

The Distribution and Heterogeneity of Mast Cells in the Cecum of Quail (*Coturnix Coturnix Japonica*)^[1]

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Abstract

The aim of the present study is to investigate the location and heterogeneity of mast cells in quail cecum. Cecum samples were fixed in basic lead acetate (BLA), Carnoy's, isotonic formaldehyde acetic acid (IFAA) and 10% neutral buffered formalin (NBF) solutions. The sections were stained with an alcian blue-critical electrolyte concentration (AB-CEC) (pH 5.8, 0.3 M MgCl₂) and safranin O (SO) (pH 1.0) used in a combined method and with toluidine blue (TB) (pH 0.5). Mast-cell population was shown in highest ratios in all layers of Carnoy fixed cecum parts within TB-stained sections. Metachromatic mast cell density was determined the most in tunica mucosa layers of the middle and distal cecum. It was seen that AB-CEC (+)/SO (-) mast-cell density was greater in tunica mucosa layers of the proximal and middle cecum in IFAA and BLA fixed tissues compare to Carnoy fixation. At the end of the study; it can be said that Carnoy solution fixed the connective tissue mast cells, as well as IFAA and BLA solutions fixed the mucosal mast cells better.

Keywords: Cecum, Fixative solution, Mast cell, Quail

Bıldırcın (*Coturnix Coturnix Japonica*) Sekumunda Mast Hücrelerinin Dağılımı ve Heterojenitesi

Özet

Sunulan çalışmanın amacı, bıldırcın sekumunda mast hücrelerinin yerleşimini ve heterojenitesini araştırmaktır. Sekum örnekleri bazik kurşun asetat (BLA), Carnoy, izotonik formaldehit asetik asit (IFAA) ve %10'luk nötr tamponlu formalin (NBF) solüsyonlarında tespit edildi. Kesitler alsiyani mavisi-kritik elektrolit konsantrasyonu (AB-CEC) (pH 5.8, 0.3 M MgCl₂)/safranin O (SO) (pH 1.0) kombine metodu ve toluidin mavisi (TB) (pH 0.5) ile boyandı. Mast hücresi popülasyonu, TB ile boyanmış kesitlerde Carnoy solüsyonu ile tespit edilen sekum bölümlerinin bütün katmanlarında en yüksek oranlarda gösterildi. Metakromatik mast hücresi yoğunluğu en fazla orta ve distal sekumun tunika mukoza katmanlarında belirlendi. AB-CEC (+)/SO (-) mast hücresi yoğunluğunun ise, Carnoy tespitine göre IFAA ve BLA ile tespit edilen dokularda, proksimal ve orta sekumun tunika mukoza katmanlarında daha fazla olduğu görüldü. Araştırma sonunda; bıldırcın sekumunda Carnoy tespit solüsyonunun bağ doku mast hücrelerini, IFAA ve BLA tespit solüsyonlarının ise mukozal mast hücrelerini daha iyi tespit ettiği söylenebilir.

Anahtar sözcükler: Sekum, Tespit solüsyonu, Mast hücresi, Bıldırcın

INTRODUCTION

The avian cecum consists of two blind-ended sacs located on the right and left of the juncture between the small and large intestines^[1,2]. It comprises three parts, the proximal, middle and distal^[3], and plays a role in various functions, including the digestion and fermentation of cellulose and the absorption of water, sodium, carbon

dioxide^[4] and nutrients^[2]. Additionally, it contains diffuse and nodular lymphatic tissues in its submucosa and lamina propria. It therefore acts as a defensive organ^[5]. These lymphatic tissues are called as cecal tonsils, and they are located in the proximal region of the cecum^[6].

In particular, mast cells are frequently found around blood vessels and nerves^[7]. They contain basophilic, cyto-



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plasmic granules, which have histamine, heparin and eosinophil chemotactic factors [8], and they can also secrete numerous cytokines that induce lymphocyte functions [9]. Moreover, mast cells play an important role in regulating the sensitivity and permeability of the gastrointestinal system [10]. Consequently, the number of mast cells increases with the presence of some diseases, such as irritable bowel syndrome [11,12].

