A Morphological and Histometrical Study on Distribution and Heterogeneity of Mast Cells of Chicken's and Quail's Digestive Tract*

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SUMMARY

This study was carried out to determine distribution and heterogeneity of mast cells in the digestive tract of the both chicken and quail. Ten Leghorn chickens and ten Japan quails were use during the investigation. Having been sacrified by decapitation, the chickens and quails' tongue, crop, esophagus, proventriculus, gizzard, duodenum, jejunum, ileum, caecum and colon tissue samples were taken. Those samples were processed and blocked following the fixation with the BLA (basic lead acetate - Mota), Carnoy's and IFAA (isotonic formaldehyde acetic acid) solutions. The histologic sections were stained with toluidine blue and alcian blue – safranine in order to determine the mast cells distribution and heterogenity in the digestive tract. Both Mast cells distribution and heterogeneity were found to be different between the digestive tract organs. In both animal species, the mast cells were found to be at very high number in the tongue, crop, esophagus and proventriculus whereas they were very few in the gizzard.In addition, it was found that BLA and Carnoy's solutions were better relative to IFAA to determine the distribution of mast cells. Positive reactions were observed against alcian blue in the staining of all mucosal mast cells in both animal species with alcian blue - safranine mix. However, safranine was reactive only to esophagus, proventriculus, gizzard, colon, and tongue mast cells. In addition, alcian blue (+) granules beside the safranine (+) granules could be observed rarely, in those mentioned organs.

Key Words: *Quail, chicken, light microscope, mast cells, digestive tract*

Tavuk ve Bıldırcın Sindirim Kanalında, Mast Hücrelerinin Dağılım ve Heterojenitesi Üzerine Morfolojik ve Histometrik Bir Çalışma

ÖZET

Bu çalışma, tavuk ve bıldırcın sindirim kanalındaki mast hücrelerinin dağılım ve heterojenitesini belirlemek amacıyla yapıldı. Çalışmada, 10'ar adet Leghorn ırkı tavuk ve Japon ırkı bıldırcın kullanıldı. Tavuk ve bıldırcınlar dekapite edilerek dil, kursak, özofagus, ön mide, mide, duodenum, jejunum, ileum, sekum ve kolondan doku örnekleri alındı. Alınan örnekler BLA (basic lead asetate – Mota), Carnoy ve IFAA (izotonic formaldehyde asetic acid) tespit solüsyonlarında tespit edilerek takip ve blokajı yapıldı. Hazırlanan histolojik kesitlere mast hücrelerinin dağılım ve heterojenitesini belirlemek amacıyla toluidine blue ve alcian blue - safranin boyamaları uygulandı. Mast hücrelerinin dağılımı ve heterojenitesinin sindirim kanalı organlarında farklılıklar gösterdiği ve bu organlar içerisinde her iki hayvan türünde de dil, kursak, özofagus ve ön midenin sayısal olarak en fazla, müsküler midenin ise en az olan organ olduğu belirlendi. Kullanılan tespit sıvılarından BLA ve Carnoy'un IFAA'ya göre mast hücrelerinin metakromatik özelliklerinin belirlenmesi ve sayısal dağılımın ortaya konmasında daha iyi sonuç verdiği kanısına varıldı. Alcian blue-safranin boyamasında, her iki hayvan türünde de, mukozal mast hücreleri alcian blue (+) reaksiyon verirken, safranin ile özofagus, ön mide, mide, kolon ve dilde pozitif reaksiyonlar gözlendi. Ayrıca bu organlarda seyrek olarak hem safranin (+) hem de alcian blue (+) hücreler de saptandı.

Anahtar Sözcükler: *Bıldırcın, ışık mikroskobu, mast hücresi, sindirim kanalı, tavuk*

INTRODUCTION

Since the digestive tract is in direct relationship with outer environment, it encounters with many foreign material throughout the life. Many of those foreign materials have antigen properties and they are eliminated in tunica mucosa by special and non-special defense mechanisms. Mast cells together with humoral and cellular defense cells have important role within that system.

They are found as small groups within connective tissues especially adjacent to the blood vessels (7,16). Mast cells having different locations have different histochemical, cytochemical, ultra structural and functional properties (18). Light microscopical appearances of those cells may change with respect to their types and locations. They are generally observed in the form of oval (13, 16), thin, long or spindle shaped (7, 26). The oval ones are generally observed in loose tissues, whereas those in the form of spindle shaped are found in compact tissues where collagen fibers are intensive (26).

