Kafkas Univ Vet Fak Derg
23 (2): 185-193, 2017
DOI: 10.9775/kvfd.2016.16131

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Effects of Different Doses of Boric Acid Injected into Chicken Egg on Bursa of Fabricius and Spleen: A Histological and Stereological Study [1]

Abit AKTAŞ [1] O. B. Burak ESENER [1] Funda YİĞİT [1] H. Hakan BOZKURT [1]
Gül İpek GÜNDOĞAN [2] İbrahim AKYAZI [3] Evren ERASLAN [3] M. Başak ULKAY [1]

[1] *This work was supported by the Research Fund of Istanbul University. Project Number: ACIP: 31619*

[1] Istanbul University, Faculty of Veterinary Medicine, Department of Histology and Embryology, TR-34320 Avcılar, Istanbul - TURKEY
[2] Istanbul Yeni Yuzyıl University, Faculty of Medicine, Department of Histology and Embryology, Zeytinburnu, Istanbul - TURKEY
[3] Istanbul University Faculty of Veterinary Medicine, Department of Physiology, TR34320 Avcılar, Istanbul - TURKEY

Article Code: KVFD-2016-16131 Received: 16.05.2016 Accepted: 19.10.2016 Published Online: 19.10.2016

Citation of This Article

Aktaş A, Esener OBB, Yiğit F, Bozkurt HH, Gündoğan Gİ, Akyazı İ, Erarslan E, Ulkay MB: Effects of different doses of boric acid injected into chicken egg on bursa of Fabricius and spleen: A histological and stereological study. *Kafkas Univ Vet Fak Derg,* 23 (2): 185-193, 2017. DOI: 10.9775/kvfd.2016.16131

Abstract

The aim of the study was to investigate the effects of different doses of boric acid injected into chicken eggs on bursa of Fabricius and spleen. The study material consisted of three treatment and one control group. On the fourth day of incubation, boric acid dissolved in 0.9% NaCl were injected into eggs at 1.000, 1.500 and 2.000 ppm doses and control group received only 0.9% NaCl injection. Chicks hatched were raised until ten weeks of age (n=10). Bursa of Fabricius and spleen were evaluated by histological, immunohistochemical and stereological methods. Weight of bursa of Fabricius, relative weight of bursa of Fabricius ($P<0.01$), volume of bursa of Fabricius, cortical thickness, follicle area and area of follicular medulla significantly reduced ($P<0.001$) by injection of 1.500 ppm boric acid compared to control group. The number of white blood cells also decreased in 1.000 ppm and 1.500 ppm groups. An increase ($P<0.001$) was recorded in hemoglobin ratio in 2.000 ppm group when compared with the other treatment groups and control group. There was an increase in 1500 ppm group in terms of spleen weight ($P<0.001$), relative spleen weight ($P<0.01$) and plasma cell count ($P<0.05$) and likewise an increase was noted in 1.000 and 2.000 ppm groups with respect to follicle count and number of apoptotic cells in comparison to control group. In conclusion, low doses of boric acid induced the bursal involution and implicitly increased plasma cell count in the spleen.

Keywords: *Boric acid, Bursa of Fabricius, Spleen, Apoptosis, Chicken*

Yumurtaya Enjekte Edilen Farklı Dozlarda Borik Asitin Bursa Fabricius ve Dalak Üzerine Etkileri: Histolojik ve Stereolojik Çalışma

Özet

Çalışmamızda farklı dozlarda yumurtaya enjekte edilen borik asitin, bursa Fabricius ve dalak üzerine olan etkileri belirlendi. Kuluçkanın 4. gününde %0.9 NaCl içinde çözülmüş 1.000, 1.500 ve 2.000 ppm dozlarında borik asit enjekte edilen deney grupları ve sadece %0.9 NaCl enjekte edilen kontrol grubu kullanıldı. Yumurta çıkımından sonra dişi civcivler 10. haftaya kadar büyütüldü (n=10). Bursa Fabricius ve dalaklar histolojik, immunohistokimyasal ve stereolojik tekniklerle değerlendirildi. 1.500 ppm borik asit uygulanan deney grubunda bursa Fabricius ağırlığı, bursa Fabricius relativ ağırlığı ($P<0.01$), bursa Fabricius hacmi, korteks kalınlığı, follikül alanı ve follikül medulla alanı ($P<0.001$) kontrol grubuna göre istatistiksel önemde düşüş görüldü. Akyuvarlarda 1.000 ppm ve 1.500 ppm grubunda kontrol grubuna göre düşüş tespit edildi. Hemoglobin oranının ise 2.000 ppm ($P<0.001$) dozundaki grupta kontrol ve diğer deney grupları göre bir artış olduğu belirlendi. Dalak ağırlığı ($P<0.001$), relativ dalak ağırlığı ($P<0.01$) ve plazma hücre sayısı ($P<0.05$) bakımından 1.500 ppm deney grubunda kontrol grubuna göre, follikül sayısı ve apoptotik hücre sayısı bakından ise 1.000 ve 2.000 ppm grubunda kontrol grubuna göre bir artış tespit edildi. Bu değerlendirmeler sonucunda borik asidin düşük dozlarda bursa Fabricius'un involasyonuna ve dolaylı olarak dalaktaki plazma hücre sayısında artışa sebep olduğunu belirlendi.

Anahtar sözcükler: *Borik asit, Bursa Fabricius, Dalak, Apoptozis, Tavuk*

İletişim (Correspondence)
+90 533 2151005
bulkay@istanbul.edu.tr

INTRODUCTION

Boron is a widely distributed element which exists in various forms and structures in nature [1]. It is obtained mainly from arid, volcanic and hydrothermal regions of Turkey and USA combined with oxygen (borates) [2]. Boron salts that are most commonly found in nature are known as boric acid and borax [3]. These are widely distributed in soil, water and foods at ppm levels [3,4]. This element was also reported to be essential for humans and animals [5]. Boric acid is a highly exposed simple inorganic compound due to its widespread commercial use [6]. Borax and boric acid are used in several industrial fields, for instance, in the manufacture of glassware and ceramic products, disinfectants, dust repellers, wooden wares and fabrics as flame retarding materials, emery products as well as in rocket fuels as additives [4].

