Iraqi Journal of Veterinary Sciences

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Morphometrical and histological effect of the crop in producing crop milk: A comparative study with blood supply between brooding and non-brooding diamond dove and Indonesian chicken

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Article information

Article history:
Received 02 May 2025
Accepted 13 August 2025
Published 30 September 2025

Keywords:
Crop
Diamond dove
Indonesian chicken
Brooding
Non-brooding

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Abstract

The current study was focused on the crop of diamond dove and Indonesian chicken to determine the location, shape, dimensions, blood supply and histological changes occurred during brooding and chick feeding and non-brooding periods to know differences led to the formation of crop milk. Twenty-four sexually mature healthy birds of the two types collected and rose until reach the brooding and feeding period divided into brooding and non-brooding groups, from August 2024 to March 2025. The crop in brooding diamond dove contained two lateral lobes with smooth central lobe, mucosal surface of the lateral lobes displayed irregular folds in female and hexagon-shaped folds in male and was packed with crop milk, while in non-brooding it was membrane-like crop of asymmetrical lobes with smooth inner surface free of folds in both sexes. Whereas Indonesian chicken crop appeared as a semicircular ring, its inner surface had irregularly shaped mucosal folds of club-like shape in brooding and non-brooding birds. Histological results in both diamond dove and Indonesian chicken revealed that crop's wall comprised four tunicae; mucosa, submucosa, muscularis and adventitia. Tunica mucosa contained well-developed mucosal folds covered with stratified squamous keratinized epithelium, that characterized in brooding diamond dove by the increase in: number of cells with peri-nuclear hallow zone that being desquamated and sloughed and undergo fatty changes forming crop milk, and the depth of rete pegs which extending from germinal layer down into lamina propria which was free of glands.

DOI: 10.33899/ijvs.2025.159538.4247,

Introduction

The oropharyngeal cavity and the glandular stomach are connected by the muscular tube known as the avian esophagus. The cervical and thoracic sections make up the majority of its division separated by the crop which is a vital part of avian digestive system, running as a temporary storing structure that tempers swallowed diet before entering the stomach (1). Despite its importance, limited studies have highlighted the differences between brooding and non-brooding birds, so, this study aims to highlight those differences and document the anatomical, morphometrical and histological adaptations associated with the brooding behavior. Depending on the diet, the avian crop's size and form vary by species, and, not all species of birds have one such as owls, gulls and penguins (2,3). The esophagus out pouching shortly before entering the thoracic cavity forming the crop which preserves the consumed food for a short time. Furthermore, the crop helps to moisten food to facilitate enzymatic breakdown in the stomach (4,5). In addition, the prolactin hormone stimulates the crops of male and female pigeons and doves to secrete crop milk during the brooding period in order to feed their squabs (6,7). Pigeon crop milk is made up of a suspension of cells that split and separate

from the crop lining (8-10). Indonesian chicken “Ayam Cemani” is a unique indigenous breed came from Java Island's Kedu Village in Temanggung City (Indonesia). The Indonesian terms "Ayam," equal to "chicken," and "Cemani," means "completely black," are combined to create the breed's moniker (11,12). Because they originated in hot places, this species thrives in harsher environments and are extremely heat tolerant (13,14). The chicken's internal organs, beak, and feathers turn primarily black due to hyperpigmentation (fibromelanosis), which caused by a dominant gene (15,16). Diamond dove (Geopelia cuneata) belongs to Columbidae family: is a small bird that brought to Puerto Rico from Australia's inland marshes and arid deserts. The common name "diamond" comes from the white dots on its wings. In female the upper parts are a shade browner than the male's, the bare eye ring is thinner and somewhat pink-red (17-19).

The current study was focused on the crop of Diamond dove and Indonesian chicken to determine the location, shape, dimensions, blood supply and histological changes occurred during brooding and chick feeding and non-brooding periods to know differences led to the formation of crop milk.

Materials and methods

Ethical approval

The research ethical approval obtained from (Research Ethics Committee)/ University of Diyala, College of Veterinary Medicine no: Vet Medicine (113); September 2024, A, and H).

