

### Avicenna Veterinary Research

Vol. 1, No. 2, Spring 2025: 27-37



ORIGINAL RESEARCH PAPER

# Comparative Histomorphologic ,Histomorphometric and Histochemistry Studies of Cecum in Adult Male and Female Black Local Turkeys of West Azarbaijan-Iran

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### Article info

# Article history: Received 2025-05-28 Received in revised form 2025-06-08 Accepted 2025-06-08

#### Keywords:

Cecal histomorphology Histomorphometric analysis Gender-specific differences in turkey cecum Lymphoid tissue distribution in turkeys

#### Abstract

Domestic fowl, including chickens, turkeys, and ducks, possess an organ known as the cecum, crucial for digestion and disease resistance due to its significant lymphoid tissue existence. However, limited information exists on the histology and histochemistry of the cecum in native breeds like the Azerbaijani Black Turkeys. This research delves into the histological examination of the cecal regions in male and female turkeys. For this purpose, to study the histology of the turkey cecum, 5 male and 5 female turkeys with an approximate weight of 4 to 6 kg were selected. After anesthetizing and euthanizing the animals, their ceca were separated and placed in a 10% formalin buffer solution. Tissue sections were prepared trough routine paraffin embedding technique and stained with hematoxylin-eosin for general examination and histomorphometry. Moreover, PAS staining was used to evaluate the dispersion of goblet cells in three parts of the male and female turkey cecum, and Masson's trichrome staining was applied for evaluating collagen fibers. Results indicated anatomical similarities in cecal positioning among birds, with males showing greater mucosal layer thickness and lymph node size compared to females. Moreover, lymph node density varied between genders, with females exhibiting higher cell counts. This study provides valuable insights into the cecal structure of turkeys, shedding light on gender-specific histological differences and lymphoid cell distribution.

#### 1. Introduction

The turkey (Meleagris gallopavo) is a species of bird belonging to the order Galliformes. It is classified under the genus Meleagris and is native to regions of Latin America. Turkeys are characterized by a fleshy wattle hanging below their beak and a distinctive fleshy protuberance (snood) extending from the top of their beak, distinguishing them from other poultry species (Fathi et al., 2009). Like many other species within the order Galliformes, male turkeys are larger and more vi-

brantly colored than females. Turkeys, like other birds, have different breeds; some are wild and cannot be domesticated, whereas others are native breeds reared for meat and egg production (Fathi et al., 2009).

The cecum plays a pivotal role in the digestive and immune systems of domestic fowl, including chickens, turkeys, and ducks, by facilitating nutrient absorption, microbial fermentation, and disease resistance (Erf, 2004, Turk, 1982). Despite its importance, native breeds of poultry, such as the Azerbaijani Black Turkey, have been the subject of limited histological

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and histochemical studies. Investigating the structural and cellular composition of the cecum in these birds can enhance our understanding of their digestive efficiency and immunological adaptations.

The avian digestive tract is structured to optimize nutrient utilization, with the cecum functioning as a key organ for cellulose digestion, water absorption, and immune surveillance (Zaher et al., 2012). Previous research has highlighted the presence of extensive lymphoid tissue within the cecum, including cecal tonsils, which contribute to pathogen defense and mucosal immunity (Rezaian and Hamedi, 2006). Additionally, the structural organization of the cecum, including mucosal folds, Lieberkühn glands, and goblet cells, plays a crucial role in maintaining intestinal homeostasis (Getty et al., 1975).

While numerous studies have examined the digestive morphology of commercial poultry species, few have focused on native turkey breeds, particularly those reared in Azerbaijan. Given their significant role in local poultry farming, understanding their digestive histology and immune function is essential for optimizing breeding programs and improving poultry health (Erf, 2004, Firth, 1977). The comparison between male and female turkeys may reveal gender-specific anatomical differences, particularly in terms of mucosal structure, lymphoid tissue distribution, and collagen fiber organization (Ciriaco et al., 2003).