It is beneficial for bone structure and functions and plays role in inflammation process as well as in regulation of the physiologic functions [1,7,8]. It was shown that boron did not exhibit toxic effects on the reproduction system of rats and was found to be effective on skeletal system at high doses [9]. In another study, boron was found to decrease spleen weight and besides, it caused arthritis in rats [10]. Bai and Hunt [11], reported that boron increased the level of antibodies. It has recently been shown that boron affected the immune response against injuries and infections and it particularly had an impact on inflammatory cell population [12]. Experimentally, boron was injected into fertilized turkey eggs and tibial length, body weight and bone ash content of hatching chicks were evaluated. On the basis of histological analysis, increase in bone mineralization and thus enhanced embryonic development were observed [13].

Bursa of Fabricius which is an organ found only in avian species is located between cloaca and sacrum. It is a primary lymphoid organ which functions in the differentiation of B-lymphocytes in birds [14,15]. The alterations in the development of bursa of Fabricius were shown to have adverse effect on antibody production [14,16]. Anlage of bursa of Fabricius arises by the 4-5th days of embryonic development and starts to regress between the following 10-12th weeks of hatching under the influence of steroid hormones [17,18].

This study was conducted in an attempt to determine how chicken was affected by the extensity of boron in the environment. For this purpose, various doses of boron were injected into the chicken eggs during embryonic development and the effects of this element on bursa of Fabricius which is the primary lymphoid organ, in association with the spleen as secondary lymphoid organ in chicken, were investigated.

MATERIAL and METHODS

Animals and Experimental Design

Fertilized eggs were obtained from a local manufacturer (Has Tavuk, Turkey). The eggs were incubated at 37.5ºC with a relative humidity of 60% and turned every three hour. A total of 120 fertilized eggs (Süper Nick) were allocated into 4 groups (3 experimental and one control). Different doses (1.000 ppm, 1.500 ppm and 2.000 ppm, respectively) of boric acid dissolved in 0.9% NaCl were injected into eggs on the 4th day of incubation, when anlage of bursa of Fabricius starts to develop. The control group received only 0.9% NaCl injection (100 µl per each egg). The injections were performed via insulin injector with disposable needles (0.6X15 mm) through a tiny hole drilled into the blind spot of the egg and the substances were injected right into the egg yolk. Then the holes were covered by paraffin and the eggs were placed back in the incubator.

Weights of females were recorded after hatching. The chicks were raised on the floor with wood shaving. Room temperature was maintained at 33ºC between days 0 and 3 and then gradually reduced by 3ºC/week. The chicks were fed on commercial diet *(ad libitum)*. Chicks and experimental conduct were in accordance with the Guidelines for Animal Experiments by the Ethical Committee of Istanbul University (Approval number: 2013/16).

Relative Weights of Bursa of Fabricius and Spleen

At 10 weeks of age, 10 birds in each group were euthanized with xylazine (1.0 mg/kg) and ketamine HCl (20 mg/kg). The body weights were recorded prior to sacrification and then necropsy was performed. Bursa of Fabricius and the spleen were harvested from each chick and weighed after dissecting the connective tissues around the organs. Relative weights of bursa of Fabricius and the spleen were calculated by the following formula:

Relative weight = organ weight (g)/body weight (g) [19].

Preparation of Samples for Histological Methods

Bursa of Fabricius and spleen were fixed in modified Davidson's solution for 24 h and then the organs were cut into 0.5-cm-thick pieces. Paraffin blocks were prepared from each piece and sectioned at 5 µm thickness and then stained with Masson's trichrome stain. Histopathological changes, apoptotic index, follicle count, follicle area, thickness of follicular cortex, area of follicular medulla, height of epithelium and plasma cell count in the spleen were stereologically evaluated.

A light microscope (Leica DM4000 B), a digital camera (MBF Bioscience) and a Stereo Investigator program compatible with this microscope were used for stereological evaluations.

The distribution of progesterone receptors (PR) was evaluated in order to determine the estrogenic activity of boric acid in the bursa of Fabricius.

Stereological Analyses

Total volume of bursa of Fabricius was calculated in accordance with *Cavelier's* principle. It is known that the volume of the regular shaped objects can be estimated by the following formula:

V = t x a;

Where (t) is the height and (a) is the base area of the object.

Slices sectioned in order to calculate the volume of bursa of Fabricius were cut at 5µm-thickness providing that whole surface area of each section has appeared on the slide. A point counting grid was used for the surface area estimation of each slice and the volume of the relevant slice was obtained by multiplying surface area by slice width. Total volume of bursa of Fabricius was calculated by adding up the volumes of all slices [20].

Follicle Count, Follicle Area, Area of Follicular Medulla, Thickness of Cortex and Height of Epithelium

For this purpose, we chose an area fraction approach with an area of an unbiased counting frame of 1.000 x 1.000 μm^2 (1 mm^2) and meander sampling of each sectioned area was done in a 4.000 x 4.000 μm^2 (4 mm^2) step size in a systemic-random manner.

Mean follicle count in an area of 1 mm^2 was determined by dividing the value (follicle count) obtained from each animal by the number of counting steps. A two-dimensional isotropic uniform nucleator was used to measure follicle area, area of follicular medulla, height of epithelium and thickness of cortex on the follicle aligned with the right top corner of the counting frame at every counting step [21].

Plasma Cell Count of Spleen

Sections of 5 µm thickness were prepared and stained with methyl green-pyronin [8]. Six animals from each group were subjected to evaluation. An unbiased counting was achieved in a systematic random manner with a counting frame area of 40 x 40 (1.600 μm^2) and a meander sampling was performed with a step size of 500 x 500 µm (250.000 μm^2). Mean plasma cell count in an area of 1 mm^2 was estimated by dividing the value (plasma cell count) obtained from each animal by the number of counting steps.