Mast cells are classified as either mucosal mast cells (MMC) or connective-tissue mast cells (CTMCs), depending on their locations, responses to applied fixative solutions and mediator compositions in rats [13,14]. Whereas MMCs are most commonly found in the lamina propria and epithelial layers of mucosal surfaces [15,16], CTMCs are located in the skin, tongue, intestinal serosa and lung parenchyma [17]. Mucosal mast-cell granules contain chondroitin sulphate [18] and rat mast-cell protease-II (RMCP-II) [17]. They are sensitive to formalin [13] and are stained with AB (+) [14]. CTMC granules, in contrast, are rich in heparin [18], contain rat mast-cell protease-I (RMCP-I) [17], are resistant to formalin [13] and are stained with SO (+) [14].

The present study sought to determine the distribution of mast cells and subtype cells using several fixative solutions and staining methods to better understand their functions in the cecum of quails.

MATERIAL and METHODS

In the present study, a sample of 21 six- to nine-week-old adult quails (*Coturnix coturnix japonica*) was used. The quails were obtained from Adnan Menderes University's Department of Laboratory Animals at Aydin, Turkey. They were kept in a storeyed cage system and fed and watered *ad libitum* under conventional conditions. All procedures were approved by the Adnan Menderes University Ethics Committee (Decision No: B.30.2.A DÜ.0.00.00.00/050.04/2010/40).

Examination with Light Microscopy

The quails were euthanized by decapitation, and samples of their cecum were removed. The right and left portions of cecum were separated. Then, right portions of first and second seven animals' cecum were divided into three parts, the proximal, middle and distal cecum. Next, they were fixed in basic lead acetate (BLA) (n=7) and 10% neutral buffered formalin (NBF) (n=7). Additionally, both right and left portions of third seven animals' cecum were divided into three parts, the proximal, middle and distal cecum. Then, the parts of the right portion in Carnoy's and the parts of left portion in isotonic formaldehyde acetic acid (IFAA) solutions were fixed (n=7). After routine histologic processing, the tissues were embedded in paraffin, and six serial sections, each 5 µm thick, were taken at 70 µm intervals. The histologic sections were then

stained with toluidine blue (TB, pH 0.5) [19] or with alcian blue-critical electrolyte concentration (AB-CEC) (pH 5.8, 0.3 M MgCl₂) and safranin O (SO, pH 1.0) in a combined method [20,21]. Finally, the sections were examined under a light microscope (Leica DMLB) equipped with an image-analysis system (Leica Q Win Standard), and appropriate locations were photographed.

Cell Count and Statistical Analyses

A subjective scoring system was used to determine mast-cell distribution in the TB- and AB-CEC/SO-stained tissues. Six sections of each animal's cecum were scored with a value from zero to four [22,23]. Mast-cell populations in the cecum's tunica mucosa (lamina propria+submucosa), tunica muscularis and tunica serosa were determined. Scores indicating the cecum's mast-cell densities [23] were assigned as follows: 0 = absent (-); 1 = weak (+); 2 = moderate (++); 3 = strong (+++); 4 = very strong (++++). Differences in mast cell-density values based on the various fixative solutions used were calculated using the SPSS 17.0 program package. Data were analysed using one-way analyses of variance (ANOVAs), and the source of the group's differences was determined *post hoc* with a Duncan's test [24]. Furthermore, mast-cell densities between the tunica mucosa folds of cecum parts were compared using paired t-tests [25]. Values are presented herein as mean ± standard error. Values for which P<0.05 (*), P<0.01 (**), and P<0.001 (***) were considered statistically significant.

RESULTS

Mast-cell distributions and densities for the proximal, middle and distal parts of the studied cecum were stained with TB or AB-CEC/SO and examined using various fixative solutions. Mast cells were observed in the cecum's lamina propria, submucosa, tunica muscularis and serosa (Fig. 1). They were especially noticeable in the connective tissue of the villi surrounding the blood vessels and between the glands in the tunica mucosa, as well as in the peripheral nerves; they were observed less frequently overall in the cecal tonsils (Fig. 2), and notably, they were most evident when BLA, Carnoy's and IFAA fixative solutions were used. But they were very few when the NBF solution was used. Therefore, the data could not be obtained from NBF fixed sections and they could not be shown in tables.