This study aims to conduct a detailed histological examination of the cecal regions of male and female turkeys, utilizing advanced staining techniques such as hematoxylin-eosin for general tissue analysis, PAS for goblet cell detection, and Masson's trichrome for collagen fiber evaluation. Through these analyses, the thickness of the mucosal layer, cecal villi height, and lymphoid tissue dispersion will be compared across different sections of the cecum (proximal, middle, and distal).

Ultimately, this research seeks to enhance our understanding of digestive physiology in native turkeys, bridging the existing gap in histological studies and providing valuable insights into avian immunology and digestion. By examining gender-specific differences in histomorphometry, these findings may contribute to future advancements in poultry health management and nutritional research.

### 2. Materials and Methods

#### 2.1. Sample Collection and Preparation

This study was conducted on ten adult turkeys (five males and five females), approximately one-year-old, naturally fed, and belonging to the local ecotype of West Azerbaijan, Iran (commonly known as Black Turkeys). Following weighing and humane euthanasia, cecal tissue samples were collected from three anatom-

ical regions: proximal, middle, and distal parts. The samples were immediately fixed in a 10% buffered formalin solution to preserve tissue integrity.

# 3. Histological Study of the Cecum

Given that the cecum in turkeys is bilaterally structured, tissue samples were obtained from three regions of each cecal section-proximal, middle, and distal. The samples were fixed in a 10% buffered formalin solution for proper tissue preservation. Following fixation, tissue specimens were processed, embedded in paraffin, and prepared for histological examination.

### 4. Histological Preparation and Analysis

For histological evaluation, the samples were stained using the hematoxylin-eosin (H&E) method, allowing for general tissue morphology and histomorphometric analysis. Additional staining protocols were employed to highlight specific cellular structures.

Periodic Acid-Schiff (PAS) staining was used to detect carbohydrate containing structures and goblet cell distribution.

Masson's Trichrome staining was performed to assess collagen fiber distribution.

### 5. Histomorphometry

Cell counts and structural assessments were conducted using a binocular light microscope equipped with calibrated grid eyepieces, with ten microscopic fields per region (proximal, middle, distal) were randomly selected for each sample. Goblet cells were counted in 0.04 mm areas under 40X.

Quantitative histomorphometric analysis was conducted using hematoxylin-eosin stained sections to measure following parameters in proximal, middle, distal parts:

Cecal mucosal layer thickness, Cecal fold height, Cecal villi height, Lieberkühn gland height, Muscular layer thickness, Dense lymphoid tissue distribution, Lymphoid nodule diameter, Lymphocyte density in dispersed and dense lymphoid tissue, Goblet cell distribution (PAS-stained sections) and Collagen fiber distribution (Masson's Trichrome-stained sections).

### 6. Statistical Analysis

Quantitative data were analyzed using ImageJ software (US National Institutes of Health), and results were expressed as Mean  $\pm$  SEM. Normality and variance homogeneity were evaluated using the Kolmogorov-Smirnov test, followed by one-way ANOVA and Duncan's multiple range test for comparative analysis

(SPSS, version 18). Statistical significance was determined at P < 0.05.

#### 7. Result

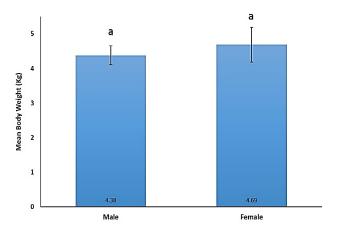
### 7.1. Clinical Observations and Body Weight Changes

In both male and female turkeys, the cecum appeared as a paired organ, located at the junction of the ileum and rectum. It consisted of three distinct regions-proximal, middle, and distal with unequal sizes. The average body weights of male and female turkeys are presented in Fig. 1.