Mast cells can be divided into two groups on the basis of their origins, locations, responses to applied fixative solutions, types of gylcoaminoglycans they have, type of intragranuler serine proteinases (1), histochemical differences, functional criteria and morphological shapes. Those groups are Connective Tissue Mast Cell (CTMC) and Mucosal Mast Cell (MMC) (12). Nomenclature of Enerabck (8) in rat intestines is used commonly for description of heterogeneity between mast cells populations. Not only staining properties and type of fixations but also their locations and functional differences within various parts of the same organ have important role in heterogeneity of mast cells (10,14). Staining properties of mast cells change according to type of fixative solutions (3,6,9), pH value and concentration

* This study is the summary of a Ph.D thesis with same title

of dye and duration of staining are effective on staining properties (9).

By this study, it was aimed to make a morphological and histometrical research on distribution of mast cells in chicken and quail's digestive tract organs and in various parts of those organs on the basis of different fixation solutions and different staining methods. Findings obtained from tissue preparations of chicken for each digestive track organ were compared. The same procedure was applied to quail as well.

MATERIALS and METHODS

In the study, 10 adult white Leghorn chicken and 10 Japanese quails (Coturnix coturnix Japonica) obtained from commercial farms were used. After they were decapitated, their abdominal region was opened and their digestive tracts were examined. Tissue samples from tongue, crop, esophagus, proventriculus, gizzard, duodenum, jejunum, ileum, caecum and colon were taken in required size. Those samples were examined according to histological methods mentioned below:

For the Examination of Light Microscopy

Three different fixative solutions, IFAA (isotonic formaldehyde), Carnoy and BLA (basic lead acetate), were used in the present study. Some tissue samples were fixed in IFAA fixative solution (formaldehyde 1.5 ml, distilled water 98 ml, glacial acetic acid 0.5 ml, pH 2.9) for 24 hours (22), some of others were fixed in Carnoy solution (absolute ethanol 60 ml, chloroform 30 ml, glacial acetic acid 10 ml) for 12 hours. Fixed samples were kept in alcohol solution of 70^0 for 12 hours. The remaining tissue samples were fixed in BLA (Mota) (basic lead acetate 1g, ethanol 50 ml, distilled water 50 ml, glacial acetic acid 0.5 ml) for 24 hours. Then, samples fixed in three different solutions were blocked in paraplast by means of routine histological techniques (8). For mast cells count and idendification; serial cross sections taken from prepared blocks and having thickness of 6 µm were stained with 0.5 % toluidine blue solution, which was prepared in Mac Ilvaine's citric acid disodiumphosphate buffer (pH 0.5), for 5-8 minutes (9). Then, they were stained in 0,2 M acetate buffer Alcian blue 8GX- Safranin O combined dyes (pH 1.42) (2, 24). Lastly, preparations were examined under light microscope (Nikon AFX-DX Optiphot-2, Japan) and appropriate locations were photographed.

Cell count and statistical analyses

Hundred-square ocular micrometer (eyepiece graticule) was used for cell count to determine mast cell distribution in the preparations stained with toluidine blue. Mast cells within ocular micrometer were counted in high power fields (x400) (17). The cells were counted within 18 areas selected from lamina propria, submucosa and tunica muscularis + tunica serosa, randomly. After serial cross sections were counted, mean of those figures was calculated. Thus, average mast cell number within the area covered by 100 square ocular micrometer was determined. The area of 100 square ocular micrometer was calculated by means of micrometrical lam by 40 objective enlargement. Then the mast cell density in each site was found and recorded as mast cell numbers/mm^2 (17). Variance analyses of mast cell numbers for both types were conducted by using SAS v.12.0 package programs. Inter-groups and intra-group differences were determined by Duncan test (21).

RESULTS

Mast cells in cross sections of 6 µm thickness taken from tissue samples belonging to various regions and fixed with three different fixation solutions were distinguished easily by metachromatic staining method. It was determined for both animals that, mast cells were dense especially near blood vessels in lamina propria (Figure 1), submukosa and tunica serosa. They were located around glands and both inside and around nerve fibers in lamina propria and submucosa. They were also observed around blood vessels between muscle groups in tunica muscularis.

