A comparative histological study of ceca and rectum in common kestrel (*Falco tinnunculus*) and white-eared bulbul (*Pycnonotus leucotis*) according to their food type

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Received: 17/11/2015; Accepted: 12/1/2016

Summary

The present study is designed to investigate the histological structure of large intestine of two species of Iraqi birds which belong to two different orders which vary in their kinds of food. Adult birds common kestrel (*Falco tinnunculus*) that belong to carnivorous and white-eared bulbul (*Pycnonotus leucotis*) which belongs to omnivorous. This study was carried on 10 birds (5 common kestrel and 5 white-eared bulbul) used for histological study of the ceca and rectum in the two species of birds that include the determination of ceca and rectum, measurements of height columnar cells, villus height, and the thickness of wall tunics. Different types of stains were used including Haematoxylin and Eosin as well as special stains including Periodic Acid Schiff, Alcian blue- Periodic Acid Schiff and Van Gieson. The present result revealed that the ceca was lymphoid type and the wall of the ceca consisted of mucosa, muscularis externa and serosa whereas the submucosa was absent in the two studied birds. The mucosa in ceca was formed by along villi, with different shapes at the proximal part of the ceca while in the middle parte it changed into folds or mounds like which were flat mucosa in the proximal part of the ceca in the two studied birds. The mucosa of ceca was lined by simple columnar cells with brush border and goblet cells; this epithelium extended to line the crypts of lieberkuhn in the bases of villi and folds. While the rectum appeared as muscular tube which consist of four basic of the digestive tract tunics; tunica mucosa, submucosa, muscularis externa and serosa. The mucosa was covered by simple columnar cells with brush border and numerous goblet cells that continue to line the crypts of lieberkuhn located between the villi and that which occupied the lamina propria.

Keywords: Large intestine, Cecum common kestrel and white-eared bulbul, Rectum common kestrel and white-eared bulbul.

Introduction

The birds need many researches to investigate the structure and function of their variable organs (1). The bodies of birds need fuel to perform their daily activities where the digestive system plays its role (2). The structure of the digestive system in birds was characterized by large individual variability as a result of an adaptation of the digestive system in these birds to environmental conditions (3 and 4). Avian species possess a variety of the adaptations for digestive processing of their diets including microbial fermentation of cellulose in hind gut (5). The avian large intestine is a part of the digestive system and it consists of cecum and rectum. The ceca are out pocketing of the alimentary canal at the junction of small and large intestine (6 and 7). There were variations in the presence, shape and size of the ceca in birds, some birds have two cecum, one cecum, but in others the cecum is absent. In large number of birds the ceca are simple tubular organ have saccular or diverticular shape (8 and 9). The ceca play an important role in bacterial fermentation, site of fluids absorption, cellulose breakdown and digestion, microbial synthesis of vitamins, osmoregula -tion and immune response (10 and11). The rectum is the other part of large intestine, generally in birds is short, straight and connect the ileum with the cloaca (12). The domestic birds are considered as an important animality fortune used for different purposes and they are very important in bioprotection from both harmful insects and rodents (13). The aims of this study was to describe the histological differences of large intestine in two species of birds common Kestrel (*Falco tinnunculus*) and white-eared bulbul (*Pycnonotus leucotis*) that

belongs to different orders according to their food habits or the different kinds of food.

Materials and Methods

Ceca and rectum of ten birds (5 common kestrel and 5 white- eared bulbul) were used. They were fixed in 10% formalin solution, and routine histological technique was done dehydrated in graded ethanol and embedded in paraffin. Sections 5-6 um thick were sectioned with rotary microtome then stained with different stains include haematoxylin and eosin for general histological structure, Van Gieson for connective tissues and muscles, periodic acid Schiff reaction (PAS) and Alcian Blue-PAS pH 2.5 for mucin and nature of mucin (acidic and neutral) respectively (14). They were examined and photographed with Olympus microscope and digital camera of 14.1 mega pixel resolution power, the histomorphometrical measurements were done using the oculometer with 40X.

Results and Discussion

Cecum in Common kestrel and white-eared bulbul in the current study showed the ceca in two studied birds were present: the histological structure of the right and left cecum was similar, lymphoid type as reported by (5) in carnivorous omnivorous birds as carvidae (15) in which the ceca may play a role in immune response and as a secondary lymphatic tissue (16) but differ from that found in chicken and goose (17), in turkey and chicken (11). It was the intestinal type. These differences might be due to the differences in the function of the ceca that are related to the food habits of these birds, the intestinal type which was histologically identical to small intestine found in herbivores and most grainevorous birds in which the function of ceca include the microbial fermentation of complex carbohydrates not digested in small intestine used for absorption (18). Also it showed that the ceca consisted of the mucosa, muscularis externa and serosa whereas submucosa was absent (Fig. 1 and 2). Similar findings were observed by (19) in *Elanus caeruleus* in which the wall of ceca lack the submucosa but disagree with (11) in the cecum of chickens and goose in which the submucosa appeared as a thin layer of connective tissue contains a blood and lymph vessels.

