean ± standard error, and when $P \leq 0.05$, differences between groups were considered significant. The SPSS 21 package program was used in the analyses (Petrie and Watson 2013).

Results

As can be seen in Fig. 1 showing the live weights of the birds before and at the end of the treatment, there was no significant difference between the body weights during the rearing period ($P > 0.05$).

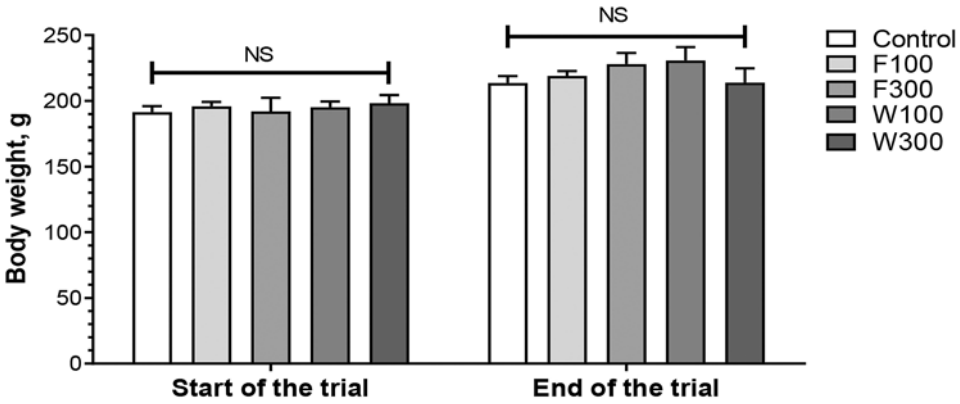

Fig. 1. The effect of boric acid supplementation on body weight of male quail.

NS: Non-significant; F100: Boric acid supplemented as 100 mg/kg into feed; F300: Boric acid supplemented as 300 mg/kg into feed; W100: Boric acid supplemented as 100 mg/l into drinking water; W300: Boric acid supplemented as 300 mg/l into drinking water.

Table 3. The effects of boric acid supplementation on spermatological characteristics and testosterone concentration.

Indicator	Control	F100	F300	W100	W300	*P* value
Right testis weight, g	3.15 ± 0.27	3.11 ± 0.17	3.07 ± 0.12	3.20 ± 0.19	3.28 ± 0.19	0.952
Left testis weight, g	2.84 ± 0.24	2.90 ± 0.16	3.15 ± 0.20	3.18 ± 0.19	3.08 ± 0.21	0.715
Testosterone, ng/ml	496.75 ± 38.57	500.00 ± 34.32	529.83 ± 35.89	434.00 ± 27.28	517.91 ± 36.40	0.111
			Indicator, million/per testis			
Spermatogonia	3.08 ± 0.46^{b}	3.33 ± 0.30^{b}	5.58 ± 0.55^{a}	4.33 ± 0.35^{ab}	5.41 ± 0.57^{a}	< 0.001
Spermatocyte	138.83 ± 8.58	147.50 ± 5.80	153.16 ± 5.26	144.83 ± 10.39	158.58 ± 7.87	0.444
Spermatid	335.58 ± 14.18	349.50 ± 8.71	359.08 ± 7.16	342.08 ± 9.20	355.66 ± 8.29	0.434
Sperm	14.08 ± 0.98^{b}	15.18 ± 0.67^{ab}	17.33 ± 0.82^{a}	14.25 ± 0.83^{ab}	16.00 ± 0.49^{a}	0.036

Values are presented as mean ± standard error of the mean. Different superscripts in the same rows show difference between the groups. $P \leq 0.05$ - significant result.

F100: Boric acid supplemented as 100 mg/kg into feed; F300: Boric acid supplemented as 300 mg/kg into feed; W100: Boric acid supplemented as 100 mg/l into drinking water; W300: Boric acid supplemented as 300 mg/l into drinking water

Testicular weights, spermatological features, and testosterone levels are presented in Table 3. The weights of the right and left testicles among the tested groups were found to be similar ($P > 0.05$). The highest spermatogonium level was observed in the F300 and W300 groups, and the lowest in the Control and F100 groups ($P > 0.001$). The W100 group was similar to other groups regarding the spermatogonium level. Spermatocyte and spermatid counts were similar among the groups ($P > 0.05$). The highest number of mature sperm was found in the F300 and W300 groups, and the lowest in the Control group ($P < 0.05$). The F100, W100 groups were found to be similar to other groups regarding the number of mature sperm.

The variables belonging to oxidative stress are presented in Table 4. The highest MDA level of testicular tissue was detected in the W300 group, followed by the W100, F300, F100 and Control groups ($P > 0.001$). The GSH level of testicular tissue was determined to be high in W300, W100, F300, Control, and F100 groups ($P < 0.001$). The differences in GSH-Px were non-significant between the groups ($P > 0.05$). The highest SOD activity of the tissue was detected in the F100 group and the lowest in the W100 group, while the activity was decreased in the Control, F300, and W100 groups ($P < 0.001$). The highest CAT activity was determined in the W300 group, followed by the F100, Control, F300 and W100 groups ($P = 0.001$). It was found that the liver MDA levels were higher in the F300 group ($P < 0.05$). The highest hepatic GSH level was found in the W300 group, and the lowest level was determined in the F100 and Control groups ($P < 0.001$).

Table 4. The effects of boric acid supplementation on lipid peroxidation and antioxidant enzyme activity in testis, liver and kidney tissues.