Study design

Twenty-four adult, sexually mature healthy birds of Diamond doves and Indonesian chicken, collected from the local markets and raised until it reaches the egg-laying stage and brooding period: (12 birds from Ayam cemani chickens and 12 birds of diamond doves) divided into two main groups brooding and non-brooding, each group contained 6 birds: (3 brooding and 3non-brooding). All birds were performed euthanasia by the use of Ketamine (10mg/kg) and xylazine (1mg/kg) intramuscular injection (20,21). Each bird was weighed before its neck and chest feathers were removed, followed by dissection of the celomic cavity to view the viscera and exposing the crop and esophagus. The lumen was cut lengthwise to check the measurements after that crop was removed from the body and cleaned under running water have been examined grossly and photographed to explain the general morphology and morphometric measurements such as color, weight, length, and diameter as well as topographical relationships (22,23). The study of blood supply of the crop was done by the use of latex mixed with carmine stain injected to the left ventricle of heart and photographed by a digital camera (24).

Statistical analysis

Statistical Packages of Social Sciences- SPSS program was used to detect the effect of different groups in parameters. The differences within the taken groups were calculated using the T test, while the differences between the parameters of groups were calculated using the ANOVA test.

Results

Morphometrical in diamond dove

According to the current study's anatomical findings, the Diamond dove's crop is an important part of the digestive system and serve as a temporary storage site for food, it appeared unilateral in position and situated in the right of the median plan, in front of the furcula, near the breast musculature and laterally to the trachea and covered it (Figure 1). The crop during the brooding (lactating period) showed an actual altered tissue in comparison with that of the non-brooding; it was larger and had a thicker wall, consisted of two symmetrical lateral lobes which were quite noticeable with a central lobe which was thin and membrane-like (Figure 1), also it was pack with grains and round-shaped pellets of crop milk that were embedded in the lateral lobes' mucosal surface (Figure 2). In contrast, in the non-brooding birds the crop had asymmetrical lobes that were smaller on the left and larger on the right with thin walls (Figure 3). Mostly the crop in both brooding and non-brooding birds was covered by skin only and its wall was closely attached to the skin, clavicle and sternum by loose connective tissue, so it could be palpable if the bird has just eaten otherwise, it was flat.

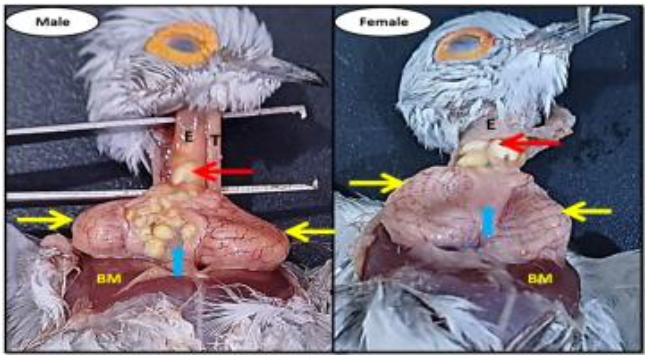

Figure 1: photograph of the brooding Diamond dove showed: crop consist of lateral symmetrical lobes right & left (yellow arrows), central lobe (blue arrow) filled with the crop milk (red arrow), esophagus (E), trachea (T), breast musculature (BM).

The inner surface of the crop in brooding birds (lactating period) was significantly thicker and well developed (Figure 4) than the inner surface of the non-brooding birds because both brooding (male and female) produced crop milk during

the lactating period; the female of brooding birds had irregularly branched folds separated by grooves, whereas the male had hexagon-shaped folds (Figure 4). While the folds were disappeared from the inner mucosal of the crop in the non-brooding birds in both sexes (Figure 5).

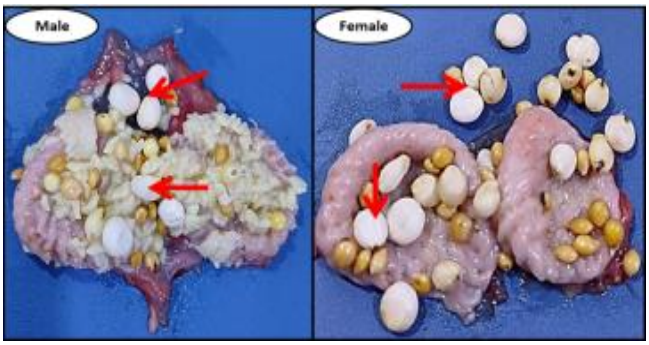

Figure 2: photograph of Diamond dove in the brooding (lactating period) showed the crop filled with crop milk (red arrows).