The mucosa of the cecum was thrown up into numerous sizes and shapes of villi which appeared as finger, leaf like shaped villi at the proximal part (Fig. 1 and 2), but it become mounds or folds at the middle part (Fig. 3 and 4) then become flattened mucosa in the distal part of the cecum. This result agreed with (20) in *Turdus migratorius* and (21) in Falconiformes, in which the cecum was lymphoid type with immune and defense function. While (19) suggest that the proximal part of cecum possibly might have an absorptive function and provide an area for use symbiotic bacteria to aid in fibrous components of food digestion. Similar observation by (8) in geese. But the present result disagreed with those reported by (22) in chicken and (17) in chickens and goose. These differences might be due to the fact that in herbivorous birds that feed on low level protein and nitrogenous materials with high level of fibrous nutrient as in galliformes and grainivorous birds the surfaces area of the ceca in these birds, considered as a functional extension for the functional surface area of the small intestine (21).

The mucosa was covered by simple columnar epithelium with striated border and numerous goblet cells (Fig. 2) that continuous to line the deep crypts of lieberkuhn which were simple branched tubular glands (Fig. 5). The columnar cells have clear cytoplasm with elongated nuclei whereas the goblet cells have rounded or swollen apical part with narrow base (Fig. 6). The goblet cells gave appositive reaction with PAS stain (present mucin) in both villi and the crypts (Fig. 7), gave a positive reaction with combines Alcian-PAS which main its secreted both type of mucin acidic and neutral mucin (Fig. 8 and 9). The mean height of epithelial cells was 26.37 ±0.55µm and 26.12±0.65µm in kestrel and white-eared bulbul respectively (Table, 1). Similar observations in *Corvus frugilegus frugilegus* by (15). The goblet cells gave a positive reaction for PAS stain in both villi and crypts of lieberkuhn in the ceca of two studied birds as reported by (17) in chicken and goose. This result showed that the goblet cells secrete the mucous that play a role in protection and

lubrication of mucosa (23). Also the goblet cells gave a positive reaction with AB-PAS which indicate that the goblet cells produce both types of mucin, an acidic and neutral mucin. This result agrees with (24) in fowl.

The lamina propria along the cecum was characterized by presence numerous crypts lieberkuhn that surrounded by abundance of dark stained lymphocyte with few of lymphatic nodules (Fig. 2 and 5). Also the lamina propria extended within the villi and folds of the cecum (Fig. 2, 4, 5 and 6). The mean thickness of the lamina propria were 497.25±2.92µm in common kestrel and 500.25±1.25µm in white-eared bulbul. (Table, 1) agreed with that observed by (15) in *Corvus frugilegus frugilegus*. The presence of the lymphocyte and lymphatic nodules in the lamina propria refers to its immune function of the ceca as falconiformes, passeriformes, and ciconiiformes were lymphoid type (5).

The muscularis mucosa observed as a very thin layer of inner circular which is difficult to distinguish and thick outer longitudinal smooth muscle fibers at the proximal part (Fig. 1) but gradually become very thin layer and interrupted in the middle and distal parts of the cecum (Fig. 5). The mean thickness of muscularis mucosa was 8.53±0.16 µm in common kestrel and 8.75±0.06 µm in whit-eared bulbul (Table, 1). The mean height of villi of mucosa was 100.25±0.85 µm in common kestrel and 152.75±1.10 µm in white-eared bulbul. The lamina propria surrounded by a layer of smooth muscle fibers for empting of the contains of crypts of lieberkuhn by its contractibility, this finding was coincides (19) in Green-winged teal *Anas crecca*. There was a significant difference in the height of villi in the cecum of the two studied birds (Table, 1). The submucosa therefore the mucosa was rest on the muscularis (Fig. 1 and 10).