Indicator	Control	F100	F300	W100	W300	*P* value
			Testis			
MDA	4.77 ± 0.62^{b}	5.10 ± 0.43^{b}	6.29 ± 0.52^{ab}	6.66 ± 0.38^{ab}	7.95 ± 0.51^{a}	< 0.001
GSH	0.83 ± 0.04^{b}	0.82 ± 0.03^{b}	1.46 ± 0.08^{ab}	1.63 ± 0.06^{ab}	2.04 ± 0.05^{a}	< 0.001
GSH-Px	30.34 ± 2.75	26.50 ± 4.43	34.63 ± 5.66	28.07 ± 4.51	41.77 ± 4.03	0.115
SOD	281.68 ± 23.60^{ab}	331.97 ± 23.74^{a}	233.65 ± 27.40^{bc}	167.68 ± 20.19^{c}	195.85 ± 15.00^{bc}	< 0.001
CAT	88.03 ± 7.83^{ab}	94.07 ± 4.93^{ab}	80.80 ± 4.22^{b}	72.51 ± 6.73^{b}	109.87 ± 6.01^{a}	< 0.001
			Liver			
MDA	40.08 ± 3.15^{b}	38.84 ± 2.05^{b}	43.08 ± 2.36^{a}	38.69 ± 2.95^{b}	40.09 ± 1.18^{b}	0.043
GSH	0.37 ± 0.03^{c}	0.44 ± 0.03^{bc}	0.85 ± 0.09^{a}	0.66 ± 0.06^{ab}	0.63 ± 0.05^{ab}	< 0.001
GSH-Px	2.51 ± 0.36	3.03 ± 0.27	2.63 ± 0.36	3.87 ± 0.41	3.75 ± 0.16	0.111
SOD	55.38 ± 4.72^{b}	65.37 ± 2.91^{ab}	64.18 ± 3.94^{ab}	73.29 ± 4.27^{a}	72.37 ± 3.52^{a}	< 0.001
CAT	103.74 ± 5.71^{b}	109.45 ± 4.93^{b}	112.80 ± 5.85^{b}	109.59 ± 8.88^{b}	154.81 ± 8.97^{a}	< 0.001
			Kidney			
MDA	35.69 ± 1.81^{b}	35.72 ± 1.31^{ab}	40.98 ± 1.28^{a}	35.14 ± 0.58^{ab}	38.36 ± 2.18^{a}	0.009
GSH	0.28 ± 0.02	0.34 ± 0.01	0.30 ± 0.09	0.35 ± 0.10	0.38 ± 0.08	0.063
GSH-Px	1.05 ± 0.13^{b}	1.33 ± 0.21^{b}	1.40 ± 0.16^{b}	2.16 ± 0.26^{a}	2.19 ± 0.28^{a}	0.001
SOD	53.56 ± 8.45^{a}	40.89 ± 6.88^{ab}	25.79 ± 4.57^{b}	36.16 ± 4.93^{ab}	23.41 ± 4.50^{b}	0.008
CAT	48.31 ± 3.19^{c}	56.25 ± 2.82^{b}	59.48 ± 3.26^{b}	70.83 ± 3.16^{a}	65.47 ± 2.67^{a}	< 0.001

Values are presented as mean ± standard error of the mean. Different superscripts in the same rows show difference between the groups. $P \leq 0.05$ - significant result.

F100: Boric acid supplemented as 100 mg/kg into feed; F300: Boric acid supplemented as 300 mg/kg into feed; W100: Boric acid supplemented as 100 mg/l into drinking water; W300: Boric acid supplemented as 300 mg/l into drinking water.

MDA: Malondialdehyde (nmol/g tissue); GSH: glutathione (µmol/g tissue); GSH-Px: glutathione peroxidase (U/g protein); SOD: superoxide dismutase (U/g protein); CAT: catalase (k/g protein)

The GSH-Px activity was similar between groups ($P > 0.05$). The W100 and W300 groups had the highest levels of liver SOD activity, whereas the Control group showed the lowest levels ($P < 0.001$). The W300 group had the highest liver CAT activity, whereas all other groups showed moderate activity ($P < 0.001$). The kidney MDA level was greater ($P < 0.01$) in F300 and W300 groups compared to the Control. Although renal GSH levels were comparable in all groups ($P > 0.05$), renal GSH-Px activity was found to be significantly higher in the W100 and W300 groups compared to other groups ($P = 0.001$). Renal CAT activity was the highest in W100 and W300 groups and the lowest in the Control group ($P < 0.01$]. The highest renal SOD activity was found in the Control group, and the lowest in the F300 and W 300 groups ($P < 0.01$).

The testicular tubule diameters and Johnsen score of the groups are given in Fig. 2. Significant differences were found in tubular diameters of testicles ($P < 0.05$). The highest tubular diameter was found in the W300 group, and the testicular tubule diameters were similar in the other groups (Fig. 2). There was no significant difference between the groups in terms of Johnsen score ($P > 0.05$) (Plate XIII, Fig. 3). The example images of groups with a Johnsen score of 10 are presented in Fig. 4 (Plate XIV). There was no difference in thickness between testicular tunica albuginea in sections stained with Masson's trichrome ($P > 0.05$), as seen in Fig. 5 (Plate XV). No major pathological changes were observed in the kidney tissues of the boric acid supplemented groups compared to the Control group. In the livers, moderate micro vesicular fat degeneration was observed in the F300 and W300 groups. In the F300 group, lymphoid granulomas were observed in the livers of some birds, especially in the portal areas as shown in Fig. 6 (Plate XVI).

