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The number and heterogeneity of mast cells in broiler ileum at different post-hatching periods

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ABSTRACT:

Mast cells are located near surfaces that contact the external environment, such as the skin, respiratory, and digestive systems. The number of mast cells in tissues can vary depending on the location and immunological status of the host. This study aimed to determine the number and heterogeneity of mast cells in the broiler ileum during the post-hatching period. The number and heterogeneity of mast cells were studied in the ileum tissue of 0-, 7-, 21- and 42-day old broilers. Mast cells were stained metachromatically with toluidine blue in all groups. Mast cells were seen in all layers of the ileum, especially in the lamina propria and submucosa. They were also observed around blood vessels and between smooth muscle cells in the tunica muscularis layer. In the broiler ileum, both subtypes of mast cells were seen: blue-colored AB (+) and red-pink colored SO (+) mast cells. AB (+) mast cells were observed in all age groups, whereas no SO (+) mast cells were found in the 0- age group. SO (+) mast cells were first detected in the ileum tissue on the seventh day after post-hatching. As a result, the number of mast cells was found to increase with age, which was statistically significant.

Kuluçka sonrası farklı dönemlerde broyler ileumdaki mast hücrelerinin sayısı ve heterojenitesi

ÖZET:

Mast hücreleri, deri, solunum ve sindirim sistemleri gibi dış çevre ile temas eden yüzeylerin yakınında bulunur. Dokulardaki mast hücrelerinin sayısı, konağın konumuna ve immünolojik durumuna bağlı olarak değişebilir. Bu çalışmada, kuluçka sonrası dönemde broyler ileumdaki mast hücrelerinin sayısının ve heterojenliğinin belirlenmesi amaçlanmıştır. 0-, 7-, 21 ve 42 günlük broylerlerin ileum dokusunda mast hücrelerinin sayısı ve heterojenitesi çalışıldı. Mast hücreleri metakromatik olarak toluidin mavisi ile tüm gruplarda boyandı. İleumun tüm katmanlarında, özellikle lamina propria ve submukozada mast hücreleri görüldü. Ayrıca kan damarların çevresinde ve tunika muskularis tabakasındaki düz kas hücreleri arasında gözlemlendi. Broiler ileumda, her iki mast hücresi alt tipi görüldü: mavi renkli AB (+) ve kırmızı-pembe renkli SO (+) mast hücreleri. Tüm yaş gruplarında AB (+) mast hücreleri görülürken, 0. yaş grubunda hiç SO (+) mast hücresi bulunmadı. SO (+) mast hücreleri ilk olarak yumurtadan çıktıktan sonraki yedinci günde ileum dokusunda tespit edildi. Sonuç olarak, mast hücre sayısının yaşla birlikte arttığı bulundu ki bu istatistiksel olarak anlamlıydı.

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1. Introduction

The gastrointestinal tract gradually establishes its mature structure and function throughout development from newborn to adult (1). A significant number of antigenic foreign substances come into close contact with the intestines (2). The intestinal mucosa is a protective barrier that allows nutrients to be selectively absorbed while preventing the entry of pathogens (3). Different components of this defensive barrier work together to resist, prevent, and, if necessary, repair injury, according to the anatomical layers of the mucosa (4). The defense role of intestinal epithelial cells depends on the variety of receptors they express in both extracellular and intracellular compartments and their capacity to communicate with the immune and nervous systems (5).

Mast cells are found close to surfaces that interface with the external environment, particularly the skin, respiratory, and digestive systems (6). Mast cells play a critical role in immunomodulatory function, especially at the mucosal interface between the body and the environment (7). The number of mast cells in tissues can vary depending on the location and immunological status of the host (8). Mast cells are classified as connective tissue mast cells (CTMC) or mucosal mast cells (MMC) based on physiological characteristics, staining features, and functional variety (9). With granule-specific dyes such as alcian blue/safranin O (AB/SO), MMC is stained with alcian blue and CTMC with safranin O (10). The granules found in their cytoplasm are classified into two types: those that were previously synthesized and stored in the granules, and those that were synthesized after stimulation (11). Mast cell mediators affect epithelial integrity and viability in the intestinal mucosa, as well as promote ion and water secretion, blood flow, coagulation, and vascular permeability (12). As, mast cells are important players in the mucosal immune response and barrier regulation in ileum (13).