The muscularis externa composed of a thick layer of smooth muscle fibers oriented into outer longitudinal layer and inner circular layer which form a sphincter at the proximal part of the cecum), then gradually become thin layer of smooth muscle fibers along the middle and distal parts of cecum (Fig. 5 and 10). Between the two layers Auerbach'splexus was present (Fig. 1 and 10). The mean thickness of muscularis externa was 37.15±0.07 µm in common kestrel and 38.02±0.25 µm in white-eared bulbul (Table, 1) similar observation by (15) The present study revealed presence of cecal sphincter of two birds this result was accordance with (24) in chicken, (25) in *Pterocles alchata caudacutus* and (17) in chickens and goose.

The outer most layer of the cecum wall was the serosa that appears as a thin layer of loose connective tissue covered by the mesothelium (Fig. 1 and 10). The mean thickness of serosa was 8.15±0.06 µm and 7.80±0.18 µm and 7.80±0.18 µm in common kestrel and white-eared bulbul respectively, there were no significant differences in mean of all parameters except the mean height of villi in the ceca of two studied birds at $P<0.05$ (Table, 1) as observed by in common quail *Coturnix coturnix* (19). Rectum in common kestrel and white-eared bulbul: The present study revealed that the wall of rectum in both studied birds composed of four basic layers (Fig. 11 and 12). The mucosa in rectum is covered by a simple columnar epithelium with brush border and large numbers of goblet cells (Fig. 12, 13 and 14). These findings agreed with that of (14) in *Corves frugileus frugileus* and *Melopsittacus undulates* by (19) in Green-winged teal.

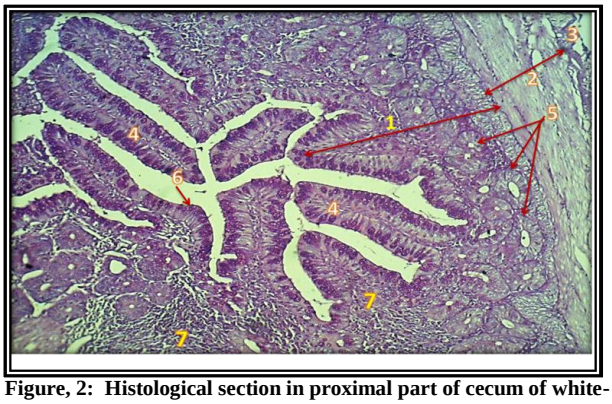
The mucosa showed a different shape of thick villi that have short finger like, tongue shaped and leaf like shape with flat apical surface (Fig. 11, 12 and 14). The epithelium covering the villi and lining the crypts of lieberkuhn was simple columnar cells with striated border with elongated or large oval nucleus and large numbers of goblet cells (Fig. 12, 13 and 14). The goblet cells showed rounded, oval shape upper part with half narrow basal part having basely located nuclei (Fig. 13, 14 and 15). The shape of the villi in two studied birds coincided with (26) in *Columba livia domestica*. The presence of villi and surface area suggest an increase in the surface area capable of greater nutrients absorption (27). The goblet cells in both villi and crypts gave a strong positive reaction with PAS stain (Fig. 13 and 16) and Alcian blue-PAS (Fig. 17). This result may be due to the production of the mucous an acidic and nuteral respectively which is a mucopoly saccharide this finding was agree with (28 and 29) in

broiler chickens this result may imply the need for increase the protection of mucosa and lubrication of faecal expulsion (30).

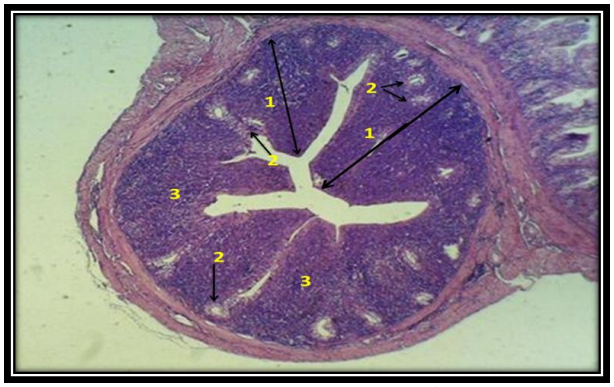
The crypts of lieberkuhn, appear as short simple branched tubular glands observed as a group of columnar cells and goblet cells around small lumen and rested on the basement membrane (Fig. 12, 15, 17 and 18). The mean height of columnar epithelium and villi were 27.0±0.20 µm and 205.26±1.25 µm respectively in common kestrel while in white-eared bulbul 27.10±0.14 µm and 208.5 ±0.64 µm respectively (Table, 2). The lamina propria composed of loose connective tissue houses the crypts of lieberkuhn with large number of lymphocyte, blood vessels and lymphatic nodules (Fig. 12 and 15) as the observation of (31) in *Columba livia* and the presence lymphatic nodules and lymphocytes act as a vigilant immunosurveillance (32) as in general carnivorous birds. The lamina propria formed the core of the villi and a bundle of smooth muscle fibers extend into the villi (Fig. 11 and 14). The mean thickness of the lamina propria was 262.75±1.10 µm in common kestrel and 264.5±1.70µm in white-eared bulbul (Table, 2). This result was parallels with (19) in Green-winged teal *Anas crecca* that are grainivorous birds and Black-Shouldered kite *Elonus caeruleus* which are carnivorous birds. The muscularis mucosa observed as a longitudinal layer of smooth muscle fibers between the mucosa and submucosa (Fig. 11). The mean thickness of muscularis mucosa were 22.45±0.10 µm and 22.57±0.23µm in common kestrel and white-eared bulbul respectively. The submucosa appeared as a thin layer of connective tissue contains blood vessels and nerve plexus. The mean thickness of submucosa was 60.25±0.85 µm and 61.85±0.48 µm respectively (Table, 2). This result agree with (33) in broilers.