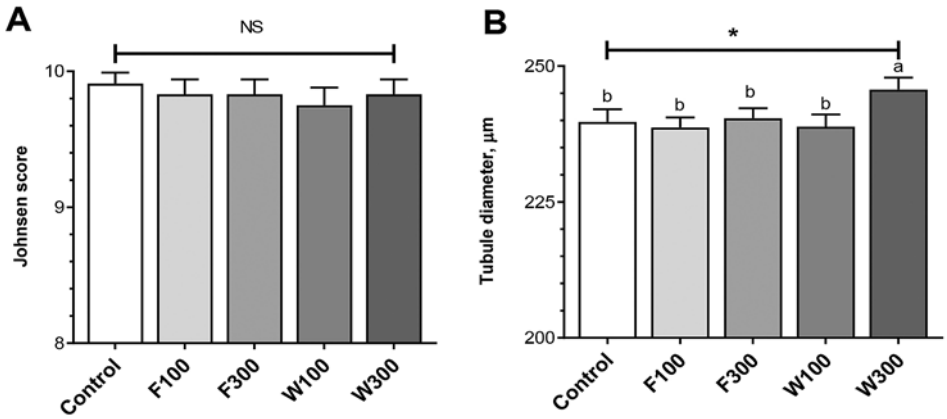

Fig. 2. The effect of boric acid supplementation on Johnsen score (A) and tubule diameters (B) of male quails.

NS: Non-significant; *: $P < 0.05$; F100: Boric acid supplemented as 100 mg/kg into feed; F300: Boric acid supplemented as 300 mg/kg into feed; W100: Boric acid supplemented as 100 mg/l into drinking water; W300: Boric acid supplemented as 300 mg/l into drinking water

Discussion

During the rearing period, a slight increase was detected in the live weights of the quails compared to the beginning of the treatment. This increase was due to the birds' aging (Alley et al. 2008). It was determined that boric acid used in different forms and at different doses did not affect adversely the live weight of male quails. There are similar studies demonstrating that boron has no negative effects on body weight (Fassani et al. 2004).

Also, prior studies have demonstrated a strong link between testicular weight and spermatological characteristics (Talebi et al. 2018; Karakus et al. 2020). Yet, there was no discernible change in the research groups' testicular weights. On the other hand, supplementation in various doses and forms was discovered to have a considerable impact on the synthesis of spermatogonium from immature cells and mature sperm cells in spermatogenesis. The process of spermatogonium self-renewal and differentiation is referred to as spermatogenesis. Testosterone is one of the elements that affect spermatogenesis. Meiosis and the development of haploid germ cells are two of the numerous stages of spermatogenesis in which it plays a role. Earlier studies (Pizzorno 2015; Białek et al. 2019) indicated that boron/boric acid increased sexual activity in males by inducing testosterone synthesis. Ibrahim et al. (2019) stated that boron supplementation increased serum testosterone and triiodothyronine (T_3) hormone levels, sperm concentration, and seminal volume, and improved semen quality by reducing sperm abnormalities. In this study, although a slight increase was detected in the F300 and W300 groups, the serum testosterone level was similar among the groups. Therefore, it was thought that a different mechanism other than testosterone might be effective in spermatogonial cell differentiation in the current study.

The seminiferous tubule widths of the study groups differ significantly from one another. The seminiferous tubules in the testicles are where spermatogenesis occurs. In this procedure, the spermatogonium in the seminiferous tubules first undergoes mitosis-mediated reproduction before going through meiosis and maturation. The quantity of cells that adhere to the tubules' walls may increase as they grow in diameter, and this could improve the sperm quality (Akpolat et al. 2018). It is thought that the high spermatogonium and sperm count in the W300 group may be related to the high tubule diameter in this group. The higher diameters of seminiferous tubules in the W300 group may be due to the quails in this group being exposed to more doses of boric acid (Simsek et al. 2020). Another factor that affected the higher mature sperm count in the F300 group may be sourced from different reasons affecting the semen quality in the high boric acid groups. Boron increases the absorption of some minerals, especially magnesium (Rondanelli et al. 2020; Iflazoglu Mutlu et al. 2021). The minerals may increase spermatogenesis by acting on different metabolisms, especially energy metabolism (Pizzorno 2015; Białek et al. 2019). On the other hand, in this study, the Johnsen score showed a value of 9–10 in all groups of this study. In other words, it seemed there was no problem in terms of spermatogenesis, tubules, and spermatozoa numbers in all groups, and this situation has been accepted as ideal and/or perfect in terms of obtaining the highest Johnsen scores. Similarly, although boron sensitivity varies in different species, no major deterioration in semen quality was found even in chronic exposures to boron (Korkmaz et al. 2011; Duydu et al. 2018).

In this study, the MDA level increased in the treatment groups depending on the dose and the supplementation method (food and drinking water). Especially in the W300 group, the increase in MDA level was found to be significant. Increased MDA level in this group may be an indicator of increased lipid peroxidation in testicular tissue and associated oxidative stress. The slight increase in MDA levels in other treatment groups also suggested that long-term use of boric acid increases lipid peroxidation in the testicles. Boric acid also increased GSH level in parallel with MDA. The increased GSH level may be due to the positive effects of boric acid on GSH metabolism. Moreover, GSH as an antioxidant agent may scavenge the MDA as an important non-enzymatic antioxidant that detoxifies lipid peroxide (Khan 2011; Rahman et al. 2014; Majid et al. 2015). Thus, higher MDA may cause higher GSH in organisms due to a correlation between MDA and GSH (Tualeka et al. 2019). In some studies (Ince et al. 2014; Cengiz et al. 2020), it has been stated that boric acid at different doses had no effect on GSH-Px enzyme activity in testis. The highest SOD activity was detected in the F100 group and the highest CAT activity in the W300 group. These findings