The aim of this study was to determine the number of mast cells and their heterogeneity in the ileum of broilers during the post-hatching period.

2. Material and Methods

Broiler eggs were obtained locally (Beypiliç A.Ş., Bolu, Turkey) and incubated in a forced-draft poultry incubator with 50-60% relative humidity 35 °C and hatched under appropriate conditions. Newly hatched (0 days old), 7, 21, and 42-day post-hatching broilers were selected as four groups of six animals each. The experimental protocol and all animal procedures were approved by the Experimental Ethics Committee (Animal Ethics Committee of Ankara University Experimental protocol number No: 2013-5-38). The ileum was sampled for histochemical examination after the animals were sacrificed under anesthesia. Tissue samples were fixed in a 10% formaldehyde solution for 24 hours. The tissues were held for 1 hour in each of 70 percent, 80 percent, and 96 percent alcohol after being stored in a running water bath for 24 hours to remove the formalin. Following that, three one-hour applications of absolute alcohol and xylol were applied. The tissue samples were then embedded in paraplast.

Mast cell histochemistry:

From the blocks, 10 serial sections of 5 µm thickness were taken at 30 µm intervals and stained with toluidine blue (0.5%, pH 0.5) prepared in McIlvaine's citric acid disodium phosphate buffer to determine and count mast cells for 10 minutes (14). Also, sections taken from blocks were stained in 0.2 M acetate buffer alcian blue (0.5%, pH 0.2) /safranin O (0.25%, pH 1.42) combined dyes to determine the subtypes of mast cells and their distributions in tissues (15).

Mast cell count:

In the serial sections prepared to find out the numerical distribution of mast cells, cell counts were performed with 100 squares ocular micrometer. The mast cells at 100 square units of the ocular micrometer were counted with a magnification of 40x. Cell count was performed at 10 randomly chosen different areas of the sections receipt from

ileum and the arithmetic mean of the results was taken. All the data obtained by calculating the square of 100 square ocular micrometer for 40x objective magnification with the help of ocular micrometer were turned into mast cell number within a unit area of 1 mm² (2).

Following staining alcian blue/safranin O, AB (+) and SO (+) distribution was evaluated semiquantitatively. In semiquantitative evaluation following criteria were used; no stained cell in the scanned area (-), 1-2 cells (\pm), 3-4 cells (+), 5-6 cells (++) , 7 and more cells (+++) (16).

Statistical analysis:

The number of mast cell were analyzed with one-way ANOVA and determination of the significance of differences between the groups were done with Duncan's test. Differences among the groups $P < 0.05$ was accepted to be significant. SPSS statistical software was used for analyses (IBM – Company, Armonk, NY-USA, version 21).

3. Results

Histochemical findings:

Toluidine blue staining: Mast cells were stained metachromatically with toluidine blue in all groups. The cells were observed in a variety of sizes and shapes, particularly round, oval, and elongated-shaped cells. Metachromatic granules were found to be homogeneously stained in the cytoplasm and could not be selected individually. Mast cell nuclei were found to be centrally and eccentrically located, and in the majority of cells, they were covered by granules. Mast cells were observed in all the ileum layers, mainly in the lamina propria and submucosa. They were also observed in connective tissue around capillaries and between smooth muscle cells in the tunica muscularis layer (Figure 1). The number of mast cells was found to increase with age, and this increase was statistically important ($P < 0.05$). However, on day 21 days, the increase in mast cell count was found to be lower than the other groups (Table 1).