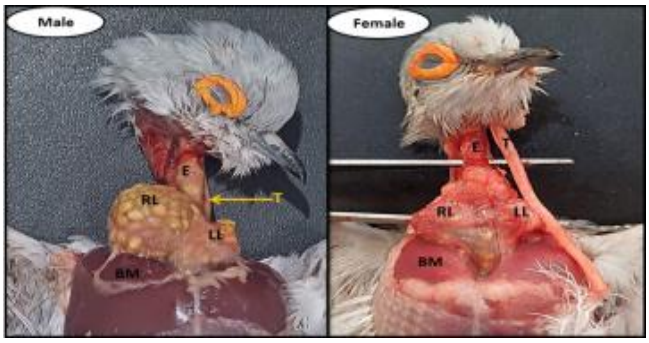

Figure 3: photograph of the non-brooding Diamond dove showed: The crop contained asymmetrical right lobe (RL) and left lobe (LL), esophagus (E), trachea (T), breast musculature (BM).

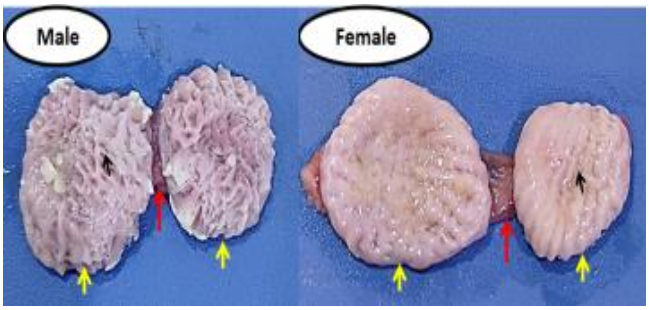

Figure 4: photograph showed the crop in the brooding Diamond dove showed: lateral symmetrical lobes right & left (yellow arrows), central lobe (red arrows), folds (black arrows).

Figure 5: photograph showed the crop of non-brooding Diamond dove (male and female).

According to the body weight, the males in the brooding and non-brooding Diamond dove weighed more than females (Table 1). The crop's weight in brooding females of Diamond dove was several times that of the brooding males; while the weights of the crops in non-brooding females and males were nearby (Table 2). Regarding crop dimensions, the statistical findings indicated that brooding males' crops were wider and longer than those of females. The crop width was roughly equal between males and females, but the crop length of non-brooding males was significantly less than that of females (Table 3).

Table 1: Comparison the body weight (gm) of diamond dove

Parameters	Body weight	T-test (P-value)
Male brooding	39.67±2.08	3.835 NS
Male non brooding	38.66±2.52	(0.782)
Female brooding	37.02±2.56	4.185 *
Female non-brooding	32.02±1.87	(0.0368)

NS: Non-Significant, * (P≤0.05).

Table 2: Comparison the crop weight (gm) of diamond dove

Parameters	Crop weight	T-test (P-value)
Male brooding	1.57±0.17	0.351 **
Male non brooding	0.37±0.01	(0.0001)
Female brooding	4.50±0.33	0.407 **
Female non-brooding	0.10±0.02	(0.0001)

** (P≤0.01).

Table 3: Comparison of the dimensions (mm) of crop diamond dove

Parameters	Length	Width
Male brooding	18.89±1.28 b	35.17±2.37 b
Male non brooding	13.28±0.71 b	36.48±2.5 b
Female brooding	15.64±0.65 a	32.14±1.87 a
Female non-brooding	22.13±2.09 b	35.92±2.15 b

Means having with the different letters in same column differed significantly.