may reveal that boric acid may also be effective in the synthesis of antioxidant enzymes. Moreover, SOD is an essential component of seminal plasma and decreased concentration of SOD has been linked to poor sperm quality and reproductive performance (Khan et al. 2012). Oxidative damage is inhibited by increasing glutathione stores in the body. Cengiz et al. (2020) found that boron protected testicular tissue against oxidative stress. In addition, the vesicula seminalis weight, spermatozoon membrane integrity, motility, viable sperm ratio, and GSH levels decreased depending on the increase of boron dose (Aktas et al. 2020). Yalcin and Abudayyak (2020) determined that boric acid is not cytotoxic and apoptotic, but it can trigger oxidative stress by acting on testicular Leydig cells at high doses. Hepatic MDA and renal MDA was influenced by high doses of boric acid. In addition, the antioxidant defense system could be activated in liver and kidney tissues similar to the testis. These findings may be an indication that long-term use of boric acid triggers oxidative stress. Kar et al. (2020) found that boric acid had anti-apoptotic, anti-inflammatory, and antioxidant effects in kidney tissue at a low dose. However, they determined that these positive properties are lost at high doses. Similarly, pathological disorders were observed especially in liver tissue in high boric acid groups in this study. This may be an indication that long-term use of high doses of boric acid causes pathological disorders by disrupting the functions of hepatocytes. It has been found that lymphoid granulomas and fat degeneration in hepatocytes, especially in the portal areas, are consistent with the findings of Dhekra and Balqees (2018). However, boron is significantly effective on adipogenic differentiation in body and liver cells and reduces lipid accumulation in tissues (Bhasker et al. 2017; Avsar Abdik et al. 2019).

In conclusion, high doses of boric acid had positive effects on spermatological activity; however; long-term administration triggered lipid peroxidation in tissues and caused pathological disorders in liver tissue. Based on the results of the current study, it is suggested that the effect of boron in organisms may vary depending on both the dose and the duration of administration.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the Firat University Scientific Research Projects Unit (FUBAP) with Grant no: VF.19.09.

References

Abdelnour SA, Abd El-Hack ME, Swelum AA, Perillo A, Losacco C 2018: The vital roles of boron in animal health and production: A comprehensive review. J Trace Elem Med Biol **50**: 296-304

Akpolat M, Kamat B, Gulle K, Bakkal BH 2018: How the prophylactic usage of curcumin affects the parp-1 immunoreactivity and spermatogenesis in scrotal radiotherapy? Med J SDU **25**: 370-384

Aktas S, Kum C, Aksoy M 2020: Effects of boric acid feeding on the oxidative stress parameters in testes, sperm parameters and DNA damage in mice. J Trace Elem Med Biol **58**: 126447

Alley DE, Crimmins EM, Karlamangla A, Hu P, Seeman TE 2008: Inflammation and rate of cognitive change in high-functioning older adults. J Gerontol A Biol Sci Med Sci **63**: 50-55

Avsar Abdik E, Abdik H, Tasli PN, Hizli Deniz AA, Sahin F 2019: Suppressive role of boron on adipogenic differentiation and fat deposition in human mesenchymal stem cells. Biol Trace Elem Res **188**: 384-392

Bhasker TV, Gowda NKS, Krishnamoorthy P, Pal DT, Sejian V, Awachat, VB, Pattanaik AK, Verma AK, 2017: Boron supplementation provides hepato-protective effect and improves performance in Wistar rats fed calcium deficit diet. Indian J Anim Res **87**: 1213-1218

Białek M, Czauderna M, Krajewska KA, Przybylski W 2019: Selected physiological effects of boron compounds for animals and humans. A review. J Anim Feed Sci **28**: 307-320

Cengiz M, Sahinturk V, Yildiz Cetik S, Kulcanay Sahin I, Bilici N, Onur Yaman S, Altuner Y, Appak-Baskoy S, Ayhanci A 2020: Cyclophosphamide induced oxidative stress, lipid per oxidation, apoptosis and histopathological changes in rats: Protective role of boron. J Trace Elem Med Biol **62**: 126574