Mast cell in sections stained with toluidine blue had various size and appearance. They were flat, oval or in the form of spindle shaped (Figure 2). Cytoplasms of mast cells taken from almost all organs and stained with metachromatic dye were homogenous. Hence, their granular structure was hardly observed by light microscope. Heterochromatic appearance in nucleus of those cells was obvious (Figure 2). It was found that mast cells were intensive near blood vessels and between glands in microscopic papilla and were intensive in compact tissues under upper surface epithelium of tongue. Mast cells in esophagus were observed around gland esophagus in a close relationship with nerve fibers and generally in the position of subepithelial. Mast cells found between muscle groups in tunica muscularis layers of digestive tract organs were generally in spindle shaped. Large cells showing good metachromasia were observed around blood vessels in tunica seroza layer of the tract wall and its subserosa (Figure 2).

In case of toluidine blue staining, mast cells were determined at most in proventriculus of chicken and in crop and tongue of quail. On the other, in both animals hand muscular stomach was found to be the organ where the least mast cells were determined (Figure 3). It was observed that mast cells of intestine were denser in villus, between crypts, around blood vessels and near nerve fibers relative to other regions of that organ. It was found for both species that while MOTA (BLA) fixation was suitable in the determination of granular structure, Carnoy's solution was better in metachromatic staining.

Throughout the digestive tract organs, examined with Alcian-blue safranine consecutive staining, mucosal mast cells (MMC) gave positive reaction with alcian blue. On the other hand connective tissue's mast cells (CTMC) of tongue, esophagus, proventriculus, and stomach in both species and those of colon only in chicken gave positive

reaction with safranine (Figure 4, 5). Mast cells giving positive reaction to safranine were mainly observed in submucosa, tunica, muscularis and tunica serosa whereas they were observed in lamina propria seldomly. In addition, few mast cells with mixed granules were determined in organs giving positive reaction to safranin, which was especially in proventriculus (Figure 4, 5).

No cell was observed in quail stomach lamina propria and tunica muscularis + tunica serosa fixed by IFAA solution. But the greatest number of mast cell was found in lamina propria of chicken proventriculus fixed by BLA. (282 cell/mm^2).

Numerical distributions and statistical analysis of mast cells in the chicken and quail's digestive tract organs with respect to different fixative solutions, organs and organ layers were given in the tables 1,2,3.

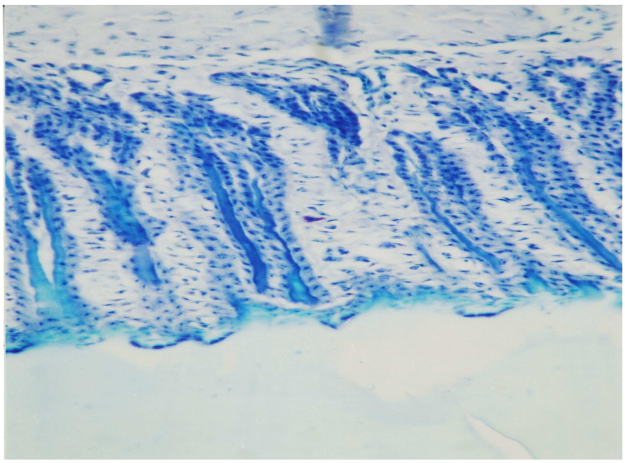

Figure 3. Mast cells observed seldomly in the lamina propria of quail's stomach, BLA, Toluidine blue, x360.

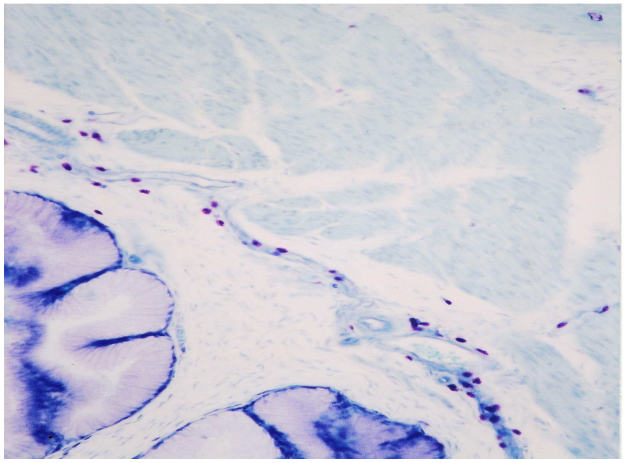

Figure 1. Mast cells located throughout the blood vessels in the lamina propria of chicken esophagus, Carnoy, Toluidine blue, x180.