Morphometrical in indonesian chicken

The Indonesian chicken males were excluded from the study because they do not undergo the brooding stage. Indonesian chicken's crop, was located at the cranial entrance of the thoraco-abdominal cavity, between the cervical and thoracic parts of the esophagus, appeared as a semicircular ring in both non-brooding and brooding females (Figure 6). On the crop's inner surface, both brooding and non-brooding birds had irregularly formed club-like mucosal folds (Figure 7) due to the inability of producing crop milk during the brooding period. Regarding body weight, the non-brooding females were larger than the brooding (Table 4), while in terms of crop weight, they showed comparable results (Table 5). According to the statistical findings of the crop dimensions, the brooding crop showed smaller dimensions than the non-brooding (Table 6).

Figure 6: photograph of Indonesian chicken (1- non-brooding female, 2- brooding female) showed: trachea (T), crop (C), esophagus (E).

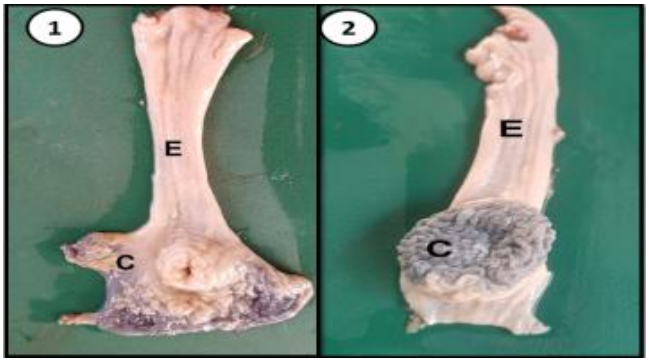

Figure 7: photograph show the inner surface of the crop in Indonesian chicken: (1) non-brooding female, (2) brooding female, showed: esophagus (E), and crop (C).

Table 4: Comparison of the body weight (gm) of indonesian chicken

Parameters	Body weight	T-test (P-value)
Female brooding	1000±32.77	84.902 **
Female non-brooding	1376±47.19	(0.0076)

NS: Non-Significant. ** (P≤0.01).

Table 5: Comparison the crop weight (gm) of Indonesian chicken

Parameters	Crop weight	T-test (P-value)
Female brooding	3.575±0.16	0.578 NS
Female non-brooding	4.011±0.27	(0.088)

Table 6: Comparison of the dimensions (mm) of crop Indonesian chicken

Parameters	Length	Width
Female brooding	2.50±0.17 c	5.00±0.32 b
Female non-brooding	28.08±2.41 a	49.78±2.72 a

Means having with the different letters in same column differed significantly.

Blood supply in diamond dove

This bird's crop received arterial blood supply from common carotid artery that originated from brachiocephalic trunk, which provided blood to the crop by two main branches (right and left) that dispersed as tiny branches over the crop's dorsal surface (Figure 8).

Figure 8: Photograph showed crop's blood supply in female Diamond dove: a- esophagus b- central attachment of crop c- lateral lobes, d- heart, e- brachiocephalic trunk f- subclavian artery g- common carotid artery (right & left), h- branches of common carotid artery, thyroid gland (yellow arrow).

Blood supply in indonesian chicken

The crop received blood supply from two sources: the left side supplied by direct branch from common carotid artery, while the right side was supplied by two branches the first branch from the right common carotid artery and the other from Subclavian artery (Figure 9).

Histological observations

According to the histological findings of the current study, the crop wall of the adult Diamond dove and Indonesian chicken was made up of four tunicae: adventitia, muscularis, submucosa, and mucosa. The wavy-looking, keratinized stratified squamous epithelium covering the

well-developed mucosal folds that make up the tunica mucosa (Figures 10-14).

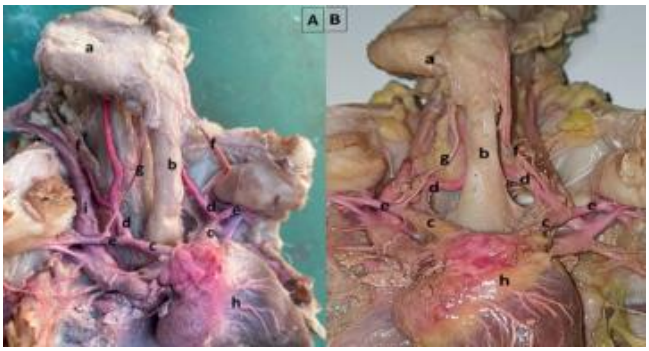

Figure 9: Photograph showed crop's blood supply in brooding female Indonesian chicken: a- crop b- esophagus c- brachiocephalic artery d- left and right common carotid artery e- subclavian artery f- branch of common carotid artery g- branch from right subclavian artery h- heart i- jugular vein.