# 8. Histological Findings

Histological analysis of the proximal cecal region showed thick mucosal folds with numerous structures. Villi were present on the folds and interfold spaces, displaying variable lengths. Some villi were finger-like, while others were sharper or absent in specific areas. Lieberkuhn glands were uniformly distributed, and

lymphoid tissue appeared both as diffuse and nodular aggregates. The epithelial layer consisted of simple columnar cells, with goblet cells and basal cells clearly identifiable (Fig. 2(a), 2(b), 2(c), Fig. 3).



**Fig. 1.** The average live weight of turkeys in kilograms. Similar superscript denotes there is no significant differences (P>0.05) in body weights between male and female turkeys.

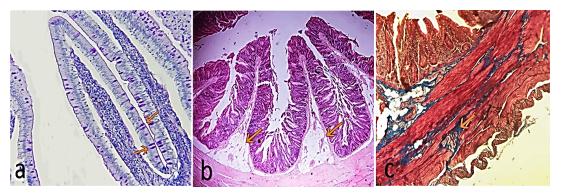


Fig. 2. a) Distribution of goblet cells (arrowhead) within the epithelium of the proximal cecal section in male turkeys. (X40) Periodic Acid–Schiff (PAS) staining. b) Cecal folds and accumulation of adipose tissue within the submucosa of the proximal cecal section in male turkeys. H&E, X20. c) Neural network of Auerbach's plexus (arrow) distributed within the muscular layer of the proximal cecal section in male turkeys. Masson's Trichrome, X40.

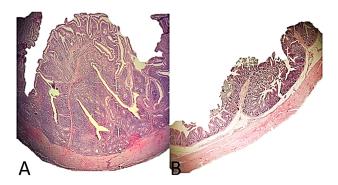


Fig. 3. Variation in the distribution of cecal folds and villi within the proximal section of the male turkey cecum. A) Presence of large folds and prominent villi. B) Smaller folds and reduced villi. H&E, 20X.

In the middle section, folds were dense and elongated, often containing adipose tissue at the base. Villi structures were less frequent and shorter, with reduced goblet cell density. Lieberkuhn glands appeared dilated, while lymphoid tissue remained diffusely scattered throughout the lamina propria (Fig. 4).

The distal cecal region exhibited fewer mucosal folds but longer, thinner villi. The epithelium showed a high density of goblet cells, especially within the Lieberkuhn glands. Lymphoid tissue was less concentrated, appearing predominantly as diffuse aggregates. Some specimens displayed shortened folds with compact villi, contrasting with the proximal and middle sections (Fig. 5).

Fig. 4. Dense mucosal folds with extended length observed in the middle section of the male turkey cecum, accompanied by the accumulation of adipose connective tissue at the base of the folds. Nodular lymphoid tissue is absent in this section. H&E, 20X.

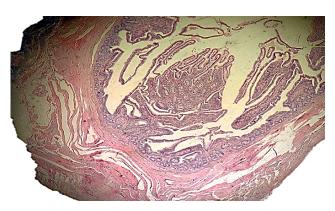


Fig. 5. Dense and elongated villi observed in the distal section of the male turkey cecum. Nodular lymphoid tissue is not seen in this region. H&E, 20X.

# 9. Histological Findings in Female Turkey Cecum

Microscopic inspection of the proximal cecum in female turkeys revealed two distinct types of villi. In some sections, thin and elongated villi extended deep into the cecal lumen, while in others, villous structures appeared shorter and thicker. Lymphoid tissue in the lamina propria was observed both as nodular clusters and diffuse forms, with cecal tonsils clearly visible in some samples (Fig. 6(P)). The distribution of Lieberkuhn glands varied significantly, with some regions showing dense glandular occurrence, while others had reduced gland disseminations. The muscular layer was composed of an inner circular and outer longitudinal layer, with the inner layer appearing thicker.

In the middle cecal section, mucosal folds became more dominant, while cecal villi were noticeably shorter. Lymphoid tissue density decreased, and the cecal lumen widened. A significant accumulation of fat-rich connective tissue was observed at the base of the folds (Fig. 6(M)).