Evaluation of Blood Parameters

Blood samples were collected from the animals by heart punctuation into EDTA tubes under anesthesia. Hematocrit value and hemoglobin level were assessed by microhematocrit and spectrophotometric methods, respectively. Additionally, blood smears were prepared, air-dried, fixed in methyl alcohol, stained with Wright stain and finally immersed in Sorensen buffer and kept at room temperature to be evaluated by light microscopy under 100x magnification. One hundred leukocytes were counted on each slide and the ratio of leukocyte subtypes was estimated.

The level of IgY (Cusabio, CSB-E09872Ch) was assessed by ELISA.

Immunohistochemical Staining Method

- Progesterone Receptor (PgR): Sections of bursa of Fabricius were mounted on slides coated with poly-l-lysine (Sigma, St. Louis, MO, USA). Three sections per animal (n = 10 animals per group) were analyzed. Intensity of immuno-labelling was assessed by examination of 10 representative high-power fields (objective 40x) [22].

- Determination of Fragmented DNA in situ: Apoptotic Index (AI) is used as a measure of the extent of apoptosis. To present the apoptotic cells, the fracture in DNA were labelled using Terminal deoxynucleotidyltransferase (TdT)-mediated nick end-labelling (TUNEL) technique in paraffin sections, following the procedure of applied kit (Apop Tag® Peroxidase In Situ Apoptosis Detection Kit, EMD Millipore). AI was detected in each section by light microscopy. For this purpose, in each case, TUNEL-positive cells and total cells were counted in 10 random areas, under 40x magnification [23].

- Apoptotic Index (AI): Percentage values of apoptotic and non-apoptotic cells were calculated. Ten different microscopic fields were determined on the sections obtained from different portions of bursa of Fabricius of each animal by systematic random sampling (Area: 50 x 75 μm^2). AI was calculated by the formula: 100x (mean number of TUNEL positive cells in 10 random fields)/(mean number of total cells in 10 random fields).

AI= 100 x (positive cell number/total cell number) [23].

Statistical Analyses

Statistical analyses were performed with Duncan's One-Way ANOVA Method by using Windows SPSS (Statistical Package for the Social Science Version 10.0).

RESULTS

Histological and Stereological Evaluation in the Bursa of Fabricius

Histologically, no difference was noted in the cortical and medullary portions of bursa of Fabricius in the control and experimental groups in accordance with the above mentioned properties except for the dose of 1.500 ppm which caused marked extracellular vacuolization particularly in the medullary regions of bursal follicles in this group *(Fig. 1)*.

Although follicle area and area of follicular medulla

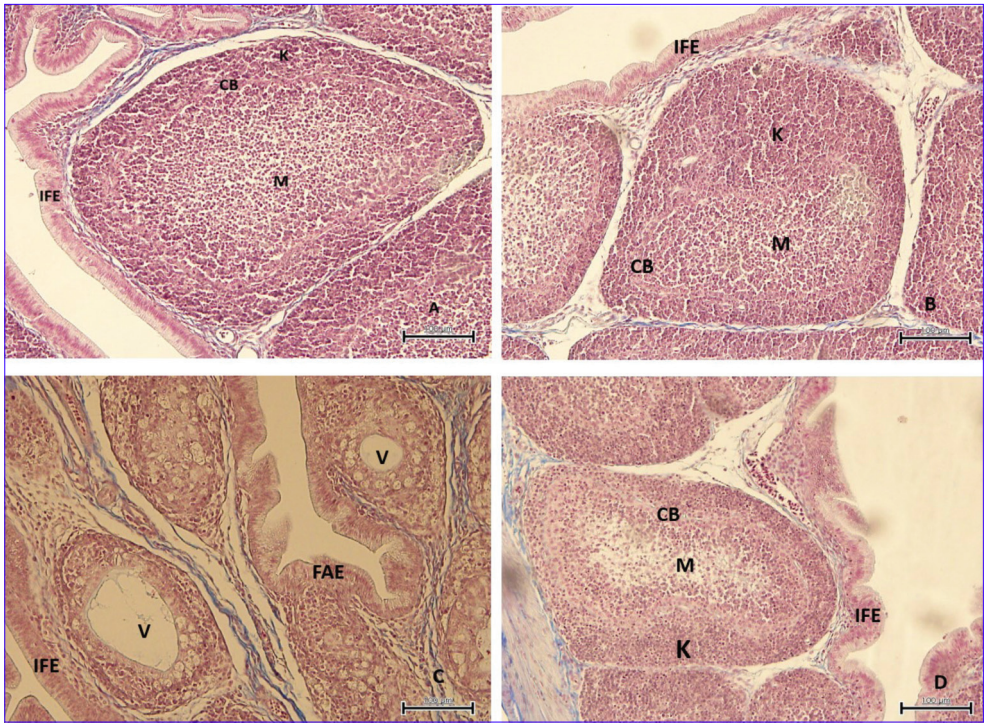

Fig 1. Histological Appearence of bursa of Fabricius in control and experimental groups, A: Control, B: 1.000 ppm, C: 1.500 ppm, D: 2000 ppm, K: Cortex, M: Medulla, CB: Corticomedullary border, IFE: Interfollicular epithelium, FAE: Follicle associated epithelium, V: Vacuolization, H&E, Bar: 100 µm

