



Histologic and Histometric Examination of Spleen in Geese (*Anser anser*)*

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Abstract: The aim of this study was to examine the histometrical and histological structures of goose (*Anser anser*) spleen. Six healthy female geese were used as material. Tissue samples taken from the spleen were processed routinely, and were then stained with H&E, Crossman's Triple stain and Toluidine blue stain. The spleen surrounded by capsules composed of connective tissue and parts of the capsules were observed increasingly thinning into the spleen as trabeculae. The red pulp area was distinguishable from the white pulp inside the organ; further, the lymph follicles appeared clearly within the white pulp. Histometric measurements revealed that the thickness of the capsules surrounding the organ ranged from 18 to 28 µm. The average thickness of the capsules was measured as 22 µm. The average number of lymph follicles was found to be 2.4 in 1.07 mm². The average width and length of the lymph follicles were measured as 113 µm and 144 µm, respectively. The average diameter of the mast cells was found to be 6 µm. The average number of mast cells was found to be 1.4 in 1.07 mm². Although the histological structures of the geese spleens seemed very similar to those of other animals in several respects, but some specific properties of geese spleens being more similar to that of mammals were also observed.

Key words: Goose, Histometry, Lymph follicle, Mast cell, Spleen.

Kaz (*Anser anser*) Dalak Dokusunda Histolojik ve Histometrik İnceleme

Özet: Bu çalışmada Kars'ta yetiştirilen kazların (*Anser anser*) dalağının histolojik ve histometrik olarak incelenmesi amaçlandı. Materyal olarak 10-12 aylık 6 adet dişi kaz kullanıldı. Dalaktan alınan örnekler rutin doku takibinden geçirilerek Crossman'ın üçlü boyaması, Hematoksilen Eosin boyama ve Toluidin mavisi ile boyandı. Kaz dalağının etrafının bağdokudan oluşmuş bir kapsül ile çevrelendiği, trabeküllerin organın içine doğru giderek incelendiği gözlemlendi. Dalak dokusunda beyaz ve kırmızı pulpa alanları birbirinden ayırt edilirken, beyaz pulpada lenf foliküllerinin belirgin olduğu görüldü. Yapılan histometrik ölçümlerde, kaz dalağının çevreleyen kapsül kalınlığı ortalaması 22 µm olarak ölçüldü. Lenf folikülü sayısı 1.07 mm² de ortalama 2.4 olarak belirlendi. Mast hücrelerinin ortalama çapı 6 µm olarak ölçüldü. Mast hücre sayısı 1.07 mm² de ortalama 1.4 olarak belirlendi. Kaz dalağının histolojik yapısı birçok yönden diğer kanatlılarla benzerlik göstermesine karşın trabeküllerin daha belirgin olması nedeniyle memeli dalağına daha benzer yapıda olduğu görüldü.

Anahtar kelimeler: Dalak, Histometri, Kaz, Lenf folikülü, Mastosit.

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INTRODUCTION

The spleen is the biggest of the lymphoid organs. Fibrous capsule surrounds the spleen that produces lymphocytes, stores and filters the blood. The capsule extends into the organ as trabeculae. Trabecular arteries and veins are located within the trabeculae. The parenchyma of the spleen called as "pulp". The pulp consists of white and red pulp (Kroese et al., 1987; Asti et al., 1997; Alabay, 2008). Red pulp is an important part of the parenchyma of organ. Furthermore, red pulp is located within the network of white pulp and amongst the trabeculae. The central artery was located mainly eccentrically in the lymph follicle within the white pulp areas (Ross and Pawlina, 2011). Lymphocytes are located in the periarteriolar lymphatic sheath (PALS) and the lymph follicles of the white pulp (Kroese et al., 1987; Asti et al., 1997; Ross and Pawlina, 2011). The spleen contains mast cells. Within the cytoplasm of mast cells, there are granules of histamine and heparin (Straus et al., 1982; Jamur et al., 2005). Except the histamine and heparin, mast cells release essential substances such as eosinophil chemotactic factor of anaphylaxis, which is the primary mediator of type I anaphylactic hypersensitivity, prostaglandin and slow reacting substance of anaphylaxis (Caughey, 2011). When these cells uptake the basic stains, they may be seen with different coloration as purple-red (metachromatic) (Jamur et al., 2005; Thangapandiyam and Balachandran, 2010). The spleen has an important defensive structure against the microorganisms because it contains a large number of phagocytic cells and is located close to the circulatory system (Alabay, 2008). In contrast to the spleen of other fowls, which is round or oval, the spleen of waterfowls is triangular (Causey Whittow, 2000)

Therefore, the aim of this study was to determine the characteristic properties of spleen of goose, as a species of waterfowl, histometrically and histologically.

MATERIALS and METHODS

This study has been approved by the Experimental Ethics Committee of Kafkas University, Faculty of Veterinary Medicine (2010/10).