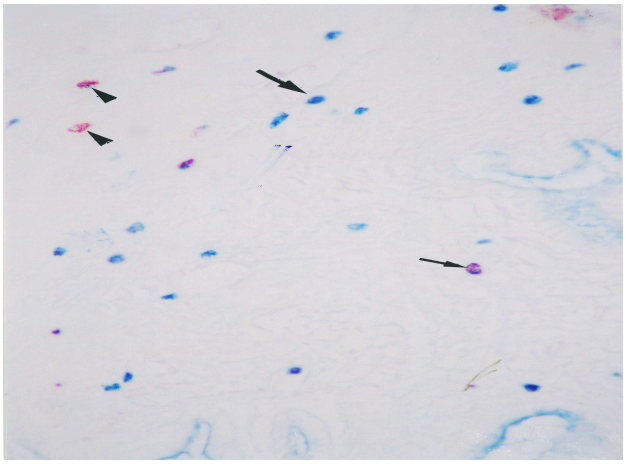

Figure 4. Mast cells including Alcian blue (+) (large arrow), Safranin O (+) (arrowheads) and mixed granule (small arrow) in the lamina propria of chicken proventiculus, Alcian blue/Safranin O, x360

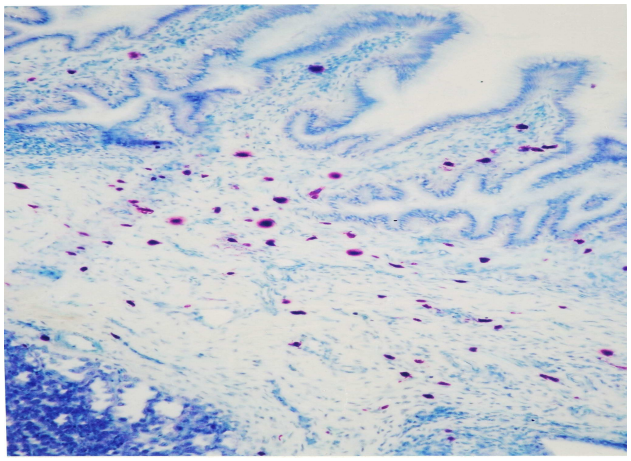

Figure 2. Mast cells observed in the lamina propria and under the foveola epithelium of chicken proventiculus, BLA, Toluidine blue, x180.

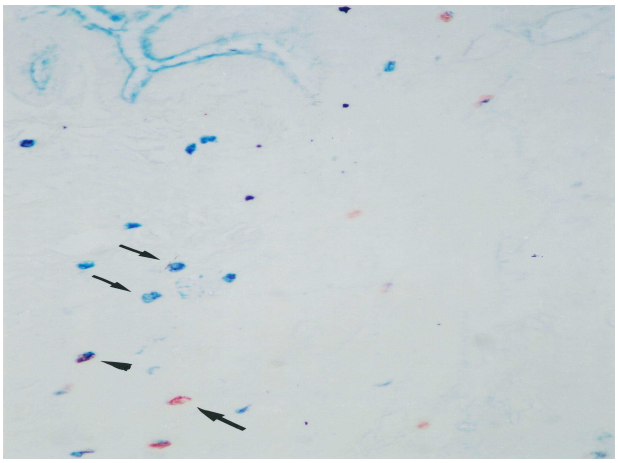

Figure 5. Mast cells including Alcian blue (+) (large arrow), Safranin O (+) (arrowhead) and mixed granule (small arrows) in the lamina propria of quail's proventiculus, Alcian blue/safranin O, x360

Table 1. Different fixations applied to chicken and quail digestive tract lamina propriya and distribution of mast cells in tissues (x ± Sx) (n=10)