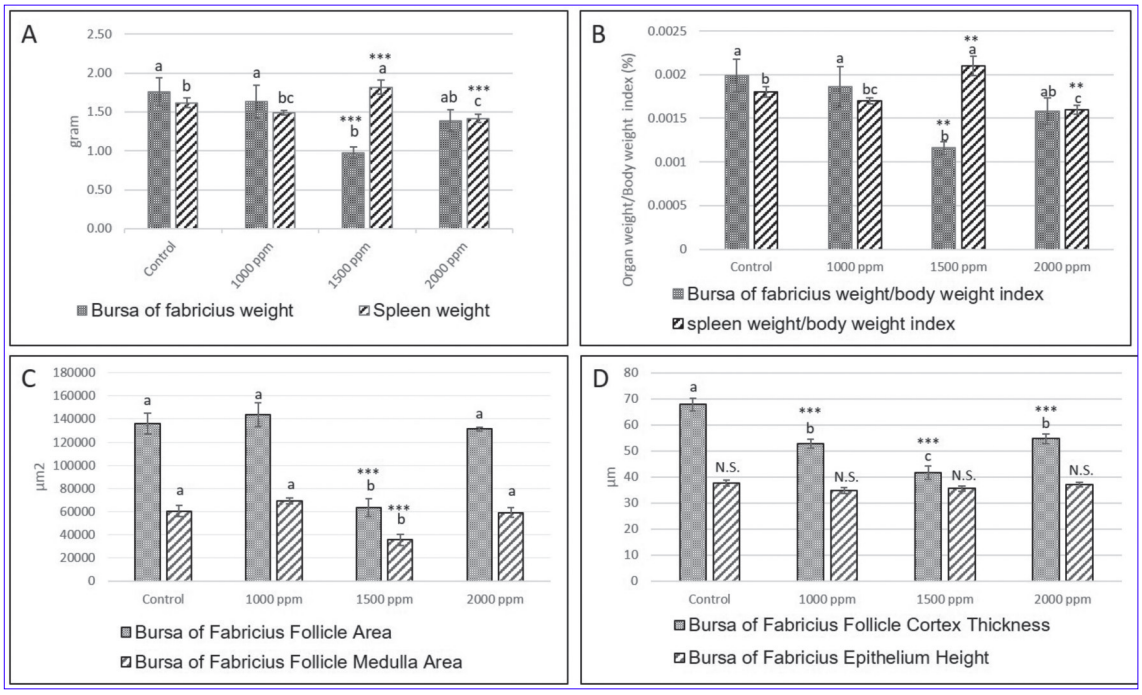

Fig 2. Bursa of Fabricius and spleen weights in control and experimental groups (A) bursa of Fabricius and spleen weight indice in control and experimental groups (B) Follicle area and area of follicle medulla of bursa of Fabricius in control and experimental groups (C) Follicle cortex and epithelium thickness values of bursa of Fabricius in control and experimental groups (D) Differences between values that doesn't share a common letter in each parameter at the graphics are statistically important (** P<0.01, *** P<0.001)

in 1.000 ppm boric acid group increased in comparison with the other groups, area of follicular medulla in groups of higher doses (1.500 and 2.000 ppm) decreased. The reduction in 1.500 ppm group was statistically significant at P<0.001 level *(Fig. 2C)*. Follicle count in 1.000 ppm (P<0.001) and 2.000 ppm groups increased when compared with control group while it decreased in 1.500 ppm group *(Fig. 3)*. Height of epithelium decreased in 1.000 and 1.500 ppm groups in comparison to control group and 2.000 ppm group (NS). A reduction was noted in the thickness of

Table 1. *Body weight, Bursa of fabricius volume, plasma cell number and blood parameter values in groups. (N.S. = Non Significant, * P<0.05, *** P<0.001)*

Parameters	Control	1000 ppm	1500 ppm	2000 ppm	Significant
Body weight (g)	873.32±19.51	874.11±9.07	834.94±19.77	874.13±12.30	NS
Bursa F volume (cm^3)	1.437±0.214^{a}	1.265±0.179^{ab}	0.627±0.063^{c}	0.883±0.094^{bc}	***
Plasma cell number	184.83±11.53^{a}	337.43±54.61^{b}	340.83±52.49^{b}	188.83±13.13^{a}	*
HCT%	31.93±0.573	30.87 ± 0.568	30.87±2.532	31.60±0.400	N.S.
HGB (g/dL)	10.1933±0.3798^{b}	9.9187±0.22132^{b}	10.2349±0.33414^{b}	11.736±0.28311^{a}	***
WBC	15.80±1.628^{a}	10.60±1.447^{b}	12.53±1.191^{ab}	15.40±1.137^{ab}	*
IgY (µg/mL)	1582.805±177.1964	1841.575±267.4685	1377.623±84.35289	1562.658±161.3952	N.S.

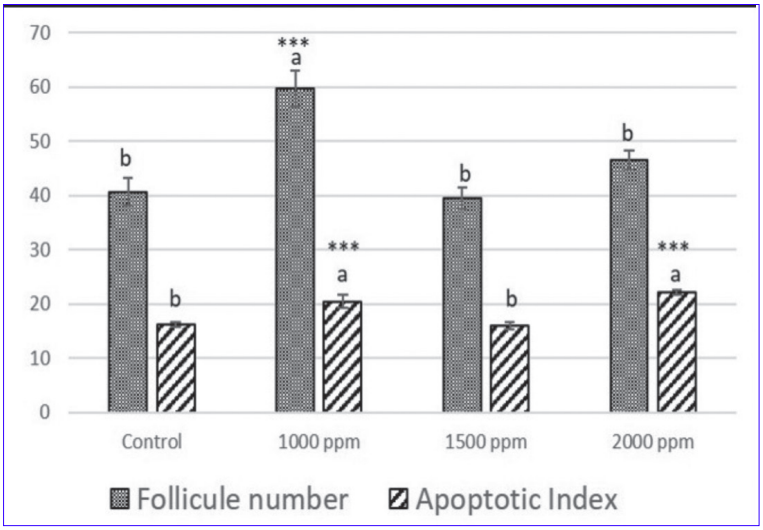

Fig 3. Follicle numbers and apoptotic index values of bursa of Fabricius in control and experimental groups. Differences between values that does not share a common letter in each parameter at the graphics are statistically important (*** P<0.001)