AB/SO combine staining: The AB/SO combined staining technique demonstrated two types of mast cells, including blue color AB (+) and red-pink color SO (+) mast cells, in the ileum sections of broilers aged 7, 21, and 42 groups. AB (+) mast cells were observed in all age groups, whereas no SO (+) mast cells were found in the 0- age group. SO (+) mast cells were first detected in the ileum tissue on the seventh day after post-hatching (Figure 2). It was found that AB (+) and SO (+) mast cells increased, especially on day 7 and 21 period, and there was no significant change in their numbers in 42 days (Table 2).

Table 1: Mast cell counts after staining with toluidine blue in four groups ($P < 0.001$). a, b, c: Differences between averages carrying different letters on the same column are important.

Tablo 1: Dört grupta toluidin mavisi ile boyandıktan sonra mast hücre sayımları ($P < 0.001$). a, b, c: Aynı sütunda farklı harfler taşıyan ortalamalar arasındaki farklar önemlidir.

Groups	n	X \pm Sx
0 days old	6	5.97 \pm 0.18 ^a
7 days old	6	9.01 \pm 0.54 ^b
21 days old	6	12.79 \pm 0.63 ^c
42 days old	6	14.12 \pm 0.81 ^c
P		***

***: $P < 0.001$

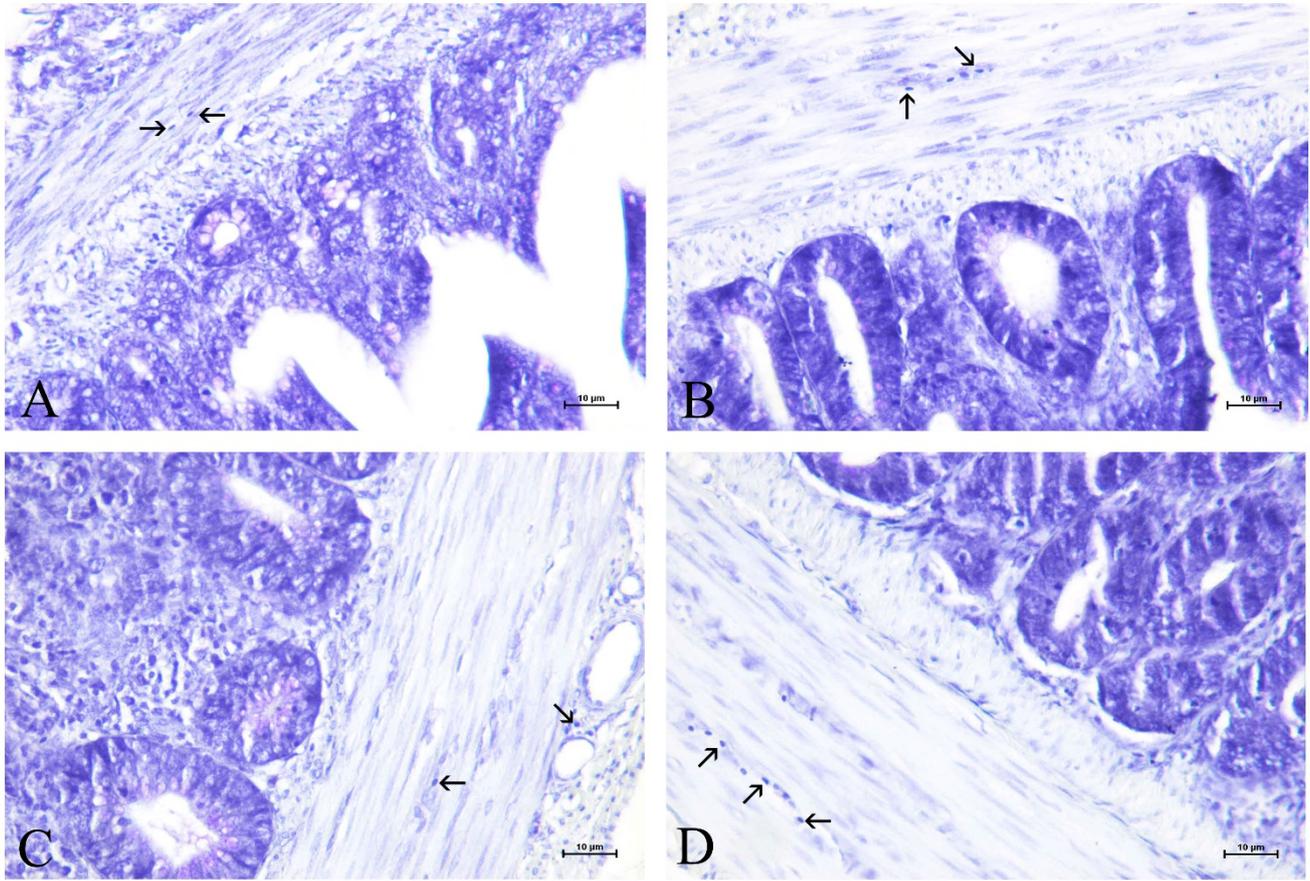


Figure 1: Toluidine blue staining. Broiler ileum. **A:** 0 days old. **B:** 7 days old. **C:** 21 days old. **D:** 42 days old. Arrow: metachromatic mast cells. Range bar, 10 µm.

Şekil 1: Toluidin mavisi boyama. Broiler ileum. **A:** 0 günlük. **B:** 7 günlük. **C:** 21 günlük. **D:** 42 günlük. Ok: metakromatik mast hücreleri. Aralık çubuğu, 10 µm.

Table 2: Mast cell counts after staining with alcian blue/safranin O combined staining. No stained cells (-), 1-2 cells (\pm), 3-4 cells (+), 5-6 cells (++), 7 and more cells (+++).

Tablo 2: Alcian mavisi/safranin O kombine boyama ile boyamadan sonra mast hücresi sayımları. Boyanmış hücre yok (-), 1-2 hücre (\pm), 3-4 hücre (+), 5-6 hücre (++), 7 ve daha fazla hücre (+++).

	0 days old	7 days old	21 days old	42 days old
AB (+)	\pm	+	++	++
SO (+)	-	\pm	+	+