Mast cells were stained metachromatically with TB, and orthochromatically stained TB (+) cells were also detected (Fig. 1). Metachromatic mast-cell densities were greater in the tunica mucosa than in the cecum's other tissue folds, regardless of the fixative solution used. Otherwise, they were most evident in the tunica muscularis and serosa when Carnoy's fluid was used in conjunction with TB stain. Of course, it was evident that metachromatic mast cell density was determined the most in tunica mucosa layers of the middle and distal cecum. Mast cell population

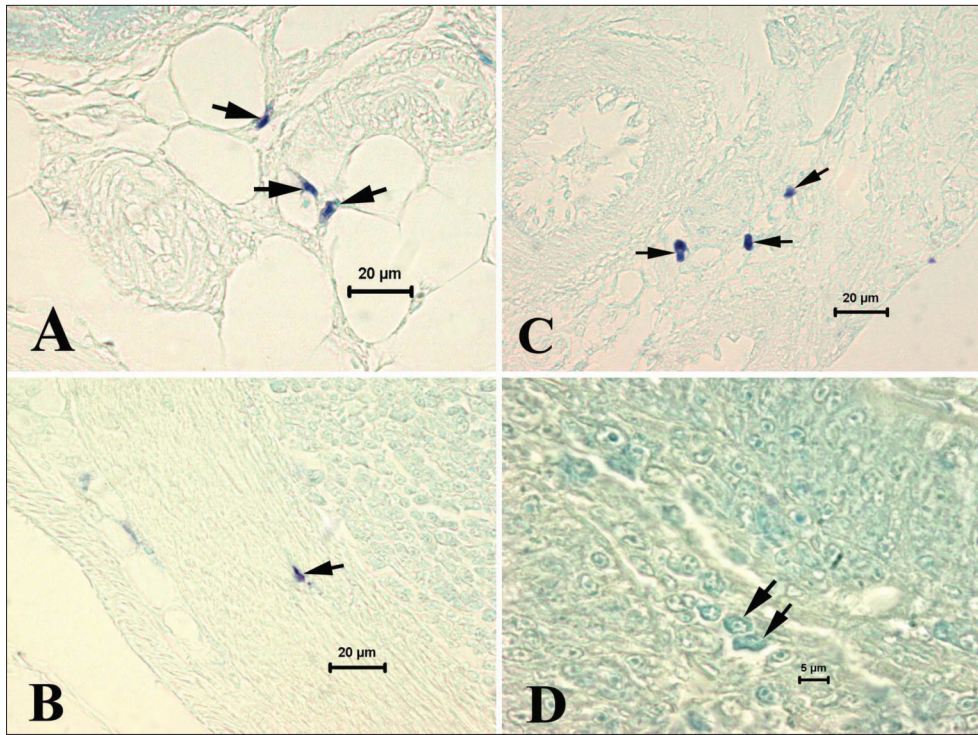


Fig 1. The metachromatic mast cells are seen in submucosa with IFAA (A), in tunica muscularis with BLA (B), in tunica serosa with Carnoy (C) and the orthochromatic TB (+) cells in villus with Carnoy fixed cecum (D). TB staining method. Bar: 20 µm (A,B,C), 5 µm (D)

Şekil 1. Submukozada IFAA (A), tunika muskulariste BLA (B), tunika serozada Carnoy (C) ile tespit edilmiş sekumda metakromatik ve villusta Carnoy (D) ile tespit edilmiş sekumda ortokromatik TB (+) mast hücreleri görülmektedir. TB boyama metodu. Bar: 20 µm (A,B,C), 5 µm (D)