Fixative Sol	BLA (Mota)		CARNOY		IFAA	
	Chicken	Quail	Chicken	Quail	Chicken	Quail
Crop	151±17.1^{3,A}	86±11.6^{y,2,C}	146±17.1^{2,A}	88±11.6^{y,2,C}	133±174.1^{3,A}	118±11.6^{x,2,B}
Esophagus	149±14.7^{a,3,A}	91±22.7^{x,2,C}	98±14.7^{b,3,C}	86±22.7^{y,2,C,D}	138±14.7^{a,3,A}	118±22.7^{x,2,B}
Proventriculus	282±14.3^{a,1,A}	111±5.9^{x,1,D}	205±14.3^{b,1,B}	124±5.9^{x,1,D}	176±14.3^{c,2,C}	76±5.9^{y,3,E}
Stomach	31±6.1^{6,A}	18±2.2^{x,5,B}	42±6.1^{4,A}	20±2.2^{x,6,B}	36±6.1^{7,A}	0±2.2 ^{y,5,C}
Duodenum	138±21.2^{b,3,4B}	107±4.2^{x,1,C}	189±21.2^{a,1,A}	100±4.2^{x,2,C}	151±21.2^{b,3,4,B}	71±4.2^{y,3,D}
Jejunum	160±8.7^{a,3,A}	84±5.3^{x,2,B}	44±8.7^{b,4,D}	68±5.3^{y,3,C}	50±8.7$^{b,6,7.,C,D}$	54±5.3$^{z.4,C,D}$
Ileum	84±11.7^{c,5,C}	47±4.1^{y,4,D}	140±11.7^{a,2,A}	55±4.1^{y,3,4,D}	100±11.7^{b,6,B}	142±4.1^{x,1,A}
Cecum	36±5.0^{b,6,C}	110±6.5^{x,1,A}	54±5.0^{a,4,B}	91±6.5^{x,2,A}	34±5.0^{b,5,C}	44±6.5^{y,4,C}
Colon	97±10.3^{a,5,A}	51±4.2^{x,4,B}	23±10.3^{b,4,5,C}	44±4.2^{x,5,B}	108±10.3^{a,5,A}	56±4.2^{x,4,B}
Tongue*	187±16.6^{b,2,B}	97±9.9^{y,2,3,D}	188±16.6^{b,1,B}	88±9.9^{y,2,D}	215±16.6^{a,1,A}	130±9.9^{x,2,C}

a, b, c: There are statistical differences between different letters used in nomenclature of the same kind taking place on the same line (P<0,001).

x, y, z: There are statistical differences between different letters used in nomenclature of the same kind taking place on the same line (P<0,001).

1,2,3,4,5,6,7: There are statistical differences between different figures used in nomenclature of the same organs taking place on the same column (P<0,05).

A,B,C,D: There are statistical differences between different letters of the same kind taking place on the same line (P<0.05).

* This location on the tongue means lamina propriya on the upper tongue surface under lamina epitalyalis.

Table 2. Different determinations applied to chicken and quail digestive tract submucosa and distribution of mast cells in tissues (x ± S $\bar{x}$) (n=10)

Fixative sol.	BLA (Mota)		CARNOY		IFAA	
	Chicken	Quail	Chicken	Quail	Chicken	Quail
Crop	208±17,1^{a,1,A}	80±11.6^{y,1,C}	198±17.1^{a,1,A}	150±11.6^{x,1,B}	158±17.1^{b,2,B}	58±11.6^{z,2,D}
Esophagus	88±14.7^{b,5,C}	79±22.7^{y,1,C}	101±14.7^{b,3,B,C}	124±22.7^{x,2,A}	138±14.7^{a,2,3,A}	95±22.7^{z,1,B}
Proventriculus$^{\&}$	173±14.3^{a,2,A}	76±5.9^{1,C}	166±14.3^{a,2,A}	65±5.9^{3,C}	116±14.3^{b,3,B}	70±5.9^{2,C}
Stomach	58±6.1^{a,4,A}	29±2.2^{y,4,C}	58±6.1^{a,6,A}	41±2.2^{x,4,B}	42±6.1^{b,5,B}	41±2.2^{x,3,B}
Duodenum	147±21.2^{2,3,B}	64±4.2^{x,2,C}	153±21.2^{2,A,B}	73±4.2^{x,3,C}	161±21.2^{2,A}	51±4.2^{y,3,D}
Jejunum	53±8.7^{b,4,B}	70±5.3^{x,1,2,A}	67±8.7^{a,6,A}	46±5.3^{y,4,B}	41±8.7^{c,5,B}	32±5.4^{z,4,C}
Ileum	54±11.7^{b,4,B}	36±4.1^{y,3,C}	80±11.7^{a,4,5,A}	40±4.1^{y,4,C}	74±11.7^{a,4,A}	56±4.1^{x,2,B}
Cecum	58±5.0^{b,4,B}	59±6.5^{y,2,B}	74±5.0^{a,5,A}	71±6.5^{x,3,A}	34±5.0^{c,5,C}	46±6.5^{z,3,B,C}
Colon	37±10.3^{b,4,B}	38±4.2^{3,B}	40±10.3^{b,6,7,B}	38±4.2^{4,B}	69±10.3^{a,4,A}	40±4.2^{3,B}
Tongue*	164±16.6^{2,A}	79±9.9^{y,1,b,C}	177±16.6^{1,A}	74±9.9^{y,3,B}	180±16.6^{1,2,A}	90±9.9^{x,1,B}