In the distal cecum, the muscular layer weakened, and mucosal folds became considerably shorter. The submucosa was densely packed with fat, while lymphoid aggregates remained prominent, appearing both as nodular and diffuse clusters. Areas with higher lymphoid concentration exhibited reduced fat deposits, and villous structures appeared sparsely distributed and very short (Fig. 6(D)).

# 10. Results of histomorphometric assessments

This study examined key morphometric differences across the three cecal sections in male and female turkeys to evaluate structural variations.

### 11. Cecal Mucosal Thickness

Dimensions of mucosal thickness remained similar in the proximal and middle cecum, but significantly decreased in the distal section in male turkeys (Fig. 7(A)). In female turkeys, mucosal thickness varied between sections, with significant differences between the middle and distal regions, though the proximal segment remained unchanged. Overall, male turkeys exhibited significantly thicker mucosal layers through all the cecal regions in comparison to females (Fig. 7(B)).



**Fig. 6.** Distribution of mucosal folds, villi, lymphoid tissue, and adipose tissue in the three sections (proximal (P), middle (M), and distal (D)) of the female turkey cecum. H&E, 20X.

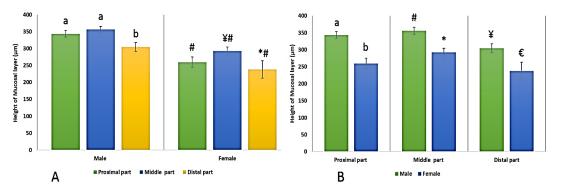


Fig. 7. A) Mean thickness of the mucosal layer across the three cecal sections (proximal, middle, distal) in male and female turkeys. B) Comparison of mucosal layer thickness in the three sections (proximal, middle, distal) between male and female turkeys. Different superscripts within each section indicate statistically significant differences (P < 0.05).

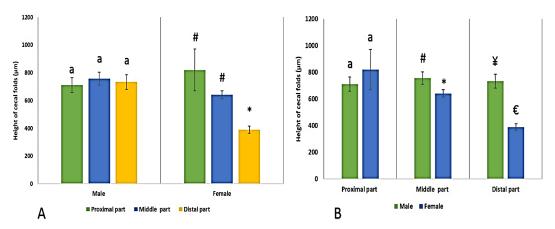


Fig. 8. A) Mean height of cecal folds in the three regions (proximal, middle, distal) of the cecum in male and female turkeys. B) Comparative analysis of cecal fold height in the three regions (proximal, middle, distal) between male and female turkeys. Different superscript letters within each region denote statistically significant differences (P<0.05).

#### 12. Cecal Fold Height

In male turkeys, fold height remained consistent across the three regions, showing no significant differences (Fig. 8(A)). However, in female turkeys, fold height gradually decreased from proximal to distal sections, showing significant variation (Fig. 8(A)). Comparisons between both sexes indicated that males had consistently taller cecal folds, with statistically significant differences in the middle and distal regions (Fig. 8(B)).

### 13. Cecal Villi Height

The distribution and height of cecal villi varied significantly among male turkeys, with large differences through all three cecal regions. In female turkeys, proximal villi appeared significantly longer, whereas middle and distal villi were markedly shorter, showing significant reductions in height (Fig. 9(A)). Comparisons between both the sexes revealed that, male turkeys

bearing taller villi through all three cecal regions, with statistically significant differences in the proximal and distal regions (Fig. 9(B)).

# 14. Histological Analysis of Lieberkuhn Glands

The measurement of Lieberkuhn gland height in the three cecal regions (proximal, middle, distal) revealed prominent differences between male and female turkeys. In male turkeys, the proximal and middle regions exhibited similar gland heights, whereas the distal regions showed a significant reduction (Fig. 10(A)). However, in female turkeys, the proximal and distal regions had similar gland sizes, while the middle regions displayed significantly longer glands. Comparative analysis between both sexes indicated that, males had larger Lieberkuhn glands overall, with significant differences in the proximal and distal regions compared to females (Fig. 10(B)).