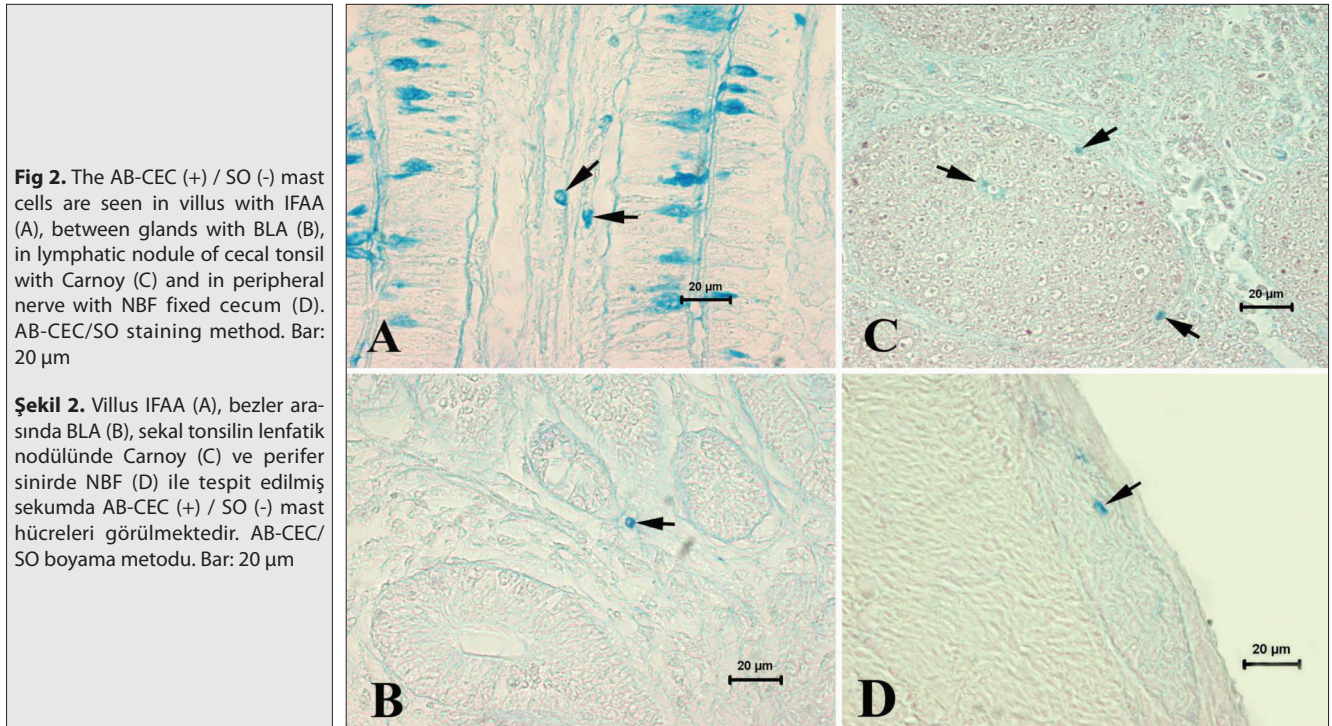


Fig 2. The AB-CEC (+) / SO (-) mast cells are seen in villus with IFAA (A), between glands with BLA (B), in lymphatic nodule of cecal tonsil with Carnoy (C) and in peripheral nerve with NBF fixed cecum (D). AB-CEC/SO staining method. Bar: 20 µm

Şekil 2. Villus IFAA (A), bezler arasında BLA (B), sekal tonsilin lenfatik nodülünde Carnoy (C) ve perifer sinirde NBF (D) ile tespit edilmiş sekumda AB-CEC (+) / SO (-) mast hücreleri görülmektedir. AB-CEC/SO boyama metodu. Bar: 20 µm

was shown in highest ratios in all layers of Carnoy fixed cecum parts within TB-stained sections ($P < 0.05$, $P < 0.01$ and $P < 0.001$). Yet, while the mast-cell densities were significantly lower in the tunica mucosa of the proximal regions of cecum fixed with Carnoy's and IFAA fixatives than they were at the most rates in the middle and distal regions of TB-stained cecum sections. All mast cell-density values from the presently described experiment are presented in [Table 1](#).

The cecum's mast cells were AB-CEC (+) and SO (-) reactive when the AB-CEC/SO staining method was used ([Fig. 2](#)). That said, mast-cell densities were greater in the tunica mucosa than they were in other folds fixed with IFAA and BLA solutions. Additionally, when Carnoy's fluid was used AB-CEC (+)/SO (-) mast cells were more evident in the tunica muscularis and serosa of the tested cecum's proximal and distal sections; thus, it was concluded that mast-cell populations increased in the presence of IFAA

Table 1. The density values of metachromatic mast cells in proximal, middle and distal parts of cecum**Tablo 1.** Sekumun proksimal, orta ve distal bölümlerinde metakromatik mast hücrelerinin yoğunluk değerleri