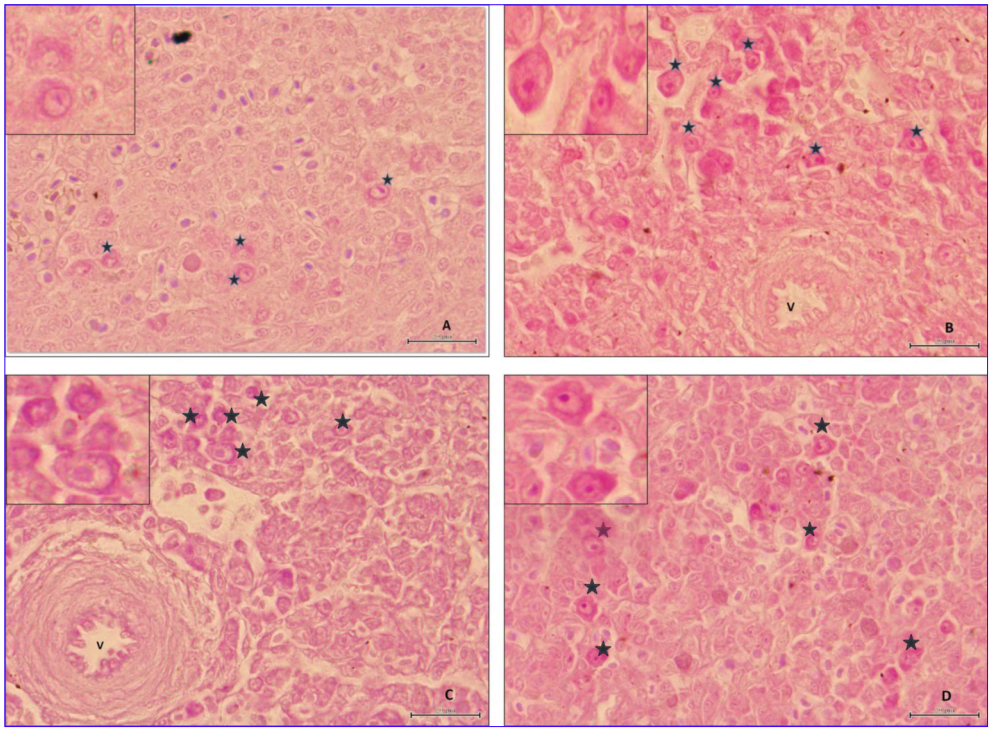

Fig 4. Plazma cell distribution and density in spleen; A: Control, B: 1000 ppm, C: 1500 ppm, D: 2000 ppm, V: vessel, *Asteriks:* Plazma cell, Methyl green-pyronin, Bar: 20 µm

follicular cortex in all experimental groups when compared with control group. A significant difference was obtained between 1.500 ppm and the other groups at P<0.001 level *(Fig. 2D)*.

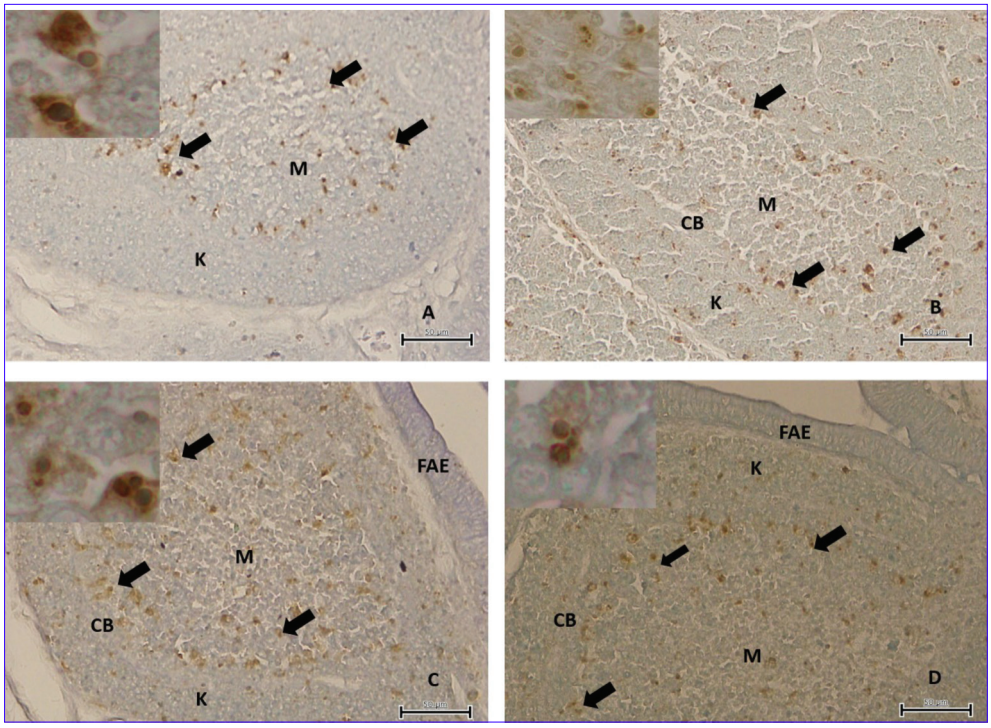

Fig 5. Apoptotic cell distribution and density in Bursa of Fabricius; A: Control, B: 1.000 ppm, C: 1.500 ppm, D: 2.000 ppm, K. Cortex, M: Medulla, CB: Corticomedullary border, FAE: Follicle associated epithelium, *Arrow:* Apoptotic cell, TUNEL, Bar: 50 µm

Body Weight, Weight of Bursa of Fabricius, Volume of Bursa of Fabricius and Relative Weight of Bursa of Fabricius

Live body weights increased by the doses of 1.000 and 2.000 ppm boric acid in comparison to control group. Although a reduction was recorded in 1500 group, the difference was not statistically significant *(Table 1)*. Bursal weights decreased in the treatment groups in comparison to control group however, the decrease was statistically significant (P<0.01) only in 1.500 ppm group *(Fig. 2A)*.