a, b, c: There are statistical differences between different letters used in nomenclature of the same kind taking place on the same line (P<0,001).

x, y, z: There are statistical differences between different letters used in nomenclature of the same kind taking place on the same line (P<0,001).

1,2,3,4,5,6,7: There are statistical differences between different figures used in nomenclature of the same organs taking place on the same column (P<0,05).

A,B,C,D: There are statistical differences between different letters of the same kind taking place on the same line(P<0.05). &:This location in proventriculus means the location region between proventriculus glands.

*This location on the tongue means the location between muscles and glands

Table 3. Distribution of mast cells in tissues which are applied different determinations in chicken and quail digestive track tunica muscularis and tunica seroz. ($x \pm S_{\bar{x}}$) (n=10)

Fixative sol.	BLA (Mota)		CARNOY		IFAA	
	Chicken	Quail	Chicken	Quail	Chicken	Quail
Crop	$90\pm17.1^{a,1,B}$	$60\pm11.6^{y,1,C}$	$61\pm17.1^{b,2,C}$	$126\pm11.6^{x,1,A}$	$55\pm17.1^{b,2,C}$	$37\pm11.6^{z,3,D}$
Esophagus	$87\pm14.7^{a,1,A}$	$57\pm22.7^{1,C}$	$75\pm14.7^{b,1,B}$	$50\pm22.7^{2,C}$	$67\pm14.7^{b,2,B}$	$45\pm22.7^{2,C}$
Proventriculus	$31\pm14.3^{c,3,C}$	$39\pm5.9^{y,2,C}$	$75\pm14.3^{a,b,1,A}$	$31\pm5.9^{y,3,C}$	$86\pm14.3^{a,1,A}$	$54\pm5.9^{x,2,4,B}$
Stomach	$52\pm6.1^{a,2,A}$	$16\pm2.2^{x,3,C}$	$37\pm6.1^{b,4,B}$	$16\pm2.2^{x,4,C}$	$35\pm6.1^{b,3,B}$	$0\pm2.2^{y,7,D}$
Duodenum	$39\pm21.2^{b,3,B}$	$24\pm4.2^{3,C}$	$49\pm21.2^{a,3,A}$	$23\pm4.2^{4,C}$	$34\pm21.2^{b,3,B,C}$	$32\pm4.2^{3,5,C}$
Jejunum	$32\pm8.7^{a,3,A}$	$26\pm5.3^{3,A}$	$31\pm8.7^{a,4,A}$	$23\pm5.3^{4,A,B}$	$22\pm8.7^{b,3,4,B}$	$19\pm5.3^{6,B,C}$
Ileum	$29\pm11.7^{3,A}$	$23\pm4.1^{3,A}$	$32\pm11.7^{4,A}$	$28\pm4.1^{3,4,A}$	$29\pm11.7^{4,A}$	$28\pm4.1^{5,A}$
Cecum	$27\pm5.0^{3,A}$	$25\pm6.5^{3,A}$	$33\pm5.0^{4,A}$	$26\pm6.5^{4,A}$	$31\pm5.0^{3,4,A}$	$22\pm6.5^{6,A}$
Colon	$30\pm10.3^{b,3,B}$	$26\pm4.2^{3,B}$	$22\pm10.3^{c,4,5,C}$	$25\pm4.2^{4,B,C}$	$40\pm10.3^{a,3,C}$	$22\pm4.2^{6,C}$
Tongue*	$90\pm16.6^{a,1,A}$	$65\pm9.9^{x,1,B,C}$	$71\pm16.6^{b,1,B}$	$41\pm9.9^{y,2,D}$	$95\pm16.6^{a,1,A}$	$75\pm9.9^{x,1,B}$