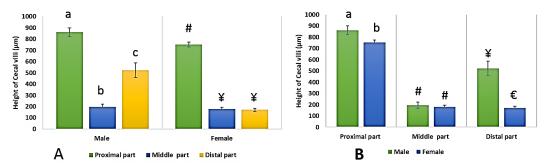


Fig. 9. A) Mean height of cecal villi across the three regions (proximal, middle, distal) of the cecum in male and female turkeys. B) Comparative analysis of cecal villi height in the three regions (proximal, middle, distal) between male and female turkeys. Different superscript letters within each region indicate statistically significant differences (P < 0.05).

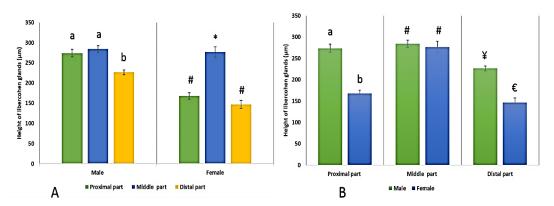


Fig. 10. A) Mean height of Lieberkuhn glands through three regions (proximal, middle, distal) of the cecum in male and female turkeys. B) Comparative analysis of the height of Lieberkuhn glands in the three regions (proximal, middle, distal) between male and female turkeys. Different superscript letters within each section indicate statistically significant differences (P<0.05).

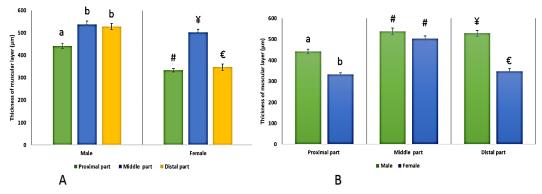


Fig. 11. A) Mean thickness of the muscular layer through the three regions (proximal, middle, distal) of the cecum in male and female turkeys. B) Comparative analysis of muscular layer thickness in the three regions (proximal, middle, distal) between male and female turkeys. Different superscript letters within each section indicate statistically significant differences (P < 0.05).

# 15. Muscular Layer Thickness of the Cecum

The thickness of the muscular layer varied between male and female turkeys. In males, muscular thickness increased regularly toward the distal region, although no significant difference was observed between the middle and distal regions (Fig. 11(A)). In contrast, female turkeys exhibited the thickest muscular layer in the middle region, with significant thinning in the proximal and distal regions (Fig. 11(A)). Once comparing male and female turkeys, males consistently had thicker muscular layers across all regions, with significant differences in the proximal and distal regions (Fig. 11(B)).

# 16. Lymphoid Tissue Distribution

The analysis of various lymphoid parameters within the three cecal regions of male and female turkeys, revealed differences in lymphoid nodule distributions as follow:

In male turkeys, nodular lymphoid structures were present only in the proximal section (Table 1).

In female turkeys, nodular lymphoid structures were abundant in both the proximal and distal regions (Table 2.)

Comparative analysis showed that, the females had significantly higher nodular lymphoid density in the proximal section (Table 3).

Further analysis of lymphoid nodule diameter revealed that although males had fewer lymphoid nodules, their nodules were significantly larger compared to those in females (Table 1, 2). Lymphocyte distribution within diffuse and nodular lymphoid tissue was quantified using 40X magnification and a calibrated grid eyepiece (Table 1, 2).

In male turkeys, diffuse lymphoid accumulation was

significantly higher in the proximal region, decreasing in middle and distal regions (Table 1).

In female turkeys, diffuse lymphoid accumulation were most concentrated in the proximal and distal regions, with lower density in the middle region (Table 1).

Comparing both sexes, significant differences in diffuse lymphoid cell accumulation were observed in the proximal and distal regions, whereas the middle region showed not at all prominent variation (Table 3).