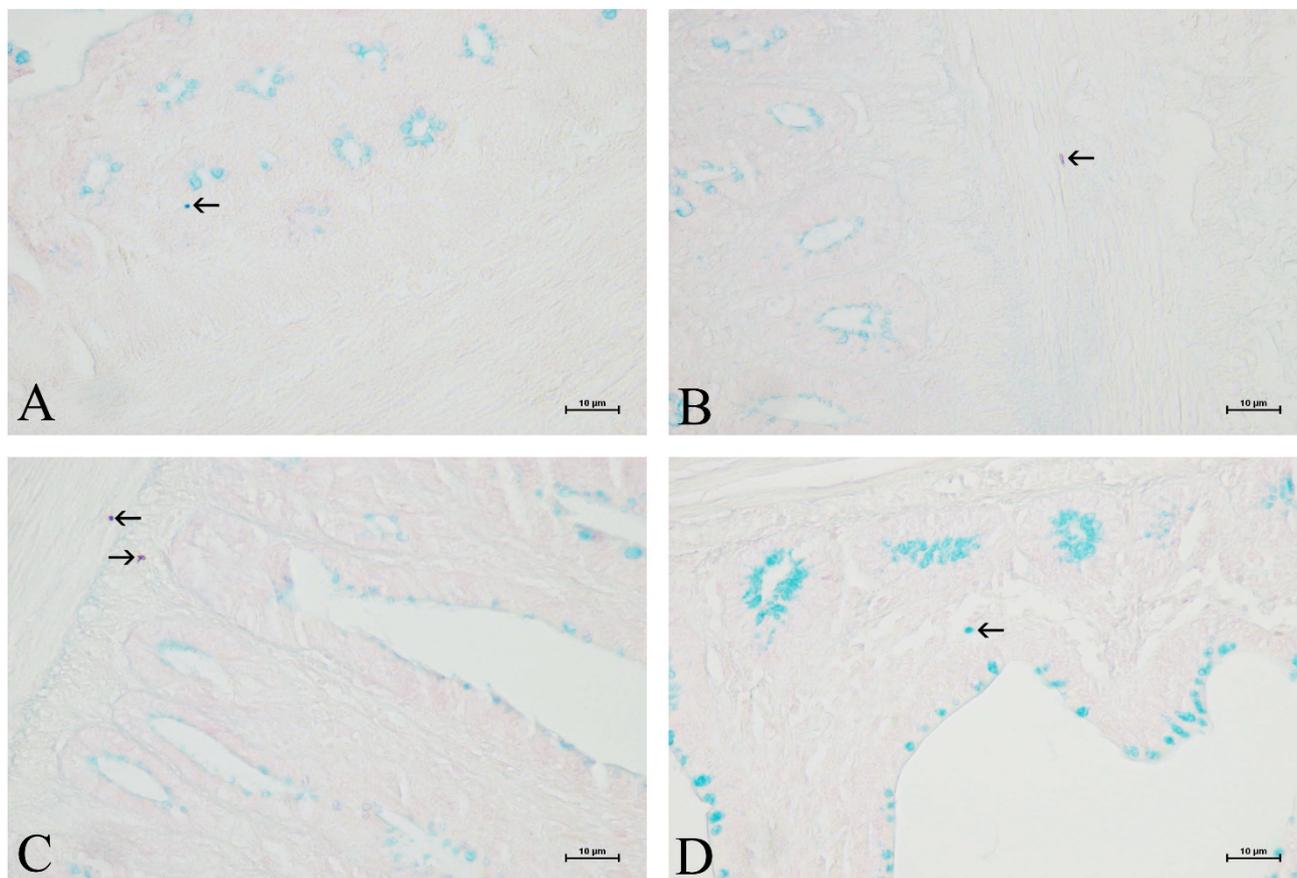


Figure 2: Alcian blue/safranin O combined staining method. Broiler ileum. **A:** 0 days old, arrow: AB (+) mast cell. **B:** 7 days old, arrow: SO (+) mast cell. **C:** 21 days old, arrow: SO (+) mast cell. **D:** 42 days old, AB (+) mast cell. Range bar, 10 µm.

Şekil 2: Alcian mavisi/safranin O kombine boyama yöntemi. Broiler ileum. **A:** 0 günlük, ok: AB (+) mast hücresi. **B:** 7 günlük, ok: SO (+) mast hücresi. **C:** 21 günlük, ok: SO (+) mast hücresi. **D:** 42 günlük, AB (+) mast hücresi. Aralık çubuğu, 10 µm.

4. Discussion and Conclusion

The intestinal mucosa is the largest interface between the inner and outer environments, and it is constantly exposed to luminal content. The ability to protect the body from harmful luminal content while regulating mucosal permeability is known as the intestinal barrier feature (17). The mucosa of the gastrointestinal tract contains a large number of immunocompetent cells such as mast cells, lymphocytes, and granulocytes (18). Mast cells in the intestine perform multiple functions necessary for homeostasis, including the regulation of epithelial activity, endothelial functions, tissue transformation, neurological functions, host defense, and innate and adaptive immunity (12).