In this study, six healthy female geese (*Anser anser*), aged 10-12 months, were used. Spleen samples were fixed in Bouin's solution for histological and histometrical examinations. The samples were embedded in paraffin. Then, the sections (5 µm thickness) were cut from the paraffin blocks and placed on the slides. Serial sections were stained with Hematoxylin and Eosin (H&E), Crossman's triple stain (Triple stain) and Toluidine blue (Luna, 1968). The slides were examined histologically by light microscopy (Olympus BX-51), and histometrically by an ocular micrometer with 100 squares, which is called as 'area', in the light microscopy. For measuring the length and width of lymph follicles, slides were selected randomly from each subject, and 100 follicles per subject were evaluated in certain areas as determined randomly from each slide selected. The follicles were counted in 100 areas, as selected per subject randomly. The mast cells were counted in 10 areas of each subject on a slide.

Statistical Analysis: The averages, standard deviation, maximum and minimum values of the histometric data were calculated using the SPSS 16.0 program.

RESULTS

In the histological examination, it was observed that the goose spleen was surrounded by capsule composed of connective tissue, similar to those of other species. It was further observed that the trabeculae formed by this capsule became increasingly thinner towards the inside of spleen (Figure 1). In the trabeculae, trabecular arteries and veins were seen side by side (Figure 2). When the areas of white and red pulp in the tissue of the spleen were distinguished from each other, the

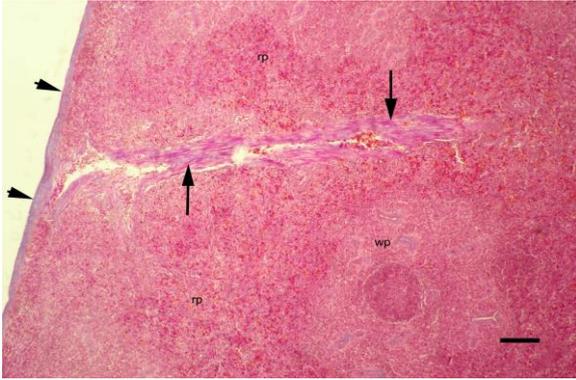


Figure 1. Histological appearance of goose spleen. White pulp (wp), red pulp (rp), capsule (arrowheads) and trabeculae (arrows). Triple stain (Bar=200 μ m).

Şekil 1. Kaz dalağının histolojik görünümü. Beyaz pulpa (wp), kırmızı pulpa (rp), kapsül (okbaşları) ve trabekül (oklar). Triple boyama (Bar=200 μ m).

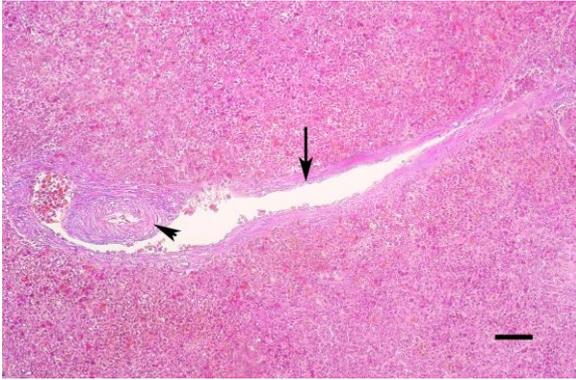


Figure 2. Trabecular artery (arrowhead) and vein (arrow) are located within the trabeculae. Triple stain (Bar=100 μ m).

Şekil 2. Trabekül içinde arteriya (okbaşı) ve vena (ok) trabekülaris. Triple boyama (Bar=100 μ m).

lymph follicles were observed clearly in the white pulp (Figure 1). The central artery in the lymph follicles was generally observed to be located eccentrically in the white pulp areas (Figure 3). Mast cells were observed in the white pulp more than in the red pulp and were never observed in the lymph follicles (Figure 4). Histometric measurements revealed that the capsule surrounding the spleen had a thickness ranging from 18.1 to 28.4 μ m. Moreover, the average thickness of the capsule was measured as 22.47 μ m.

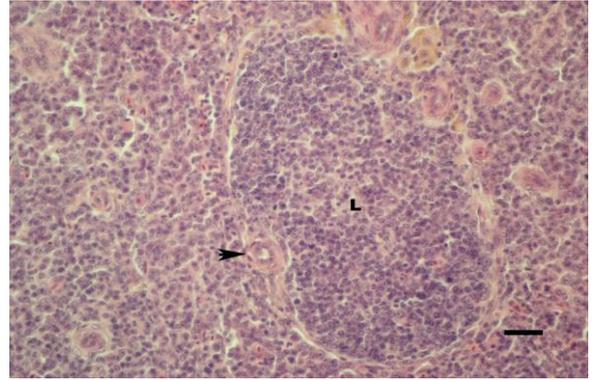


Figure 3. Central artery (arrowhead) and lymph follicle (L) in goose spleen. H&E (Bar=50 μ m).

Şekil 3. Kaz dalağında arteria sentralis (okbaşı) ve lenf folikülü (L). H&E (Bar=50 μ m).