A marked reduction was recorded in 1.500 ppm group in terms of relative body weight and this reduction was statistically significant (P<0.01) when compared with 1.000 ppm group and control group *(Fig. 2B)*. Moreover, a marked decrease in the volume of bursa of Fabricius was noted in treatment groups in comparison to control group and the difference was statistically significant (P<0.001) between 1.500 ppm and 1.000 ppm and the control group *(Table 1)*.

Spleen Weight, Relative Spleen Weight and Plasma Cell Count

A reduction was recorded in the treatment groups in terms of spleen weight and relative spleen weight in comparison to control group. The difference with respect to spleen weight was statistically significant in 1.500 ppm group at P<0.001 level and likewise a statistically significant reduction (P<0.01) was noted also for relative spleen weight in the same group *(Fig. 2A, 2B)*.

Plasma cell count increased by the injection of different doses of boric acid in the treatment group when compared with control group. The differences between 1.000 ppm and 1.500 ppm groups and 2.000 ppm and control group were statistically significant at P<0.05 level *(Table 1)*. Plasma cells were most abundant in the vicinity of the blood vessels *(Fig. 4)*.

Changes in Apoptotic Index in the Bursa of Fabricius

In our study, no evaluation was carried out in the embryonic development stage. However, an increase was noted in apoptotic cell count by injection of 1.000 ppm and 2.000 ppm boric acid on 10 weeks of age in comparison to 1.500 ppm group and control group (P<0.001) *(Fig. 3)*. Apoptotic cells were mostly detected in corticomedullary regions *(Fig. 5)*.

Changes in Blood Parameters

The number of white blood cells reduced by injection of 1.000 ppm (statistically significant at P<0.05 level) and 1.500 ppm boric acid compared to 2.000 ppm and control groups.

Although hematocrit percentage values decreased in 1.000 ppm and 1.500 ppm groups, no statistically significant change was noted between the treatment groups and the control. Hemoglobin (g/dl) levels increased in control group and in 2.000 ppm group (statistically significant at P<0.001 level). On the other hand, serum IgY levels increased in 1.000 ppm while decreased in 1.500 ppm groups compared to control group (NS) *(Table 1)*.

Progesterone Receptor Expression

In our study, progesterone receptor was not expressed in the bursa of Fabricius during 10 weeks of experimental period.

DISCUSSION

In a study in which the effects of Boron on body weight were investigated, body weight of the chicks which were given 100 mg/L in drinking water for 2 weeks were reduced but those that received the same amount for six weeks gained weight. However, boron at the doses of 200 and 400 mg/L decreased their body weights [24]. Hunt [7] reported that different doses of boron with vit D caused an increase in the body weight of the chicks. King et al.[13], reported that different doses of boron injected into the egg had no effect on the body weight.

Jin et al.[24] indicated that 200 mg/L boron increased the weight of bursa of fabricius in six-week-old chicks but boron at the doses of 100 and 400 mg/L decreased the same parameter in two and four-week-old chicks.

Spleen weight was lower in the chickens which received 200 mg/ L boron in drinking water for two and six weeks when compared with the control group [24]. In a study carried out with rats boron increased weight and relative weight of the spleen [10]. The difference was considered to be associated with the difference in species.

When the results we obtained were compared with other studies, boron similarly, caused a reduction in the size and weight of bursa of fabricius in the chickens. Likewise, spleen weight increased at the dose of 1.500 ppm but decreased with other doses. Divergent results obtained for quails depend on the type of the species. Bursa of fabricius is permanent during life time in quails unlike other avian species [25].

Other criterion regarding the development of bursa of fabricius is apoptosis. Apoptosis is an important control mechanism in organogenesis [26]. Cell dead through apoptosis is linked with immune suppression [27]. Biochemical and morphological manifestations of apoptosis primarily occur at the 18th day of incubation and after hatching it occurs with sexual maturation [28]. Motyka and Reynolds [29], reported that apoptosis occurred at 4, 7 and 10 weeks of age.

On the basis of our results that the number of apoptotic cells was lower in 1.500 ppm treatment group, in which lowest values were recorded for bursal weight and volume, than those of 1.000 and 2.000 ppm groups, was considered to be associated with putative initiation of apoptosis at an early phase.

T-lymphocytes differentiate in timus and B-lymphocytes differentiate in the bursa of fabrius which is an organ found only in avian species [30]. Calander et al.[31], indicated that spleen is the organ where plasma cells proliferate and memory B cells are located. Kurtoglu et al.[8], reported that 25 mg/kg boron statistically increased plasma cell count. In a study carried out with rats antibody levels were increased by boron [11].

Therefore plasma cell count was performed only in the spleen. On the basis of data obtained, plasma cell count increased in 1.500 ppm group, in which involution was prominent. This showed that certain doses of boron led to early development of bursa of fabricius in chickens.

Likewise, Fairbrother et al.[32], evaluated the effects of boron, arsenic and selenium on immune function, development and maturation of avocet chicks. For this purpose, they investigated white blood cell count (WHC) of the chicks younger than 5 weeks of age that hatched from the eggs collected from different environments containing different levels of boron, arsenic and selenium under laboratory conditions and found no statistically significant difference. Kurtoğlu et al.[8], reported that different doses of boron (5 and 25 mg/kg) taken with food in combination with vit D_3 slightly increased WBC in 45-day-old chicks but the increase was not statistically significant.

In our study, WBC in 1.000 ppm ($P<0.05$) and 1.500 ppm groups were decreased in comparison to 2.000 ppm and the control groups. The difference in our findings which contrasted with those of Fairbrother et al.[32] and Kurtoğlu et al.[8] was considered to be associated with age and species.

In parallel with the findings obtained for plasma cell count, WBC was lower in the groups with higher plasma cell count. This was associated with the early occurrence of humoral immunity because it is known that white blood cells rather take place in cellular response. Likewise, Ig-y levels were higher in the groups of low doses.

Involution period of the bursa of Fabricius varies among different species. Period for bursal regression may not be determined only by age at least in some species but by the beginning of egg production.