# 17. PAS Staining and Goblet Cell Distribution

PAS-stained sections revealed PAS-positive reactions within goblet cells, indicating intracellular mucin accumulation (Fig. 12). PAS staining also highlighted basal lamina structures surrounding epithelial layers, blood vessels, and smooth muscle fibers, particularly within muscle-separating membranes (endomysium, perimysium, epimysium).

Table 1
Comparison of various parameters of diffuse and nodular lymphoid tissue in different regions of the male turkey cecum.

Region of	Mean Number	Mean Diameter	Distribution of	Distribution of
Cecum	of Lymphoid	of Lymphoid	Lymphocytes in Lymphoid	Lymphocytes in Diffuse
	Nodules (N)	Nodules $(m\mu)$	Nodules $(0.04 \text{ mm}^2)$	Lymphoid Tissue $(0.04 \text{ mm}^2)$
Proximal	$8.0 \pm 0.894$	$361.33 \pm 20.867$	$1964.26 \pm 19.57$	$1379.4 \pm 114.4^a$
Middle	0	0	0	$899.11 \pm 184.9^b$
Distal	0	0	0	$855.22 \pm 154.3^b$

Table 2
Comparison of various parameters of diffuse and nodular lymphoid tissue in different regions of the female turkey cecum.

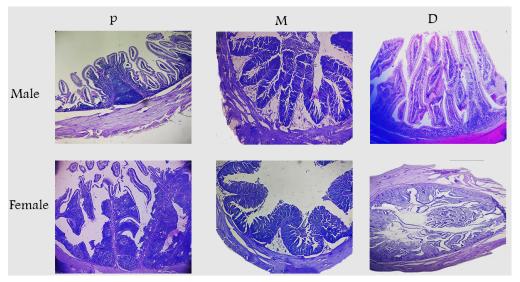
Region of	Mean Number	Mean Diameter	Distribution of	Distribution of
Cecum	of Lymphoid	of Lymphoid	Lymphocytes in Lymphoid	Lymphocytes in Diffuse
	Nodules (N)	Nodules $(\mu)$	Nodules $(0.04 \text{ mm}^2)$	Lymphoid Tissue $(0.04 \text{ mm}^2)$
Proximal Section	$122.0 \pm 0.57^a$	$260.5 \pm 44.561^a$	$1699.52 \pm 105.23^a$	$1061.5 \pm 107.6^a$
Middle Section	0	0	0	$885.32 \pm 116.6^{ab}$
Distal Section	$8.33 \pm 1.55^b$	$195.64 \pm 28.229^{\circ}$	$^{a}2044.45 \pm 71.11^{b}$	$1292.2 \pm 209.3^{a}$

Dissimilar superscripts within each column indicate statistically significant differences (P < 0.05).

**Table 3**Comparative Analysis of Various Parameters of Diffuse and Nodular Lymphatic Tissue in Different Cecal Regions between Male and Female Turkeys.

Male/Female	Mean Number	Mean Diameter	Distribution of	Distribution of
	of Lymphoid	of Lymphoid	Lymphocytes in Lymphoid	Lymphocytes in Diffuse
	Nodules (N)	Nodules $(\mu m)$	Nodules $(0.04 \text{ mm}^2)$	Lymphoid Tissue $(0.04 \text{ mm}^2)$
Proximal Male	$0.8 \pm 0.894^{\#}$	$361.33\pm867/20^{\#}$	$1964.26 \pm 19.57^{\#}$	$1379.4 \pm 114.4^a$
Proximal Female	$0.22 \pm 0.57^*$	$260.5\pm44.561^*$	$1699.52 \pm 105.23^{\#}$	$1061.5 \pm 107.6^b$
Middle Male	0	0	0	$899.11 \pm 184.9^{\#}$
Middle Female	0	0	0	$855.32 \pm 116.6^{\#}$
Distal Male	0	0	0	$855.22 \pm 154.3^{\fmathbf{Y}}$
Distal Female	$8.33 \pm 1.55$	$195.64\pm28.229$	$2044.45\pm71.11$	$1292.2\pm209.3$

Dissimilar superscripts within each column indicate statistically significant differences (P < 0.05).