TB	n	Proximal			Middle			Distal		
		TM	TMs	TS	TM	TMs	TS	TM	TMs	TS
IFAA	7	0.45±0.07 ^{Bb}	0.23±0.05 ^c	0.29±0.05 ^b	1.52±0.10 ^{Aa}	0.54±0.08 ^b	0.09±0.04 ^b	1.52±0.14 ^{Aa}	0.45±0.07 ^b	0.14±0.05 ^b
BLA	7	1.02±0.15 ^a	0.54±0.09 ^b	0.26±0.06 ^b	1.11±0.09 ^b	0.50±0.08 ^b	0.16±0.05 ^b	1.16±0.07 ^b	0.57±0.09 ^b	0.21±0.06 ^b
CARNOY	7	1.12±0.12 ^{Ba}	0.87±0.11 ^a	0.91±0.10 ^a	1.70±0.10 ^{Aa}	0.81±0.07 ^a	0.65±0.09 ^a	1.76±0.12 ^{Aa}	1.00±0.09 ^a	1.07±0.09 ^a
P		***	***	***	***	*	***	**	***	***

^{a,b,c} Different superscripts in the same column indicate the significant difference. ^{A,B} Different superscripts in the same row indicate the significant difference. NS: Non-significant, * P<0,05, ** P<0,01, *** P<0,001, TB: Toluidine blue, IFAA: Isotonic formaldehyde acetic acid, BLA: Basic lead acetate, TM: Tunica mucosa, TMs: Tunica muscularis, TS: Tunica serosa

Table 2. The density values of AB-CEC (+) / SO (-) mast cells in proximal, middle and distal parts of cecum**Tablo 2.** Sekumun proksimal, orta ve distal bölümlerinde AB-CEC (+) / SO (-) mast hücrelerinin yoğunluk değerleri

AB-CEC/SO	n	Proximal			Middle			Distal		
		TM	TMs	TS	TM	TMs	TS	TM	TMs	TS
IFAA	7	2.01±0.07 ^{A,C,a}	0.44±0.06 ^b	0.51±0.06 ^b	2.16±0.10 ^{Aa}	0.83±0.10	0.40±0.09 ^b	1.85±0.11 ^C	0.88±0.08 ^{a,b}	0.45±0.08 ^b
BLA	7	2.09±0.11 ^a	0.97±0.09 ^a	0.57±0.08 ^b	1.95±0.09 ^{a,b}	0.79±0.08	0.62±0.08 ^{a,b}	1.78±0.08	0.71±0.07 ^b	0.54±0.08 ^b
CARNOY	7	0.85±0.11 ^{Bb}	1.08±0.09 ^a	1.10±0.10 ^a	1.77±0.12 ^{A,b}	0.93±0.06	0.77±0.11 ^a	1.68±0.12 ^A	1.08±0.10 ^a	1.10±0.08 ^a
P		***	***	***	*	NS	*	NS	*	***

^{a,b} Different superscripts in the same column indicate the significant difference. ^{A,B,C} Different superscripts in the same row indicate the significant difference, NS: Non-significant, * P<0,05, *** P<0,001, AB-CEC/SO: Alcian blue-critical electrolyte concentration/safranin O, IFAA: Isotonic formaldehyde acetic acid, BLA: Basic lead acetate, TM: Tunica mucosa, TMs: Tunica muscularis, TS: Tunica serosa

and BLA solutions to the tunica mucosas of a cecum's proximal and middle portions when compared with the introduction of Carnoy's solution to the same (P<0.05, P<0.001). It was also concluded that mast-cell density is significantly lower for the tunica mucosa of a cecum proximal region to which Carnoy's fixative has been introduced than it is for middle and distal cecum regions stained with AB-CEC/SO. All values associate with mast-cell population are presented in [Table 2](#).

DISCUSSION

Rodent mast cells are classified into two types, MMCs and CTMCs [13,14]. MMCs are stained with AB (+), and CTMCs are stained with SO (+) [14]. Uslu and Yoruk [19] state that MMCs stain blue and CTMCs stain red with a combined AB/SO staining method. And yet, the aforementioned researchers also report that three kinds of mast cells were found to be AB (+), SO (+) and AB/SO (+) when AB/SO staining was introduced to some digestive-tract organs in chickens (*gallus gallus domestica*) [23], quails [26] and rats [27]. Moreover, Harem and Liman [21] have demonstrated that rat and quail mast cells have varied glycosaminoglycan types with regards to both combined AB-CEC (pH 5.8, 0.3 M MgCl₂) and aldehyde fuchsin (AF) tissue-staining techniques. In the present study, the tested cecum mast cells were AB-CEC (+) and SO (-) reactive when the AB-CEC/SO staining method was used. For this reason, it was concluded that quail-cecum mast cells were MMCs. Furthermore, in the

present study, orthochromatic TB (+) cells were observed in the TB-stained sections. Mendonca et al. [28] report that immature mast cells contain orthochromatic granules. They also state that the granules become metachromatic when fully developed. Based on this information, it was concluded that the orthochromatically stained TB (+) cells observed in the present study may be immature quail-cecum mast cells.