In diamond dove the crop wall of Diamond dove during the brooding (lactating period) was very thicker in comparison with that of the non-brooding (Figure 10 and 11). The epithelium's papillary hyperplasia was growing. The first characteristic of this was the rise in the quantity and depth of rete pegs that extended into the lamina propria from the germinal (basal) layer of the epithelium. The Diamond doves' crop was distinguished by its well-developed rete pegs and superficial squamous cells with peri-nuclear hollow zone. Alongside the deeper rete pegs, the germinal layer proliferated and folded extremely far upward into the superficial epithelium (Figures 13). Moreover, the superficial layer of the epithelial cells may desquamate and sloughed and undergo fatty change to form a substance called crop milk, which play a specific role in squab growth (Figure 13). The lamina propria in both brooding and birds that did not brood were made of loose, uneven connective tissue with small amounts of collagen, reticular, elastic, and fine blood capillaries and interrupted in nature and being continuous with tunica sub-mucosa which had fewer amounts of loose irregular connective tissue and devoid of glands (Figure 12). An outer longitudinal and inner circular smooth muscle fibers were composed the tunica muscularis. The tunica adventitia was seen to have fatty tissue and loose, uneven connective tissue (Figures 10 and 11).

Indonesian chicken the crop of both brooding and non-brooding Indonesian chicken revealed that tunica mucosa presented primary and intermediate well-developed papillae of varying shapes and sizes covered with keratinized stratified squamous epithelium resulting in irregular luminal surface (Figures 14 and 15). The basal surface was also irregular because of the presence of inter-papillary pegs. Lamina propria was very well developed loose irregular connective tissue, comprised of collagen bundles along with elastic and few reticular fibers, with fine blood capillaries, muscularis mucosae was thin, it also devoid of mucous glands (Figure 14). Tunica sub mucosa was increased in thickness having more distribution of blood capillaries and small sized blood vessels. The outer circular layer and inner longitudinal layer made up the tunica muscularis, and there was tunica adventitia (Figure 14).

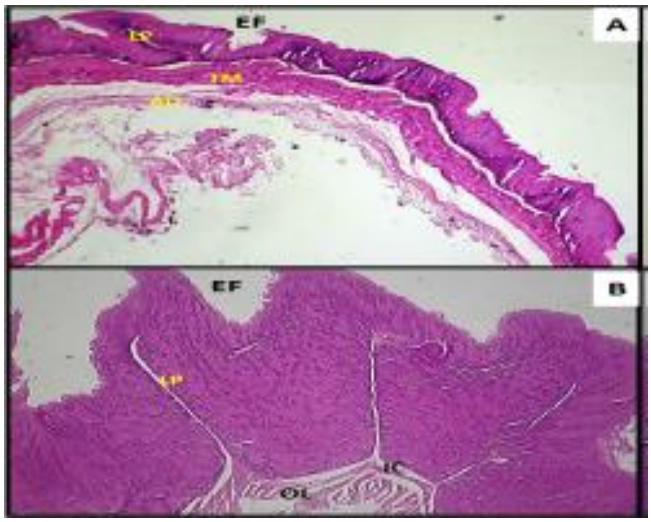

Figure 10: Photomicrograph of the crop in Diamond dove (A: non-brooding male, B: brooding male) showed: Epithelial Folds (EF), Lamina propria (LP), Tunica Muscularis (TM) consisted of: inner circular (IC) & outer longitudinal (OL) smooth m., adventitia (AD) (H&E stain: X4).