Chavan S, Sava L, Saxena V, Pillai S, Sontakke A, Ingole D 2005: Reduced Glutathione: Importance of specimen collection. Indian J Clin Biochem **20**: 150-152
Cortés S, Espinoza-Navarro O, Ferreccio C 2017: High exposure to boron in drinking water and sperm parameters in Chilean young people. Int J Morphol **35**: 99-104
Dhekra MM, Balqees HA 2018: Impact of boron and nano-boron on the Heterophil/Lymphocyte ratio and histopathological changes of liver and kidney in broiler chickens infected with Escherichia coli. Basra J Vet Res **17**: 290-306
Donoiu I, Militaru C, Obleagă O, Hunter JM, Neamţu J, Biţă A, Romulus Scorei I, Constantina Rogoveanu O 2018: Effects of boron-containing compounds on cardiovascular disease risk factors - A review. J Trace Elem Med Biol **50**: 47-56
Duydu Y, Basaran N, Aydin S, Ustundag A, Yalcin CO, Anlar HG, Bacanli M, Aydos K, Atabekoglu CS, Golka K, Ickstadt K, Schwerdtle T, Werner M, Meyer S, Bolt HM 2018: Evaluation of FSH, LH, testosterone levels and semen parameters in male boron workers under extreme exposure conditions. Arch Toxicol **92**: 3051-3059
Estevez-Fregoso E, Kilic A, Rodríguez-Vera D, Nicanor-Juárez L, Romero-Rizo CEM, Farfán-García ED, Soriano-Ursúa MA 2023: Effects of Boron-Containing Compounds on Liposoluble Hormone Functions. Inorganics **11**: 84
Fassani EJ, Bertechini AG, Brito JAG, Kato RK, Fialho ET, Geraldo A 2004: Boron supplementation in broiler diets. Braz J Poult Sci 6: 213-217
Foot NC 1933: The Masson trichrome staining methods in routine laboratory use. Stain Technol **8**: 101-110
Góth LA 1991: Simple method for determination of serum catalase activity and revision of reference range. Int J Clin Chem **196**: 143-152
Grote K, Niemann L, Selzsam B, Haider W, Gericke C, Herzler M, Chahoud I 2008: Epoxiconazole causes changes in testicular histology and sperm production in the Japanese quail (Coturnix coturnix Japonica). Environ Toxicol Chem 27: 2368-2374
Hunter JM, Nemzer BV, Rangavajla N, Biţă A, Constantina Rogoveanu O, Neamţu J, Romulus Scorei I, Bejenaru LE, Rău G, Bejenaru C, Mogoşanu GD 2019: The fructoborates: Part of a family of naturally occurring sugar-borate complexes-biochemistry, physiology, and impact on human health: A review. Biol Trace Elem Res **188**: 11-25
Ibrahim TB, Abdel-Wahab A, Abdel Aziz RL, El-Anwar AH, Ibrahim SS 2019: Dietary boron supplementation and its impact on testicular function, thyroid activity and serum calcium in rams. Small Rumin Res 174: 156-162
Iflazoglu Mutlu S, Simsek UG, Iflazoglu S, Yilmaz A, Mutlu M, Tatli Seven P 2021: Impact of dietary calcium tetraborate supplementation on the mineral content of egg and eggshell of laying quails. GSC Biol Pharm Sci **15**: 18-26
Ince S, Kucukkurt I, Demirel HH, Acaroz DA, Akbel E, Cigerci IH 2014: Protective effects of boron on cyclophosphamide induced lipid peroxidation and genotoxicity in rats. Chemosphere **108**: 197-204
Kabu M, Uyarlar C, Żarczyńska K, Milewska W, Sobiech P 2015: The role of boron in animal health. J Elementol **20**: 535-541
Kar F, Hacioglu C, Senturk H, Burukoglu Donmez D, Kanbak G 2020: The role of oxidative stress, renal inflammation, and apoptosis in post ischemic reperfusion injury of kidney tissue: The protective effect of dose-dependent boric acid administration. Biol Trace Elem Res **195**: 150-158
Karabag Coban F, Ince S, Kucukkurt I, Demirel HH, Hazman O 2015: Boron attenuates malathion-induced oxidative stress and acetylcholinesterase inhibition in rats. Drug Chem Toxicol **38**: 391-399
Karakus K, Aygun T, Celik S, Tariq MM, Ali M, Rafeeq M, Bukhari FA 2020: Comparison of models for some testicular characteristics in Karakas male lambs. Pak J Zool **52**: 2047-2055
Khaliq H, Zhong Z, Ke-Mei P 2018: The physiological role of boron on health. Biol Trace Elem Res. **186**: 31-51
Khan RU 2011: Antioxidants and poultry semen quality. Worlds Poult Sci J **67**: 297-308
Khan RU, Laudadio V, Tufarelli V 2012: Semen traits and seminal plasma biochemical parameters in White Leghorn layer breeders. Reprod Domest Anim **47**: 190-195
Korkmaz M, Yenigun M, Bakirdere S, Ataman OA, Keskin S, Muezzinoglu T, Lekili M 2011: Effects of chronic boron exposure on semen profile. Biol Trace Elem Res **143**: 738-750
Krishnan BB, Selvaraju S, Gowda NKS, Subramanya KB, Pal D, Archana SS, Bhatta R 2019: Dietary boron supplementation enhances sperm quality and immunity through influencing the associated biochemical parameters and modulating the genes expression at testicular tissue. J Trace Elem Med Biol **55**: 6-14
Kuru R, Yarat A 2017: Boron and a current overview of its effects on health citation metadata. Clin Exp Health Sci **7**: 107
Majid A, Qureshi MS, Khan RU 2015: In vivo adverse effects of alpha-tocopherol on the semen quality of male bucks. J Anim Physiol Anim Nutr **99**: 841-846
Matkovics B, Szabo I, Varga IS 1988: Determination of enzyme activities in lipid peroxidation and glutathione pathways. Laboratoriumi Diagnosztika **15**: 248-249
Nielsen FH, Meacham SL 2011: Growing evidence for human health benefits of boron. Evid Based Complementary Altern Med **16**: 169-180
Petrie A, Watson PF 2013: Statistics for Veterinary and Animal Science. 3rd ed. Wiley-Blackwell Publishing, Hoboken, UK

Pizzorno L 2015: Nothing boring about boron. Integr Med **14**: 35-48
Rahman HU, Qureshi MS, Khan RU 2014: Influence of dietary zinc on semen traits and seminal plasma antioxidant enzymes and trace minerals of beetal bucks. Reprod Domest Anim **49**: 1004-7
Rondanelli M, Faliva MA, Peroni G, Infantino V, Gasparri C, Iannello G, Perna S, Riva A, Petrangolini G, Tartara A 2020. Pivotal role of boron supplementation on bone health: A narrative review. J Trace Elem Med Biol **62**: 126577
Scialli AR, Bonde JP, Bruske-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
Shihab HM, Khaleel AMN 2023: Synthesis of New Homogeneous Amino Acids Compound with Boron and Some of Its Metal Complexes. Chem Methodol **7**: 137-155
Simsek UG, Aslan S, Birben N, Altundal B 2020: Examining the effects of adding boric acid at different doses into mixed feed on fattening performance, carcass characteristics, and bone quality of Japanese quails. GSC Biol Pharm Sci **13**: 1-9
Sun Y, Oberley LW, Li Y 1988: A simple method for clinical assay of superoxide dismutase. Clin Chem **34**: 497-500
Talebi AR, Ghasemzadeh J, Khalili MA, Halvaei I, Fesaha F 2018: Sperm chromatin quality and DNA integrity in partial versus total globozoospermia. Andrologia **50**: 1-6
Tualeka AR, Martiana T, Ahsan A, Russeng SS, Meidikayanti W 2019: Association between malondialdehyde and glutathione (L-gamma-Glutamyl-Cysteinyl-Glycine/GSH) levels on workers exposed to benzene in Indonesia. Open Access Maced J Med Sci **7**: 1198-1202
Turker FS, Dogan A, Ozan G, Kıbar K, Erisir M 2016: Change in free radical and antioxidant enzyme levels in the patients undergoing open heart surgery with cardiopulmonary bypass. Oxid Med Cell Longev 1783728
Yadav A 2023: Insights on the enhanced hydrogen sulfide adsorption on X (X = Cr, Ni, Al, C, Si, O, S) doped boron nitride nanotubes. Comput Theor Chem **1220**: 114005
Yalcin CO, Abudayyak M 2020: Effects of boric acid on cell death and oxidative stress of mouse TM_3 Leydig cells *in vitro*. J Trace Elem Med Biol **61**: 126506