The effects of fixative solutions on various species' mast-cell counts and distributions have been previously reported. For example, it was found that BLA solution was better than Carnoy's and IFAA solutions in fixing the mast cells in turkey digestive systems [19]. Karaca and Yoruk [26] have reported as well that the mast-cell distributions in chicken and quail digestive tracts are best determined with BLA and Carnoy's solutions. Similarly, Carnoy's fixative solution is noted as the most effective fixative for identifying mast cells in chicken intestinal mucosa [29], and IFAA solution is seen as the most suitable fixative for determining mast-cell density in the same. BLA solution, meanwhile, has been shown comparatively superior to other fixatives in helping to identify the granule structures in the lower respiratory tracts and lungs of local ducks and geese [30], as for identifying human MMCs, Strobel et al. [31] recommend using either Carnoy's or BLA solution. Similarly, both Asti et al. [32] and Eren [33] have showed that more mast cells are identified in dog skin when the tissue is fixed with IFAA solution than when it is fixed with 10% formaldehyde solution; it has been stated as well that

a greater number of mast cells is found in Bouin-fixed esophagus^[34] and mast-cell density is greater in IFAA-fixed rat duodenum^[27] and cow uterus^[22]. On the other hand, while MMCs are commonly found in the lamina propria and epithelial layers of mucosal surfaces^[15,16], CTMCs are found in skin, muscle and peritoneal cavity^[35]. For its part, the present study showed that mast-cell population was in highest ratios in all layers of Carnoy fixed cecum parts within TB-stained sections. Furthermore, it showed that AB-CEC (+)/SO (-) mast-cell density was greater when IFAA and BLA solutions were introduced into the tunica mucosa of the tested proximal and middle cecum. In the study, it showed that metachromatic mast cells are more evident in the tunica muscularis and serosa folds of all parts of the cecum when Carnoy's fluid is used. Based on these results, the present study's authors have concluded that different fixative solutions and staining methods affect the distribution of mast cells in quail cecum. Also, it can be said that Carnoy solution fixed the CTMCs, as well as IFAA and BLA solutions fixed the MMCs better. However, the density values of mast cell were determined using a semi-quantitative scoring method in the study. For this reason, it is needed the studies that count of mast cells will be revealed with stereological methods in order to improve the reliability of the study data.

The cecum contains diffuse and nodular lymphatic tissues^[5], and these are located in the cecum's proximal region^[6]. Kiernan^[36] has stated that mast-cell granules containing heparin are stained with TB metachromatically and AB 8GX. The present study showed that mast-cell density was significantly lower in the tunica mucosa of the cecum's proximal region when Carnoy's and IFAA fixatives were used than it was in other cecum regions stained with TB, and these same circumstances were observed when Carnoy's solution was used to fix cecum sections stained with AB-CEC/SO. It is therefore apparent that fewer mast cells containing heparin are located in the tunica mucosa of the cecum's proximal region if Carnoy's and IFAA fixatives are applied to it. Also, lymphatic tissues that are called as cecal tonsils are located in the proximal part of the cecum. That's why, fewer mast cells may be found in proximal part of the cecum. Furthermore, in the present study mast cells were rarely observed with two-stain methods when NBF solution was used as a fixative. This is consistent with other studies showing that mast-cell counts are lower in NBF-fixed tissue than in tissues fixed with other solutions^[29,31,34]. As with previous studies, the present study shows that MMCs are sensitive to formalin. It may therefore be concluded that they are not sufficiently preserved in NBF solution.

Mast-cell densities vary in different poultry digestive-tract folds. Uslu and Yoruk^[19] report that mast-cell numbers are highest in the submucosa, and they were similar in lamina propria and tunica muscularis + tunica serosa of turkey digestive tract. Additionally, Karaca and Yoruk^[26]

state that lamina propria and submucosa have greater numbers of mast cells than do tunica muscularis and serosa in quail cecum. In the present study, metachromatic mast cell density was greater in the tunica mucosa than it was in other folds of the cecum. Based on these results, it can be asserted that mast cells are more often located in the mucosal folds of quail cecum than they are in their tunica muscularis and serosa.