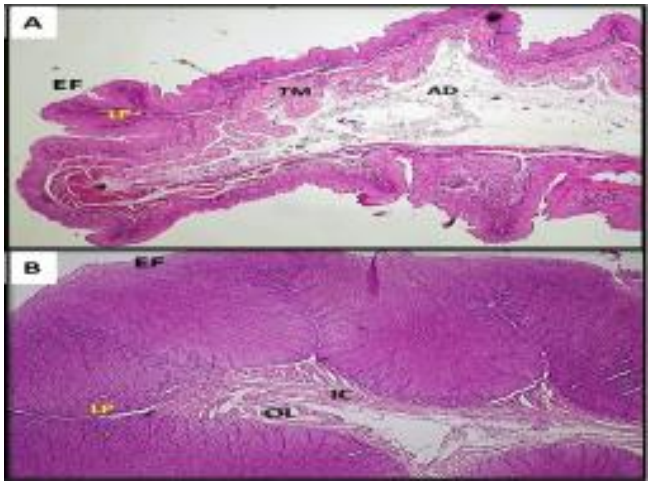

Figure 11: Photomicrograph of the crop in Diamond dove (A: non-brooding female, B: brooding female) showed: Folds (EF), Lamina propria (LP), Tunica Muscularis (TM) consisted of: inner circular (IC) & outer longitudinal (OL) smooth m., adventitia (AD) (H&E stain: X4).

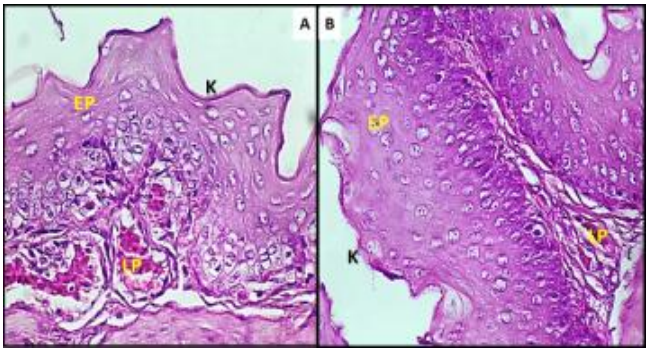

Figure 12: Photomicrograph of the crop in non-brooding Diamond dove (A: male, B: female) showed: St. Sq. Epi.(EP), Kratin (K), Lamina propria (LP), (H&E stain: X40).

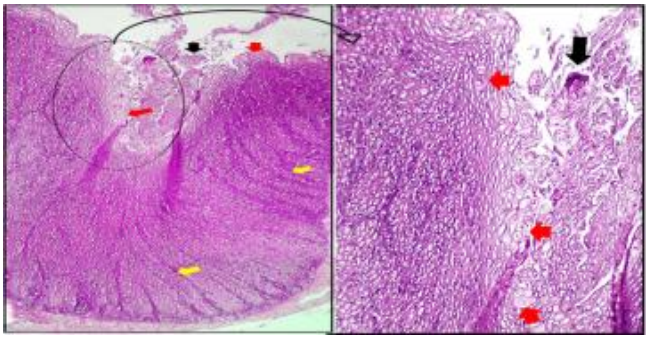

Figure 13: Photomicrograph of crop in brooding Diamond dove showed: st. sq. epithelium with well-developed rete pegs (yellow arrows) and perinuclear hallow zone (red arrows) were detected only in dove's epithelium with desquamated and sloughed epithelial cells (black arrows) (H&E stain: X4, X20).

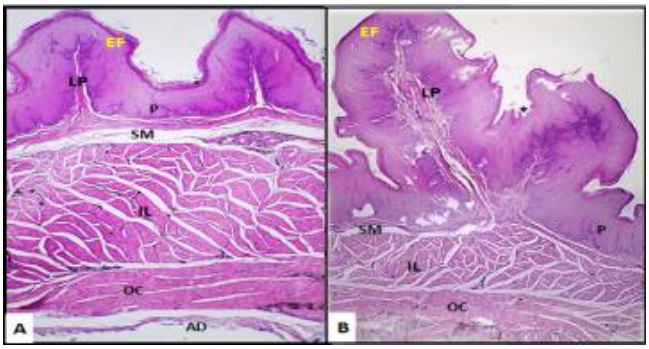

Figure 14: Photomicrograph of crop in Indonesian chicken (A: non-brooding female, B: brooding female) showed: Epithelial Folds (EF), inter-papillary pegs (P), Lamina propria (LP), Sub mucosa (SM), inner longitudinal (IL) & outer circular (OC) smooth m., adventitia (AD) (H&E stain: X4).