**Fig. 12.** Distribution of tissues with PAS-positive reactions in the three regions of the cecum: proximal (P), middle (M), and distal (D) in male and female turkeys. PAS, 10X.

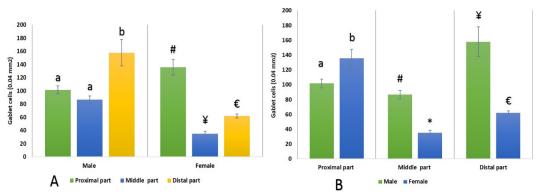


Fig. 13. A) Mean distribution of goblet cells in the three regions (proximal, middle, distal) of the cecum in male and female turkeys in  $0.04 \text{ mm}^2$ . B) Comparison of the mean distribution of goblet cells in the three regions (proximal, middle, distal) of the cecum between male and female turkeys in  $0.04 \text{ mm}^2$ . Different superscripts in each region indicate a significant difference (p<0.05).

# 18. Quantification of goblet cell distribution revealed an inconsistent pattern across cecal regions as follow

In male turkeys, goblet cell distribution increased progressively toward the distal region of the cecum (Fig. 13(A)).

In female turkeys, goblet cell distribution was highest in the proximal region of the cecum (Fig. 13(A)).

# 19. Comparative analysis showed significant differences between genders as follow

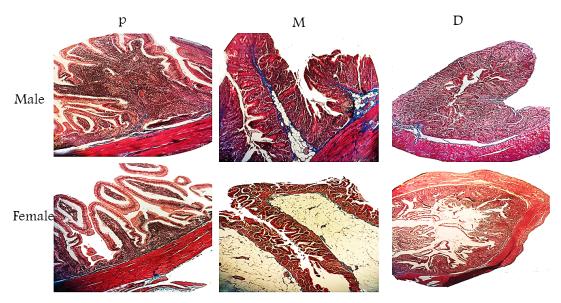
In the proximal region of the cecum cecum, males had more goblet cells than females (Fig. 13(B)).

In the middle and distal regions of the cecum, females had higher goblet cell counts than males (Fig. 13(B)).

# 20. Masson's Trichrome Staining Findings

Masson's Trichrome staining was used to evaluate collagen fiber distribution within the cecal tissue. In this staining, collagen fibers appeared blue, as a clearly distinguishing connective tissue structures. Typical stained sections are showed in Fig. 14.

Regions with dense fibrous connective tissue showed strong blue staining, indicating a higher collagen occurrence. However, areas where connective tissue was intermixed with nodular and diffuse lymphoid aggregates or adipose tissue exhibited lower collagen density, resulting in less intense blue pattern. Additionally, blue staining was observed along the basal lamina of blood vessels, connective tissue surrounding neural ganglia, and muscle fibers, further highlighting collagen distribution in the cecal architecture. Collagen occurrence was also prominent in the serous layer.



**Fig. 14.** collagen fibers distribution in three regions of the cecum: proximal (P), middle (M), and distal (D) in male and female turkeys. Masson Trichrome, 20X.

# 21. Quantitative Analysis of Collagen Fiber Distribution

Collagen fiber dispersion was examined in three anatomical regions (proximal, middle, distal) of the cecum in male and female turkeys using Masson's Trichrome-stained sections and Image J software for digital analysis. Statistical comparison revealed no significant differences in collagen fiber distribution between males and females through cecal regions (Fig. 15).

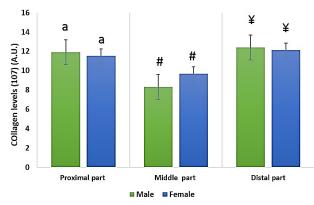


Fig. 15. Comparison of collagen fiber distribution in the three regions (proximal, middle, distal) of the cecum between male and female turkeys. Different superscripts in each region indicate a significant difference (p<0.05).