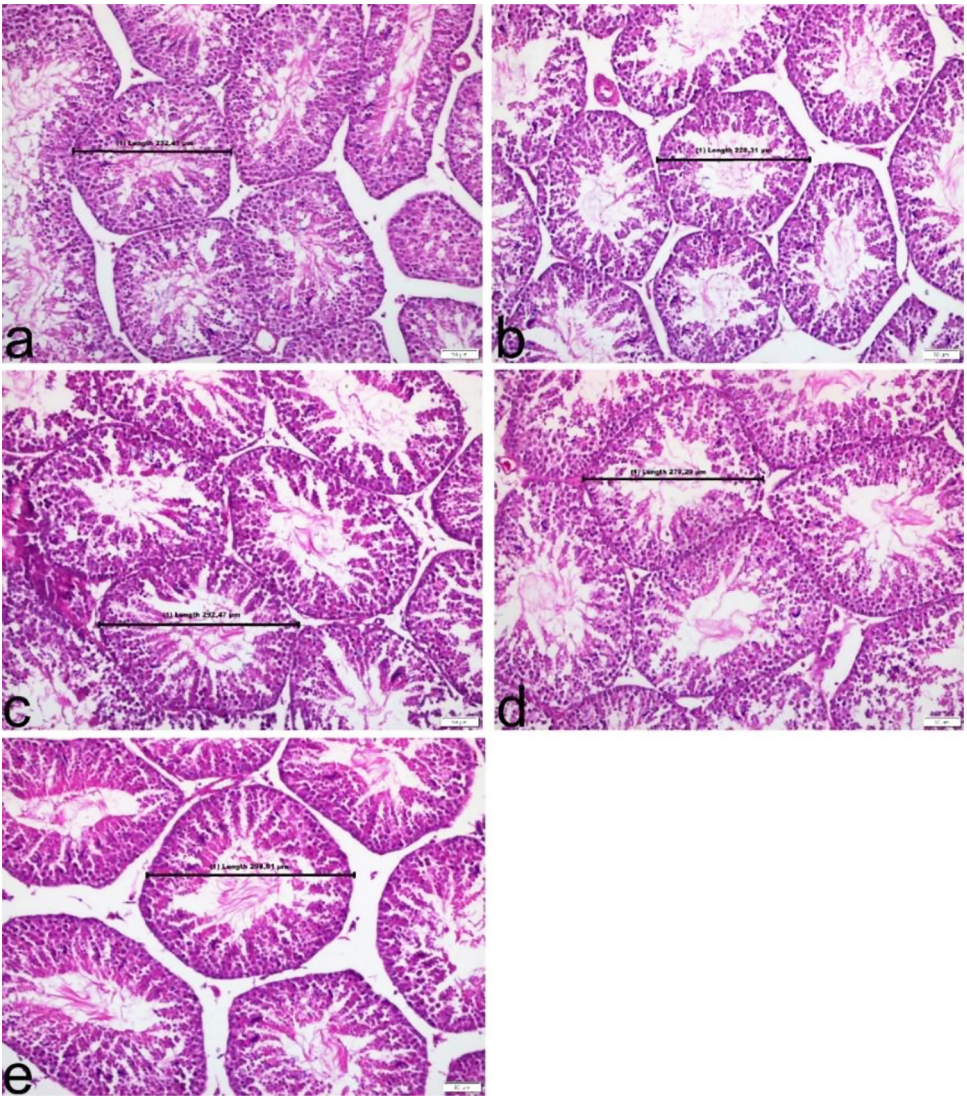

Fig. 3. Measurement of seminiferous tubule diameters, haematoxylin-eosin staining (× 200).

(a) Control; (b) F100; (c) F300; (d) W100; (e) W300. F100: Boric acid supplemented as 100 mg/kg into feed; F300: Boric acid supplemented as 300 mg/kg into feed; W100: Boric acid supplemented as 100 mg/l into drinking water; W300: Boric acid supplemented as 300 mg/l into drinking water.