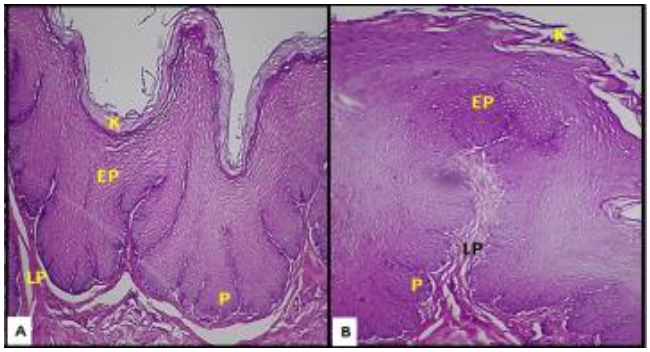

Figure 15: Photomicrograph of crop in Indonesian chicken (A: non-brooding female, B: brooding female) showed: St. Sq. Epi. (EP), Kratin (K), inter-papillary pegs (P), Lamina propria (LP) (H&E stain: X10).

Discussion

The crop represents a musculomembranous pouch, and the bottom portion is attached to the proventriculus (25-27) and further during the anatomical work, the results demonstrated that the size and shape of the crop of two birds (the Indonesian chicken and the diamond dove) appeared differently in each species. These findings were consistent with the statement made by (28,29) that the size and shape of the crop constitute species-specific features. Additionally, the crop's shape, structure, and topography vary according on the species (30). In Diamond dove: the anatomical findings of the current study in Diamond Dove demonstrated that the crop location, who stated that the crop of Galliformes and Falconiformes is located at the thoracic inlet; in Psittaciformes, it is stretched transversely across the neck (31,32). The crop shape in Diamond dove throughout the brooding period, these findings are consistent with those in common homing pigeons and wood pigeons (33-35). There were some irregular folds on the mucosal surface of the lateral lobes, but the middle lobe seemed smooth, these results concur with observations made about common wood pigeons (35) and there were two sources of the crop's blood supply: one from the subclavian artery and the other from the common carotid artery (36,37). In Indonesian chicken: the current study's anatomical findings revealed that the crop in Indonesian chickens appeared as a semicircular ring, the crop anatomy of house sparrows resembles a bag, it was situated between the esophageal cervical and thoracic segments, in close proximity to the thoraco-abdominal cavity's cranial pole (38,39). In chickens, the crop is a ventral diverticulum of the esophagus. There are asymmetrical folds on the inner surface. These results are consistent with data of common wood pigeons (35) and the two branches of the common carotid artery provided the crop with its blood supply (36).

In the current study, the histological results of adult Diamond dove and Indonesian chicken revealed that the crop wall was made up of four tunicae: mucosa, submucosa,

muscularis and adventitia. The keratinized stratified squamous epithelium covering the mucosal folds analogous to that pigeon and in wood pigeon (40-43). While epithelium was non-keratinized (35) in barn owl, (44) in laughing dove. In diamond dove, the crop wall during lactating period was very thicker in comparison with that of the non-brooding with papillary hyperplasia, peri-nuclear hollow zone and well-developed rete pegs (26) in pigeon and (45) in ducks, cattle egrets, and pigeons. The desquamated and sloughed superficial layer of epithelial cells to form crop milk (8,46,47) in pigeon. The lamina propria in both brooding and non-brooding birds was continuous with sub-mucosa and their connective tissue was of loose irregular type and devoid of glands (48) in Rose-ringed Parakeet, Rock Dove and Collared Dove), (44) in Laughing Dove and (35) in wood pigeon. The outer longitudinal smooth muscle and inner circular smooth muscle's tunica muscularis (35) in wood pigeon (49,50) in chickens and contrary to what we found in Indonesian chicken in the current study consisting of outer circular smooth muscles and interior longitudinal muscles (45) in ducks, cattle egrets, and pigeons. In Indonesian chicken: the tunica mucosa of both brooding and non-brooding birds presented primary and intermediate papillae of varying shapes covered with keratinized epithelium and the basal surface was irregular due to the presence of inter-papillary pegs. Lamina propria of loose irregular connective tissue, devoid of mucous glands (48-50) in chickens. While lamina propria contained plenty of compound tubuloalveolar glands (40) in the Linnet and Kestrel, (35) in barn owl. The outer circular layer and inner longitudinal layer of the tunica muscularis (45) in ducks, cattle egrets, and pigeons, but this result disagree to what we found in Diamond dove (35) in barn owl who found it consisted of inner circular and outer longitudinal skeletal muscle bundles.