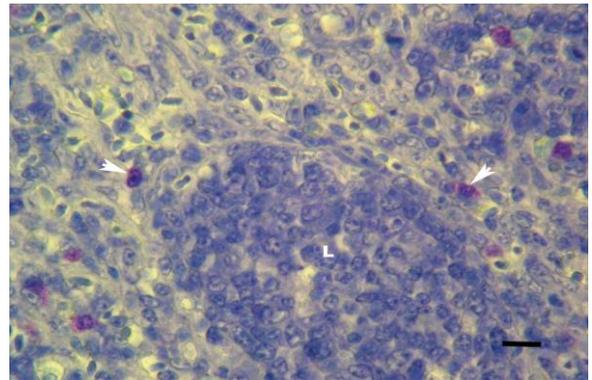


Figure 4. Mast cell (arrowheads) and lymph follicle (L) in goose spleen. Toluidine blue (Bar=20 μ m).

Şekil 4. Kaz dalağında mast hücresi (okbaşları) ve lenf folikülü (L). Toluidine blue (Bar=20 μ m).

The average number of lymph follicles was found to be 2.36 in 1.07 mm². The width of the lymph follicles varied between 20.6 and 248.6 μ m (with an average of 113 μ m), while the length of lymph follicles varied between 25.8 and 310.8 μ m (with an average of 144.3 μ m). The ratio of lymph follicles' width to length (W/L) was determined as 0.79. The diameters of mast cells varied between 5.2 and 7.2 μ m (with an average of 5.97 μ m). On average, 1.35 mast cells were counted in 1.07 mm² (Table 1).

Table 1: Results of histometric measurement in geese spleen.**Tablo 1:** Kaz dalağında histometrik ölçüm sonuçları.

	N	Min.	Max.	Mean ± SD
Thickness of capsule (µm)	6	18.06	28.38	22.47±2.60
Number of follicles (1.07 mm ²)	6	1.00	8.00	2.36±1.34
Width of follicles (µm)	6	20.64	248.64	113.00±44.48
Length of follicles (µm)	6	25.80	310.80	144.33±56.67
Ratio of width/length of follicles (%)	6	0.48	1.00	0.79±0.10
Diameter of mast cells (µm)	6	5.17	7.24	5.97±0.66
Number of mast cells (1.07 mm ²)	6	0	6	1.35±1.31

SD: Standard Deviation.

DISCUSSION and CONCLUSION

Kozlu et al. (2011) reported that the capsule surrounding the spleen did not form trabeculae entering into the organ of ostriches or kestrels but it did so in ospreys. Bradley (1915) reported in fowls that the trabeculae entering into the spleen were composed of fibrous tissue. Tischendorf (1985) reported that while trabeculae were not seen in many species of poultry, they were found a small extent in geese. Fitzgerald (1969) reported that the spleen in quail had clear trabeculae. Liman and Bayram (2011) reported that the trabecular artery, trabecular vein and central artery were quite obvious in quails. In this study, trabeculae were observed quite clearly. It was concluded that the histologic structure of the goose spleen resembles that of mammals more closely than that of poultry. In some of previous studies (Thorbecke et al., 1957; Payne, 1971; Hodges, 1974; Jeurissen et al., 1988), it

has been stated that the boundary between the white and red pulps was not distinguishable in poultry. On the other hand, the boundary between the white and red areas was distinguishable in some others (Starck and Riclefs, 1998; Causey Whittow, 2000). In this study, the boundary between the white and red pulps was distinguishable in geese. Tishendorf (1985) reported that the thickness of capsule surrounding the spleen of duck (*Anas platyrhynchos domestica*) ranged from 23 to 40 µm. Biljana et al. (2008) reported that the average width of lymph follicles was 76 µm. However, in this study, it was found that the thickness of capsule was slightly thinner, and the average width of lymph follicles was longer. Karaca et al. (2006) reported that the mast cells were encountered within the lymph follicles. Balcan et al. (2009) reported that these cells were seen mainly in red pulp and that the number of mast cells increased during development. In our study, the cells were encountered within the lymph follicles and they were mainly found in the white pulp apart from the lymph follicles of the spleen. It has been reported that the diameter of mast cells was 30 µm in humans and ranged from 3.5 to 22 µm in rodents (Galli et al., 1984). Uslu and Yoruk (2013) reported that the number of mast cells in the lower respiratory tract and in the lung of goose (average 20 in mm²) was lower than that of duck (average 30 in mm²). Herein, the average diameter of these cells was 5.97 µm and the average number of mast cells was 1.35 in 1.07 mm² in the spleen. We could not find any data in the literature about the diameter of mast cells of poultry so no comparison could be made among poultry species.

In conclusion, although the histological structure of goose spleen was very similar to that of other animals in several respects, some specific properties of goose spleen (related with the histological structure, histometric values and the distribution of mast cells) were also observed in the present study. Hence, it was considered that the

data presented herein might make a useful contribution to the literature.

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