Mast cells may produce, store, and release a large number of bioactive and vasoactive mediators that continuously modulate the tissues in which they are located (19). The number and density of mast cells in tissues vary depending on factors such as age and pathogens (20). Mast cells can reside in all layers of the gastrointestinal tract, but most of them are found in the lamina propria of the mucosa and the submucosa (21). A positive correlation between organ growth and mast cell count has been observed in the avian's spleen and thymus studies (20). Mast cells were first seen on the 9th day of incubation, as mentioned in Keleks's study, and their number continuously increased until the 15th day of incubation on quail skin (22). Furthermore, experimental studies in bursa Fabricius in Turkey show that mast cells vary statistically between age groups (23). The number of mast cells was found to increase in parallel with

the development of the chicken lungs during the postnatal growth period in a study (24). In our study, an increase in the number of mast cells in the post-hatching period was observed. Likewise, our findings were similar to those of the studies above in which mast cells vary numerically with age-related. During this time, we thought that mast cell counts could be affected by factors like pathogens exposure and connective tissue development.

Different developmental patterns of CTMCs and MMCs are thought to be influenced by different factors such as fibroblasts and cytokines in the tissues where they are located (25). Mast cells are classified into subgroups based on histochemical staining features, morphological features, enzymatic content, and responses to the mediators and secretory agents they produce (26). When granule-specific dyes like AB/SO are applied to mast cells, the cytoplasm of MMCs that react positively to alcian blue stains blue. CTMC, on the other hand, reacts positively to safranin and has a red-pink cytoplasm (2). It has been reported to be observed in the chick embryo lung from the 15th day of incubation for MMCs and the 18th day of incubation for CTMCs (27). Mast cells containing SO (+) granules are known to appear in the digestive system on the 18th day after hatching (28). From the 12th day of incubation, it was found that the amount of SO (+) mast cells in the glandular gastric mucosa of *gallus domesticus* increased regularly (29). At all developmental stages, from new hatching to 120 days after hatching, the presence of AB (+) mast cells was observed in avian lymphoid organs (20). In a histochemical study performed in Japanese quails' lungs, the post-hatching period was studied at 7-day intervals from the first day to the 60th day, and both subtypes of mast cells were observed in all age groups (30). In a study of *gallus domesticus* kidneys at various ages, it was observed that AB (+) mast cells were found more in the developmental stage than SO (+) mast cells (31). We observed two subtypes of mast cells in the ileum of broilers, which agrees with previous histochemical studies documenting mast cells' staining properties with AB/SO. As a prominent finding, while AB (+) mast cells were found in all age groups, SO (+) mast cells started to be seen after 7 days. The findings suggest that mast cell heterogeneity during the post-hatching period may be linked to ileum development or foreign matter contact.

Unique products such as cytokines and growth factors are secreted by mast cells. They can also function as antigen-presenting cells by processing bacteria and antigens, modulating the immune system. After post-hatching, exposure to various environmental stimuli and pathogens may increase mast cell count. According to this study, the number of mast cells increased consistently between the ages of 0 and 42. In conclusion, this study's findings show that the number and heterogeneity of mast cells can vary depending on age-related changes in the broiler ileum. The increase in the number of mast cells with age suggests that substances released from their granules may contribute to the ileum's development process after post-hatching.

Conflict of Interest

The author declared no conflict of interest.

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Authors' Contributions

Idea / concept: Tuğrul ERTUĞRUL
Experiment design: Tuğrul ERTUĞRUL
Supervision / Consultancy: Tuğrul ERTUĞRUL
Data collecting: Tuğrul ERTUĞRUL
Data analysis and interpretation: Tuğrul ERTUĞRUL
Literature search: Tuğrul ERTUĞRUL
Writing the article: Tuğrul ERTUĞRUL
Critical review: Tuğrul ERTUĞRUL

Ethical Approval

An ethical statement was received from the authors that the data, information and documents presented in this article were obtained within the framework of academic and ethical rules, and that all information, documents, evaluations and results were presented in accordance with scientific ethics and moral rules. Also, the tissue samples used in our study were obtained from the project named "The effect of synbiotic application in egg and post-hatch feeds on performance parameters, tibia ash and intestinal histomorphology and microflora in broilers" which is approved by the Animal Ethics Committee of Ankara University (no: 2013-5-38).

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