a, b, c: There are statistical differences between different letters used in nomenclature of the same kind taking place on the same line ($P<0,001$).

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A,B,C,D: There are statistical differences between different letters of the same kind taking place on the same line ($P<0.05$).

*This location on the tongue means lamina propriya on the upper tongue surface under lamina epitalyalis.

DISCUSSION

In this research; distribution, location and staining properties of mast cells within the digestive tract organs and between organ layers of chicken and quail were determined by using different fixative solutions (Carnoy, Mota (BLA) and IFAA). Following the evaluation about morphological and statistical findings, comparisons were made with respect to organs, organ layers and species. In literature search no study on distribution and location of mast cells within digestive system in quail was met but very few were found for chicken. Therefore, our findings were compared with the data belongimg other species in literature.

Toluidine blue was used to determine mast cells in preparations taken from tongue, crop, esophagus, proventriculus, gizzard, duodenum, jejunum, ileum, caecum and colon tissues belonging to digestive track of chicken and quail (9). The most useful determinant property for those cells at the level of light microscope was metachromatic staining (6, 19).

When each organ was examined for both animals it was determined that mast cells are extensive in lamina propria and they are denser in areas close to lamina epithelialis. It has been also observed that mast cell density was extremely low in tunica muscularis and tunica serosa layers in both species. However, they were available mainly around blood vessels in all organs and layers. Locations of them were close to glands in esophagus and tongue. They were also found around and inside of nerve fibers in almost all organs studied. For the mentioned animals, the greatest numbers of mast cells were found in tongue, crop and proventriculus. Results for chicken were parallel with Wang's study (24). Namely, Wang obtained the highest number of mast cell in proventriculus and lowest one in intestines.

According to a study conducted on ducks; most mast cells had found in proventriculus and located closely to blood vessels and to neck of glands in lamina propriaa under epithelial layers. The same study had also showed that mast cells in intestines were located in villuse depths and around the intestinal crypts (23). Our results about proventriculus but not for esophagus comply with results of studies conducted in ducks.

Kurtdede and Yörük (15) have observed that mast cells are found in the forms of ovoid, round and flatted. In our research we have seen that shapes of mast cells under the light microscope are very variable and they can be different with respect to their location. While mast cells locating in lamina propria were oval cells, those closed to lamina epithelialis were elongated and spindle shaped. On the other hand mast cells locating between muscle fibers within tunica muscularis were oval and elongated.

According to Wang's study (24), Carnoy's solution was the optimum fixative fluid for the chicken mast cells. That fluid was found to be the most suitable also for ducks by another study (23). In our study BLA, Carnoy and IFAA solutions were used, and it was decided that the best fixative solutions for mast cells in digestive tract of chicken and quail are BLA and Carnoy's

solutions. Mast cells were stained with TB metachromatically well in both fixative solutions and it was concluded that BLA is the most suitable fixative solution for determination of mast cells granules in digestive system of chicken and quail under light microscope.

Wang (24) has declared that AB-S combined stain is effected by fixation type. In that study it has been determined that some mast cells have only blue granules, some have only red granules and rest have both red and blue granules. In the study where we have fixed tissue samples by BLA and IFAA, mast cells had only blue, only red or both colored granules in tongue, crop, esophagus, proventriculus, gizzard, duodenum, jejunum, ileum, cecum and colon of both chicken and quails.This result is parallel with Wang's findings.

In a study conducted on rats (20), it has been determined that there are extensive typical mast cells (CTMC) in tongue, esophagus, and in the stomach's part having cutaneous mucosa, while atypical mast cells (MMC) were more extensive in glandular stomach, intestines and in cecum. In our study we have determined that atypical mast cells (MMC) were extensive in glandular stomach and less typical mast cells were found in large intestine. On the other hand, it wasn't found any atypical mast cell (CTMC) in small intestines stained by safranine.