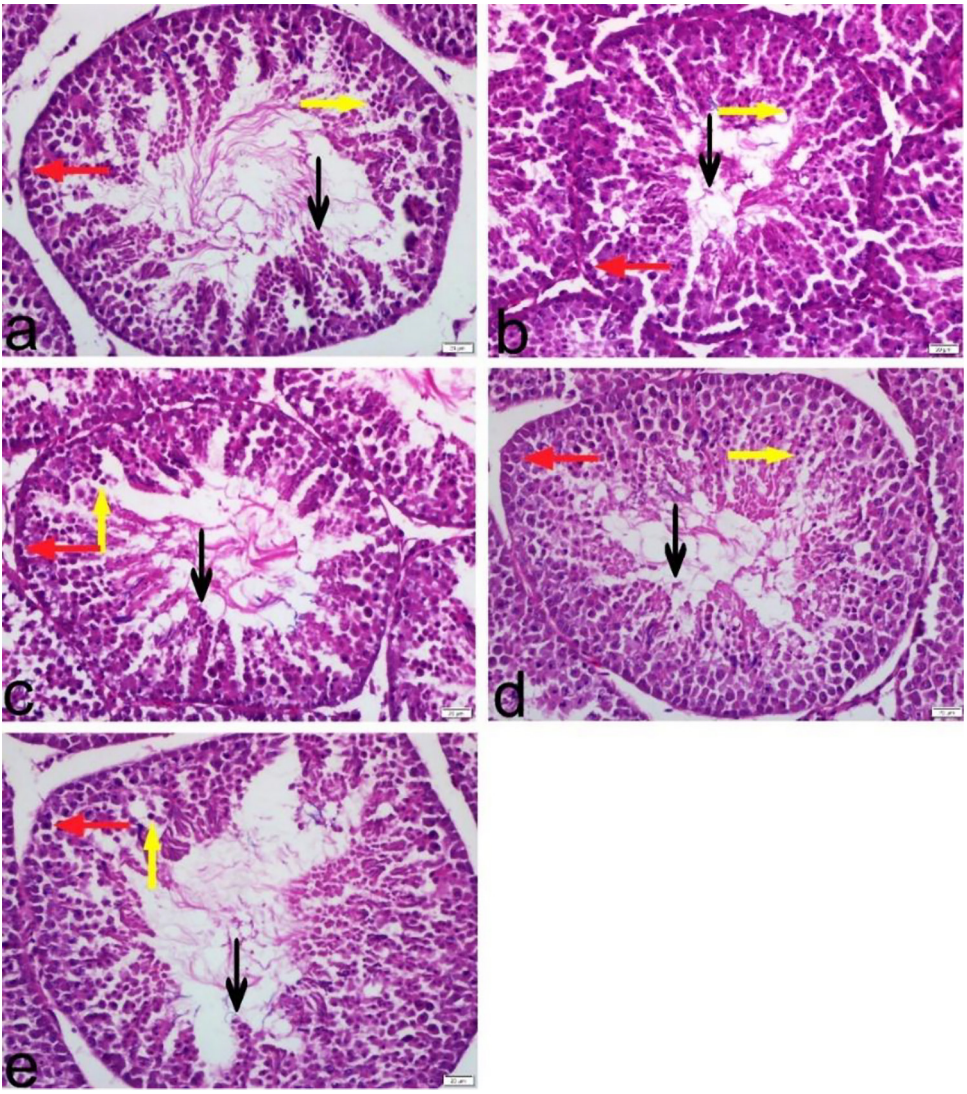

Fig. 4. The groups with a Johnsen score of 10; tubules showing complete spermatogenesis, red arrows: spermatogonium, yellow arrows: spermatids, black arrows: spermatozoa, haematoxylin-eosin staining (× 400).

(a) Control; (b) F100; (c) F300; (d) W100; (e) W300. F100: Boric acid supplemented as 100 mg/kg into feed; F300: Boric acid supplemented as 300 mg/kg into feed; W100: Boric acid supplemented as 100 mg/l into drinking water; W300: Boric acid supplemented as 300 mg/l into drinking water.