Involution of the bursa starts at approximately 8 weeks of age in both sexes of white leghorns. In reality, scattered, atrophic or cystic follicles occur at 20 weeks of age and they become definitive by 24th week and the involution process is completed by 26th week and finally cicatrized bursal remnants were found at 28 weeks of age [15]. Vacuolization was present in the bursal medullary regions in the chickens that received 400 mg/L boron in drinking water for 6 weeks [24] and in antiandrogen injected quails [25] which was compatible with our experimental design.

Many studies showed that boron affected the mechanism of steroid hormone activity and particularly supported the hypothesis, stating that boron was essential for hydroxylation step in steroid synthesis [25,33]. Besides, boron was reported to regulate glucose, fat and protein metabolism by its enzymatic properties [33,34]. Jin et al.[24], administered 200 mg/dL of boron to the broilers in drinking water Follicle area and follicle count decreased in the chicks which were fed on this diet between 2 to 4 weeks of age and in 2-week-old chicks when compared with the control group but no difference was noted at 6 weeks of

age. In the same study, boron at 400 mg/dL level reduced the area of follicular nodules and follicle count in 2-6 week old and 4-6 week old chicks, respectively.

It has been reported in previous studies regarding the hormonal mechanisms of bursal involution that bursa of fabricius of the quails which received estrogen were smaller than those of the control group; however estradiol administration during embryonic period increased their sizes unlike the situation in adult quails [16]. In our previous study we have seen that Bisphenol A and Diethylstilbestrol caused involution of bursa of fabricius in chickens [35].

It was reported that progesterone receptor expression was not detectable in the chicken bursa until 10 weeks of age, however the expression started to appear between 12 to 15 weeks and expression status of progesterone receptor increased by maturation [36,37]. In our study, we found no evidence of progesterone receptor expression during a period of 10 weeks. Occurrence of bursal involution despite the lack of progesterone receptor expression in this period was associated with the influence of extrinsic boron administration.

Taking into account the changes in the thickness of follicular cortex, area of follicular medulla, follicle area, follicle count and height of epithelium, boron had an impact on the involution of bursa of Fabricius particularly at the dose of 1.500 ppm, as indicated by above mentioned studies. It may be associated with its effectiveness on the metabolism of steroid hormones as well as that on fat, glucose and protein metabolism by regulating the enzymatic activity.

When results obtained were compared with the parameters of previous studies the majority of data were compatible and ultimately boron treatment at low doses had positive impact on the development of bursa of fabricius which functions as the principle organ of avian immune system but it had no effect at high doses. In this study, alternatively, we consider that application of stereological techniques pioneering unbiased evaluations and presentation of new parameters will contribute to future researches.

REFERENCES

1. Naghii MR, Samman S: The role of boron in nutrition and metabolism. *Prog Food Nutr Sci*, 17, 331-349, 1993.

2. Woods WG: An introduction to boron - History, sources, uUses, and chemistry. *Environ Health Persp*, 102, 5-11, 1994.

3. Howe PD: A review of boron effects in the environment. *Biol Trace Elem Res*, 66, 153-166, 1998. DOI: 10.1007/BF02783135

4. Robbins WA, Xun L, Jia J, Kennedy N, Elashoff DA, Ping L: Chronic boron exposure and human semen parameters. *Reprod Toxicol*, 29, 184-190, 2010. DOI: 10.1016/j.reprotox.2009.11.003

5. Nielsen FH: Boron in human and animal nutrition. *Plant Soil*, 193, 199-208, 1997. DOI: 10.1023/A:1004276311956

6. Ku WW, Chapin, RE, Moseman RF, Brink RE, Pierce KD, Adams KY: Tissue disposition of boron in male Fischer rats. *Toxicol Appl Pharmacol*, 111,145-151, 1991. DOI: 10.1016/0041-008X(91)90143-3

7. Hunt CD: The biochemical effects of physiological amounts of dietary boron in animal nutrition models. *Environ Health Persp*, 102, 35-43, 1994.

8. Kurtoğlu F, Kurtoğlu V, Celik I, Kececi T, Nizamlioglu M: Effects of dietary boron supplementation on some biochemical parameters, peripheral blood lymphocytes, splenic plasma cells and bone characteristics of broiler chicks given diets with adequate or inadequate cholecalciferol (vitamin D-3) content. *Brit Poult Sci*, 46, 87-96, 2005. DOI: 10.1080/00071660400024001

9. Chapin RE, Ku WW, Kenney MA, McCoy H: The effects of dietary boric acid on bone strength in rats. *Biol Trace Elem Res*, 66, 395-399, 1998. DOI: 10.1007/BF02783150

10. Hu QQ, Li SH, Qiao EM, Tang ZT, Jin EH, Jin GM, Gu YF: Effects of boron on structure and antioxidative activities of spleen in rats. *Biol Trace Elem Res*, 158, 73-80, 2014. DOI: 10.1007/s12011-014-9899-5

11. Bai Y, Hunt CD: Dietary boron alleviates adjuvant-induced arthritis (AIA) in rats. *FASEB J*, 9 (3): A576-A576, 1995.

12. Samman S, Naghii MR, Wall PML, Verus AP: The nutritional and metabolic effects of boron in humans and animals. *Biol Trace Elem Res*, 66, 227-235, 1998. DOI: 10.1007/BF02783140

13. King N, Odom TW, Sampson HW, Pardue SL: *In ovo* administration of boron alters bone mineralization of the chicken embryo. *Biol Trace Elem Res*, 30, 47-58, 1991. DOI: 10.1007/BF02990341

14. Pedernera EA, Romano M, Besedovsky HO, Aguilar MC: The bursa of Fabricius is required for normal endocrine development in chicken. *Gen Comp Endocrinol*, 42, 413-419, 1980. DOI: 10.1016/0016-6480(80)90172-0

15. Ciriaco E, Pinera PP, Diaz-Esnal B, Laura R: Age-related changes in the avian primary lymphoid organs (thymus and bursa of Fabricius. *Microsc Res Tech*, 62, 482-487, 2003. DOI: 10.1002/jemt.10416