### 22. Discussion

The turkey (Meleagris gallopavo) belongs to Galliformes, and similar to other galliform birds, males exhibit larger body size and distinct anatomical features compared to females. Despite extensive studies on commercial poultry breeds, research on native turkeys, particularly the Azerbaijani Black Turkey, is scarce. This study aimed to bridge that gap by investigating histological and histomorphometric differences between sexes in the cecum.

The paired ceca, essential for nutrient absorption and immune regulation, are present in most galliform birds but absent in certain avian groups, such as parrots, woodpeckers, owls, hummingbirds, and falcons (Bezuidenhout, 1993, Hassan and Moussa, 2012, Pourrhaji and Hashemi, 2019). Findings confirmed that Azerbaijani Black Turkeys possess paired ceca, structurally consistent with other poultry species, situated at the ileocecal junction and divided into three anatomical regions including proximal, middle, and distal.

Histological examination demonstrated structural variations across cecal regions, holding digestive competence and microbial activity. The presence of mucosal folds and villi enhances fermentation of complex carbohydrates, notably cellulose and hemicellulose, similar to observations in ostriches (Bezuidenhout, 1993). According to results of this study, in male turkeys, thick mucosal folds with abundant villi were identified, whereas in females, fold height decreased progressively toward the distal section. Moreover, males exhibited significantly thicker mucosal layers, reinforcing the hypothesis that sex-related anatomical differences influence digestive function.

Beyond digestion, the avian cecum plays a major role in immune defense. It serves two primary functions: Microbial fermentation, generating short-chain fatty acids (acetic acid, butyric acid, propionic acid) for gut health, and Immune surveillance, supported by cecal tonsils and lymphoid aggregations (Hussein and Reshag, 2019).

Lymphoid structures within the cecum exhibit species-specific differences. In chickens, cecal tonsils are highly structured, whereas in kiwi birds, they are absent (Potter et al., 2006). In ostriches, lymphoid nodules are densely packed within the proximal cecum, whereas in geese and ducks, they are less prominent (Warui et al., 2009). According to the findings of this study, recommendation of sex-related variations in lymphoid tissue distribution in Azerbaijani Black Turkeys is sound, as in male turkeys there were fewer lymphoid nodules, nevertheless their nodules were significantly larger, suggesting a localized immune defense complex; but female turkeys exhibited greater nodule density, particularly in the distal section, a pattern not previously reported in other avian species. This proposes that, female turkeys may depend on enhanced immune activity in distal cecal regions, potentially due to higher pathogen exposure in the lower digestive tract.

PAS staining confirmed the presence of goblet cells, essential for mucin production and intestinal barrier maintenance (Al-Agele et al., 2020). Our findings indicated sex-specific differences in goblet cell dispersal. In male turkeys, goblet cell records increased toward the distal section, suggesting progressive mucin secretion. In female turkeys, goblet cell records were highest in the proximal cecum, potentially reflecting local protective adaptation. Males exhibited more goblet cells in the proximal region, whereas females had greater goblet cell counts in the middle and distal regions.

Masson's Trichrome staining highlighted collagen fiber presence, notably around blood vessels, neural ganglia, and muscle layers, reinforcing structural stability within the cecum. However, no significant differences in collagen fiber distributions were observed between male and female turkeys, suggesting similar connective tissue organization exists between genders.

#### 23. Conclusion

This study provides important insights into the histomorphologic and histomorphometric deviations in male and female Azerbaijanian black turkeys, particularly concerning lymphoid tissue disseminations, mucosal thickness, and digestive tract morphology. The findings highlight sex-related differences in immune constructions and digestive adaptations, emphasizing the need for further research into their physiological relevance.

By integrating advanced histological and molecular techniques, future research can provide a comprehensive perspective on avian digestion and immunity, contributing to improvements in poultry breeding science, health management, and nutritional optimization.

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