Conclusions

The increase in crop weight and size in brooding Diamond Doves, especially in females, is due to the production and storage of crop milk, the crop undergoes physiological and structural changes, such as epithelial thickening, fatty changes, and increased cellular activity, all of which contribute to the enlargement and increased weight of the crop. In contrast, female Indonesian chickens don't produce crop milk, and their feeding role during brooding is less directly dependent on crop function. As a result, when their feeding activity may decrease and energy is redirected toward incubation, the crop reduces in size and weight due to lower food intake and less use, reflecting reduced functional demand. Crop's weight of brooding dove females was lager from males, whereas it was nearby in non-brooding. While in Indonesian chicken crop's weight was nearby in brooding and non-brooding of both sexes.

Acknowledgment

The author is grateful to the University of Dyala, College of Veterinary Medicine for all the facilities to achieve this study.

Conflict of interest

There is no conflict of interest.

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التأثير الشكلي القياسي والنسيجي للحوصلة على إنتاج حليب الحوصلة: دراسة مقارنة مع التجهيز الدموي بين الحمام الماسي والدجاج الإندونيسي في فترة الرقود وعدم الرقود

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الخلاصة

أجريت الدراسة الحالية لمقارنة الحوصلة بين الحمام الماسي والدجاج الإندونيسي من حيث الموقع والشكل والأبعاد وإمدادات الدم والتغيرات النسيجية والشكلية التي تحدث خلال فترات الرقود على البيض وزق الأفراخ وعدم الرقود، لمعرفة الفروقات التي أدت إلى تكوين حليب الحوصلة. تم جمع أربعة وعشرين طائرا ناضجا جنسيا من كلا النوعين من الأسواق المحلية وتمت تربيتها حتى الوصول إلى فترة الرقود والزق، قسمت إلى مجموعتين راقدة وغير راقدة، للفترة من أب ٢٠٢٤ الى اذار ٢٠٢٥. ظهرت الحوصلة في الحمام الماسي الراقد في فترة الزق على شكل فصين جانبيين مع فص متوسط أملس وكانت معبأة بحليب الحوصلة، واحتوى السطح المخاطي للفصين الجانبيين طيات غير منتظمة الشكل في الإناث وسداسية الشكل في الذكور وكانت ممتلئة بحليب الحوصلة، بينما في الطيور غير الراقدة كانت تركيبا شفافا يشبه الغشاء، وكان السطح الداخلي أملسا وخاليا من الطيات في كلا الجنسين. أما حوصلة الدجاج الإندونيسي فظهرت على شكل حلقة نصف دائرية وكان سطحها الداخلي يحتوي على طيات مخاطية غير منتظمة الشكل تشبه الهراوات في إناث الطيور الراقدة وغير الراقدة. كشفت النتائج النسيجية في كل من الحمام الماسي والدجاج الإندونيسي أن جدار الحوصلة يتكون من أربعة غلالات: الغلالة المخاطية، الغلالة تحت المخاطية، الغلالة العضلية والغلالة البرانية. احتوت الغلالة المخاطية على طيات مخاطية متطورة مغطاة بظهارة حرشفية طبقية متقرنة، والتي تميزت في الحمام الماسي الراقد بزيادة عدد الخلايا ذات المنطقة الشفافة المحيطة بالنواة التي كانت تتقشر وتنسلخ وتخضع لتغيرات دهنية مشكلة حليب الحوصلة، وكذلك زيادة عمق الأوتاد الظهارية المنبثقة من الطبقة الجرثومية إلى الصفيحة المخصوصة التي كانت خالية من الغدد.