16. Quinn MJ, Jr McKernan M, Lavoie ET, Ottinger MA: Effects of estradiol on the development of the bursa of Fabricius in Japanese quail. *J Exp Zool A Ecol Genet Physiol*, 311, 91-95, 2009. DOI: 10.1002/jez.504

17. Quinn MJ, Jr Lavoie ET, Ottinger MA: Reproductive toxicity of trenbolone acetate in embryonically exposed Japanese quail. *Chemosphere*, 66, 1191-1196, 2007. DOI: 10.1016/j.chemosphere.2006.07.085

18. Ratcliffe MJ: The ontogeny and cloning of B cells in the bursa of Fabricius. *Immunol Today*, 6, 223-227, 1985. DOI: 10.1016/0167-5699(85)90039-8

19. Chen K, Fang J, Peng X, Cui H, Chen J, Wang F, Chen Z, Zuo Z, Deng J, Lai W, Zhou Y: Effect of selenium supplementation on aflatoxin B(1)-induced histopathological lesions and apoptosis in bursa of Fabricius in broilers. *Food Chem Toxicol*, 74, 91-97, 2014. DOI: 10.1016/j.fct.2014.09.003

20. Sahin B, Emirzeoglu M, Uzun A, Incesu L, Bek Y, Bilgic S, Kaplan S: Unbiased estimation of the liver volume by the Cavalieri principle using magnetic resonance images. *Eur J Radiol*, 47, 164-170, 2003. DOI: 10.1016/S0720-048X(02)00152-3

21. Piskin A, Kaplan S, Aktas A, Ayyildiz M, Raimondo S, Alic T, Bozkurt HH, Geuna S: Platelet gel does not improve peripheral nerve regeneration: An electrophysiological, stereological, and electron microscopic study. *Microsurg*, 29, 144-153, 2009. DOI: 10.1002/micr.20599

22. Gonzolez-Moran MG: *İmmunohistochemical* localization of progesterone receptor isoforms and estrogen receptor alpha in the chicken oviduct magnum during development. *Acta Histochem*, 117, 681-687, 2015. DOI: 10.1016/j.acthis.2015.10.003

23. Yildirim F, Sonmez K, Ozyogurtcu H, Sennazli G, Gurel A, Gunduz MC, Sontas BH: Evaluation of Bcl-2, Bcl-X-L and Bax expression and apoptotic index in canine mammary tumours. *Kafkas Univ Vet Fak Derg*, 20, 513-520, 2014. DOI: 10.9775/kvfd.2013.10450

24. Jin EH, Gu YF, Wang J, Jin GM, Li SH: Effect of supplementation of drinking water with different levels of boron on performance and immune organ parameters of broilers. *Ital J Anim Sci*, 13, 205-214, 2014. DOI: 10.4081/ijas.2014.3152

25. Quinn MJ, Summitt CL, Ottinger MA: Effects of androgen disruption by DDE on the development and functioning of the immune system in

Japanese quail. *Immunopharmacol Immunotoxicol*, 28, 535-544, 2006. DOI: 10.1080/08923970600927850

26. Ghobrial IM, Witzig TE, Adjei AA: Targeting apoptosis pathways in cancer therapy. *CA Cancer J Clin*, 55, 178-194, 2005. DOI: 10.3322/canjclin.55.3.178

27. Sun EW, Shi YF: Apoptosis: The quiet death silences the immune system. *Pharmacol Ther*, 92, 135-145, 2001. DOI: 10.1016/S0163-7258(01)00164-4

28. Reynaud CA, Bertocci B, Dahan A, Weill JC: Formation of the chicken B-cell repertoire: Ontogenesis, regulation of Ig gene rearrangement, and diversification by gene conversion. *Adv Immunol*, 57, 353-378, 1994. DOI: 10.1016/S0065-2776(08)60676-8

29. Motyka B, Reynolds JD: Apoptosis is associated with the extensive B-cell death in the sheep ileal peyers patch and the chicken bursa of Fabricius - A possible role in B-cell selection. *Eur J Immunol*, 21, 1951-1958, 1991. DOI: 10.1002/eji.1830210825

30. Sharma JM: The Structure and function of avian immune system. *Acta Vet Hung*, 45, 229-238, 1997.

31. Calander D, Stanberg J, Larsson A: Chicken Antibodies. *Ups J Med Sci*, 104, 179-189, 1999. DOI: 10.3109/03009739909178961

32. Fairbrother A, Fix M, O'Hara T, Ribic CA: Impairment of growth and immune function of avocet chicks from sites with elevated selenium, arsenic, and boron. *J Wildl Dis*, 30, 222-233, 1994. DOI: 10.7589/0090-3558-30.2.222

33. Devirian TA, Volpe SL: The physiological effects of dietary boron. *Crit Rev Food Sci Nutr*, 43, 219-231, 2003. DOI: 10.1080/10408690390826491

34. Li SH, Li WC, Zhou JX, Jin GM, Gu YF, Chen HI, Zhao J, Huang J: Effects of drinking boron on the growth and the biochemical indexes and antioxidant ability in serum of rats. *Chin J Vet Sci*, 3, 486-492, 2013. DOI: 10.4081/ijas.2014.3152

35. Yiğit F, Aktas A, Daglioglu S: Effects of bisphenol A and diethylstilbestriol on the involution of bursa Fabrisius in the hens. *J Fac Med Istanbul Univ*, 39, 168-174, 2013.

36. Pasanen S, Ylikomi T, Palojoki E, Syvala, H., Pelto-Huikko, M, Tuohimaa P: Progesterone receptor in chicken bursa of Fabricius and thymus: Evidence for expression in B-lymphocytes. *Mol Cell Endocrinol*, 141, 119-128, 1998. DOI: 10.1016/S0303-7207(98)00086-0

37. Ylikomi TJ, Isola JJ, Gasc JM, Tuohimaa PJ: Sexual maturation-associated and estrogen-induced progesterone receptor expression in the bursa of Fabricius. *J Immunol*, 138, 3174-3178, 1987.