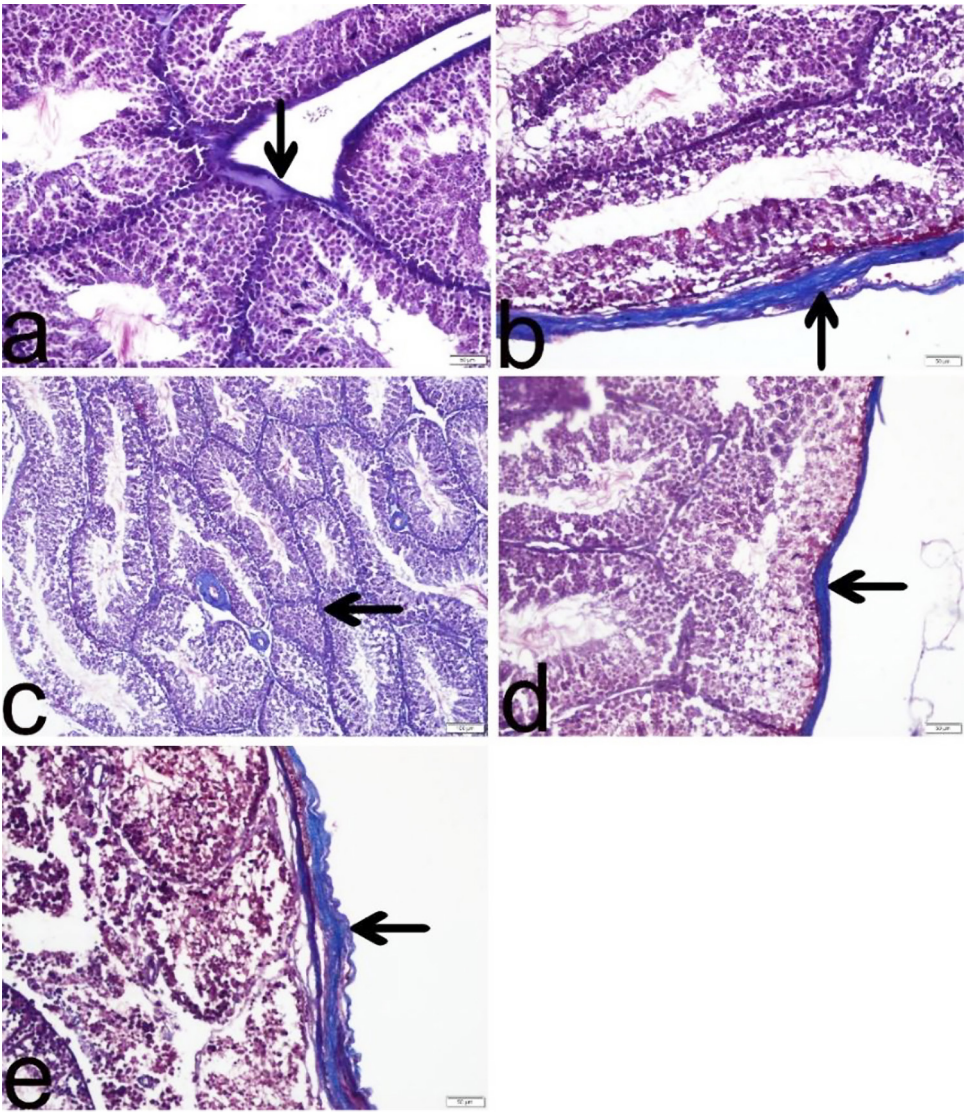

Fig. 5. In Masson trichrome staining, tunicae albugineae showing similar thickness (arrows), Masson trichrome staining (a, b, d, e: × 200, c: × 100)

(a) Control; (b) F100; (c) F300; (d) W100; (e) W300. F100: Boric acid supplemented as 100 mg/kg into feed; F300: Boric acid supplemented as 300 mg/kg into feed; W100: Boric acid supplemented as 100 mg/l into drinking water; W300: Boric acid supplemented as 300 mg/l into drinking water.

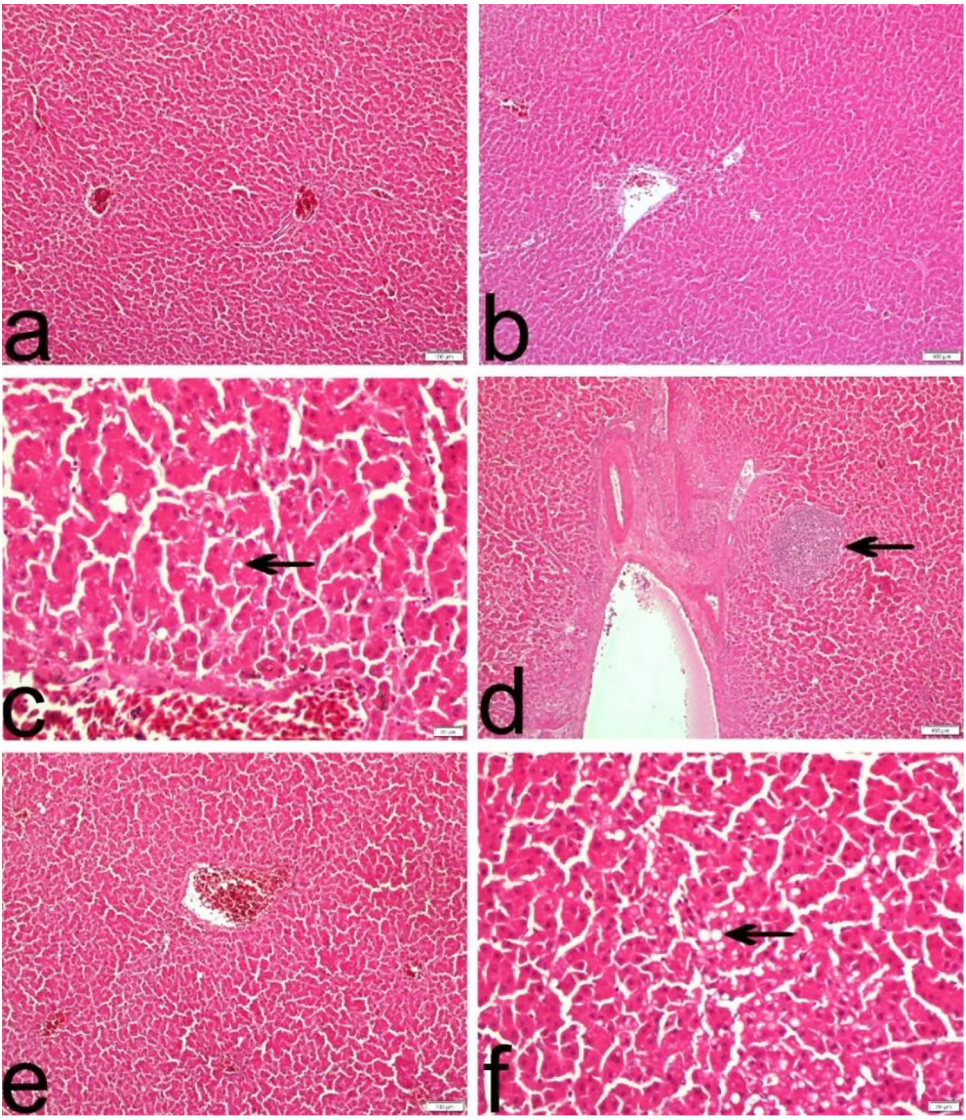

Fig. 6. Liver images: (a) control group, normal appearance; (b) F100 group, normal appearance; (c) F300 group, moderate micro vesicular lubrication in hepatocytes (arrow); (d) F300 group, lymphoid granuloma in the portal area; (e) W100 group, normal appearance; (f) moderate hepatocytes, micro vesicular steatosis (arrow); (a, b: × 100, c, f: × 400, d, e: × 200)

F100: Boric acid supplemented as 100 mg/kg into feed; F300: Boric acid supplemented as 300 mg/kg into feed; W100: Boric acid supplemented as 100 mg/l into drinking water; W300: Boric acid supplemented as 300 mg/l into drinking water.