In conclusion, the distribution of mast cells for quail cecum was determined in the present study by applying TB and AB-CEC/SO staining methods to all regions and folds of said cecum. Moreover, it was revealed that the type of fixative solution used when testing quail cecum tissue affects mast-cell distribution.

REFERENCES

- 1. McLelland J:** Anatomy of the avian cecum. *J Exp Zool Suppl*, 3, 2-9, 1989. DOI: 10.1002/jez.1402520503
- 2. Clench MH:** The avian cecum: Update and motility review. *J Exp Zool*, 283, 441-447, 1999. DOI: 10.1002/(SICI)1097-010X(19990301/01)283:4/5<441::AID-JEZ13>3.0.CO;2-8
- 3. Strong TR, Reimer PR, Braun EJ:** Avian cecal microanatomy: A morphometric comparison of two species. *J Exp Zool Suppl*, 3, 10-20, 1989. DOI: 10.1002/jez.1402520504
- 4. Goldstein DL:** Absorption by the cecum of wild birds: Is there interspecific variation? *J Exp Zool Suppl*, 3, 103-110, 1989. DOI: 10.1002/jez.1402520517
- 5. Rezaian M, Hamed S:** Histological study of the caecal tonsil in the cecum of 4-6 months of age white leghorn chicks. *Am J Anim Vet Sci*, 2, 50-54, 2007. DOI: 10.3844/ajavsp.2007.50.54
- 6. Akter SH, Khan MZI, Jahan MR, Karim MR, Islam MR:** Histomorphological study of the lymphoid tissues of broiler chickens. *Bangl J Vet Med*, 4, 87-92, 2006. DOI: 10.3329/bjvnm.v4i2.1289
- 7. Metcalfe DD, Baram D, Mekori YA:** Mast cells. *Physiol Rev*, 77, 1033-1079, 1997.
- 8. Parwaresch MR, Horny HP, Lennert K:** Tissue mast cells in health and disease. *Pathol Res Pract*, 179, 439-461, 1985. DOI: 10.1016/S0344-0338(85)80184-9
- 9. Henz BM, Maurer M, Lippert U, Worm M, Babina M:** Mast cells as initiators of immunity and host defense. *Exp Dermatol*, 10, 1-10, 2001. DOI: 10.1034/j.1600-0625.2001.100101.x
- 10. Ramsay DB, Stephen S, Borum M, Voltaggio L, Doman DB:** Mast cells in gastrointestinal disease. *Gastroenterol Hepatol*, 6, 772-777, 2010.
- 11. Guilarte M, Santos J, de Torres I, Alonso C, Vicario M, Ramos L, Martínez C, Casellas F, Saperas E, Malagelada JR:** Diarrhoea-predominant IBS patients show mast cell activation and hyperplasia in the jejunum. *Gut*, 56, 203-209, 2007. DOI: 10.1136/gut.2006.100594
- 12. Weston AP, Biddle WL, Bhatia PS, Miner PB Jr:** Terminal ileal mucosal mast cells in irritable bowel syndrome. *Dig Dis Sci*, 38, 1590-1595, 1993. DOI: 10.1007/BF01303164
- 13. Enerbäck L:** Mast cells in rat gastrointestinal mucosa. I. Effect of fixation. *Acta Pathol Microbiol Scand*, 66, 289-302, 1966.
- 14. Enerbäck L:** Mast cells in rat gastrointestinal mucosa. 2. Dye-binding and metachromatic properties. *Acta Pathol Microbiol Scand*, 66, 303-312, 1966.
- 15. Miller HR:** Mucosal mast cells and the allergic response against nematode parasites. *Vet Immunol Immunopathol*, 54, 331-336, 1996. DOI: 10.1016/S0165-2427(96)05696-6
- 16. Huntley JF, McGorum B, Newlands GF, Miller HR:** Granulated intraepithelial lymphocytes: their relationship to mucosal mast cells and globule leucocytes in the rat. *Immunology*, 53, 525-535, 1984.