Wang (24) has examined numerical distribution of mast cells throughout the chicken digestive track and he has evaluated it qualitatively. According to him mast cells are more extensive in proventriculus, at a medium density in tongue and esophagus, lesser in small intestines and the least in cloacae and cloacae-rectum area. In our study, mast cells were extensive in proventriculus of chicken and in crops of quails, although they might vary with fixation.

In his study about chicken mast cells' distribution and their ultrastructural properties, Wight (25) has evaluated those cells in all body areas and has determined that they are located most extensively in oviduct infundibulum, ovarian, mouth ceiling of digestive tract and in peripheral nerves. Mast cells mainly locate in lamina propria of infundibulum and digestive tract. It has been reported that mast cells are located in submucosa, sometimes between crypts and at the deep parts of villus. It has been reported by the same study that, they are also present in smaller numbers among the circular and longitudinal muscles in association with blood vessels and nerves and in the tunica adventitia. In a study conducted by Gibson et al. (11) on rat's digestive system, the greatest number of mast cell has been determined in intestinal mucosa, among tongue, esophagus, proventriculus, submucosa and tunica muscularis. While we have met mast cells in connective tissue between gland groups, we did not observe any between glands in proventriculus.

Chen et al. (5) have met mast cells in different shapes in lower respiratory tract (LRT). It has been found in the same study that granules in cross sections fixed by IFAA were stained more extensively relative to those fixed by FA and it is more difficult to distinguish them. Among the fixative solutions used in this study (BLA, Carnoy and IFAA), it has been observed that BLA and Carnoy's solutions are more suitable for determination of mast cells and BLA solution gives better results in distinguishing granules. In that studies, in AB-S combined staining only AB (+) cells has been observed regardless of location, fixations used or age but safranine positive cells are not found (5). In our study, just a few numbers of safranine (+) cells were determined in cross sections of tongue, esophagus, proventriculus, stomach and colon where BLA and IFAA fixative solutions were applied.

In a study on normal, gnotobiotic and parasitical pigs, much more mast cells have been determined in cross sections fixed by BLA compared to tissue sections fixed by routine formalin fixation (17). Mast cells located in lamina propria, were generally mucosal mast cells.

In a study on human duodenum, mast cells have been shown in both mucosa and connective tissue sections fixed by formalin (19). The same study has reported that mast cell granules which are less in number are dark blue and numbers of granules in cells are lower. On the contrary, it is specified by the present study that mast cells in connective tissues have typical connective tissue mast cells properties. When it is compared to the literature, from this point of view, it is similar to findings of Pabts et al. (17) and Ruitenberg and et. al. (19).

In another study on human intestine, formalin, Carnoy and BLA fixations have been used (3). When they stained sections with alcian blue (pH 0.5) they had found the greatest number of mast cell in BLA fixation. In the same study morphologies of mast cells fixed by BLA have been found to be the best. They have found less number of mast cells in sections fixed by formalin. In sections fixed by Carnoy's solution they have received results like in BLA fixation. Though we have found similar results in our study, desired results were not obtained in samples fixed in Carnoy solution and stained with AB-S inconsistently with Befus et al. (3).

Befus et al.(1985) have reported that more mast cell were found in lamina propria fixed by BLA relative to samples fixed by formaldehyde but they have found similar results for submucosa and tunica muscularis. They have observed 70 % lesser mast cell in submucosa and 80 % lesser mast cell in muscularis relative to lamina propria in samples fixed by BLA (3). In this study, it has been demonstrated that mast cells are found intraepitelially in only one sample, and no mast cell is found intraepitelially in formalin fixed tissue samples. In addition, we have not found any mast cell located in epithelium in tissue samples.

Consequently, light microscopic morphologies, location areas, numerical distributions, and heterogeneity of mast cells in chicken and quail digestive tract organs found to be similar. Our findings about chicken are in conformity with the literature. Since there is not any study about quail digestive tracts before, we think that our findings will contribute to related literature. We believe

that it is necessary to conduct biochemical and physiological researches in order to explain numerical distribution, heterogeneity and location area differences of mast cells in organs and between organ layers.

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