- 17. Gibson S, Miller HR:** Mast cell subsets in the rat distinguished immunohistochemically by their content of serine proteinases. *Immunology*, 58, 101-104, 1986.
- 18. Pipkorn U, Karlsson G, Enerbäck L:** Phenotypic expression of proteoglycan in mast cells of the human nasal mucosa. *Histochem J*, 20, 519-525, 1988. DOI: 10.1007/BF01002650
- 19. Uslu S, Yoruk M:** Morphological and histometrical studies on distribution and heterogeneity of mast cells in turkey digestive system. *Van Vet J*, 19, 47-51, 2008.
- 20. Scott JE, Dorling J:** Differential staining of acid glycosaminoglycans (mucopolysaccharides) by Alcian blue in salt solutions. *Histochemie*, 5, 221-233, 1965.
- 21. Harem MK, Liman N:** Histochemical method for demonstrating quail mast cell types simultaneously. *Biotech Histochem*, 84, 275-282, 2009. DOI: 10.3109/10520290902991394
- 22. Eren U, Asti RN, Kurtdede N, Sandikci M, Sur E:** The histological and histochemical properties of the mast cells and the mast cell heterogeneity in the cow uterus. *Tr J Vet Anim Sci*, 23, 193-201, 1999.
- 23. Aksoy A, Cinar K:** The ontogeny, distribution and histochemistry of mast cells in the proventriculus of gallus gallus domestica in pre- and postnatal periods. *Van Vet J*, 19, 25-29, 2008.
- 24. Park JY, Hong SM, Klimstra DS, Goggins MG, Maitra A, Hruban RH:** Pdx1 expression in pancreatic precursor lesions and neoplasms. *Appl Immunohistochem Mol Morphol*, 19, 444-449, 2011. DOI: 10.1097/PAI.0b013e318206d958
- 25. Grzybowska KK, Letourneau R, Kempuraj D, Donelan J, Poplawski S, Boucher W, Athanassiou A, Theoharides TC:** IL-1 Induces vesicular secretion of IL-6 without degranulation from human mast cells. *J Immunol*, 171, 4830-4836, 2003. DOI: 10.4049/jimmunol.171.9.4830
- 26. Karaca T, Yoruk M:** A morphological and histometrical study on distribution and heterogeneity of mast cells of chicken's and quail's digestive tract. *Van Vet J*, 15, 115-121, 2004.
- 27. Demirbag E, Cinar K, Kutlar MH, Eroglu G, Sari SM:** Distribution and heterogeneity of mast cells in small intestine of rat (*Rattus rattus*). *SDU J Sci*, 7, 92-99, 2012.
- 28. Mendonca VO, Vugman I, Jamur MC:** Maturation of adult rat peritoneal and mesenteric mast cells. *Cell Tissue Res*, 243, 635-639, 1986. DOI: 10.1007/BF00218072
- 29. Fei ACY, Chi Lee YC:** The fixation effects of mast cells in chicken intestinal mucosa. *Bull Inst Zool Academia Sinica*, 22, 119-122, 1983.
- 30. Uslu S, Yoruk M:** Morfological and histometric studies on mast cell distribution and heterogeneity, present in the lower respiratory tract and in the lung of local duck (*Anas platyrhynchos*) and goose (*Anser anser*). *Kafkas Univ Vet Fak Derg*, 19, 475-482, 2013. DOI: 10.9775/kvfd.2012.8064
- 31. Strobel S, Miller HR, Ferguson A:** Human intestinal mucosal mast cells: evaluation of fixation and staining techniques. *J Clin Pathol*, 34, 851-858, 1981. DOI: 10.1136/jcp.34.8.851
- 32. Asti RN, Kurtdede A, Kurtdede N, Ergun E, Guzel M:** Mast cells in the dog skin: Distribution, density, heterogeneity and influence of fixation techniques. *Ankara Univ Vet Fak Derg*, 52, 7-12, 2005. DOI: 10.1501/Vetfak_0000000022
- 33. Eren U:** Mast cells in the skin of dog. *Ankara Univ Vet Fak Derg*, 47, 167-175, 2000. DOI: 10.1501/Vetfak_00000000500
- 34. Kelek S, Cimenoglu N, Cinar K:** The determination of mast cell density with different fixatives in the esophagus of the goose (*Anser anser*). *SDU Fen Bil Enst Derg*, 15, 102-104, 2011.
- 35. Enerback L:** The gut mucosal mast cell. *Monogr Allergy*, 17, 222-232, 1981.
- 36. Kiernan JA:** A comparative survey of the mast cells of the mammalian brain. *J Anat*, 121, 303-311, 1976.