The muscularis externa arranged in a thick inner circular layer and thin outer longitudinal layer of smooth muscle fibers, blood vessels and Auerbach's plexus present within the connective tissue between the two layers (Fig. 18). The mean thickness of the muscularis externa was 299.50±2.10 µm in common kestrel and 211.25±4.26 µm in white-eared bulbul (Table, 2). There were significant differences between the two studied birds as finding of (28) in Pea-fowl and (19) in *Anas crecca* and *Colurnix colurnic* birds which they are herbivorous and granivorus birds respectively. In the current study the muscularis externa in common kestrel was thicker than that in the white-eared bulbul, this finding may be due to that the solid fecal materials in the rectum of common kestrel were a large masses therefore it needs a thick muscular layer fecal expulsion also this may help in antiprestatic movement which occur in the rectum (16 and 34). The outer most layers of rectum were the serosa which observed as a thin layer of loose connective tissue covered by mesothelium (Fig. 18). The mean thickness of serosa was 25.05±0.41 µm in common kestrel and 25.12±0.31µm in white-eared bulbul. There were no significant differences in mean of all parameters except the mean thickness of muscularis externa. The serosa of the rectum in the two studied birds was loose connective tissue covered by mesothelium as reported in *Columba livia domestica* and in broiler chicken by (33).

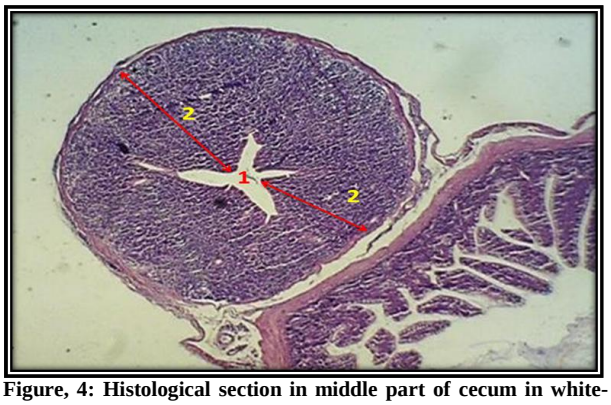
Figure, :1 Histological section in the proximal part of cecum wall in common kestrel, 1.Tunica Mucosa, 2.Tunica muscularis, 3.Tunica serosa, 4.Goblet cell, 5.Muscularis mucosa, 6.Villi, 7.Auerbach's plexus, H and E X100.



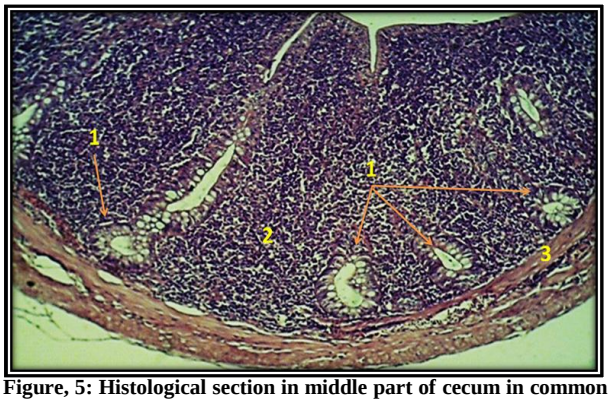
Figure, 2: Histological section in proximal part of cecum of white-eared bulbul in cecum, 1.Mucosa 2.Muscularis externa, 3.Serosa, 4.Villi, 5.Crypt of lieberkuhn, 6.Goblet cells, 7.Lamina propria, PAS X100.



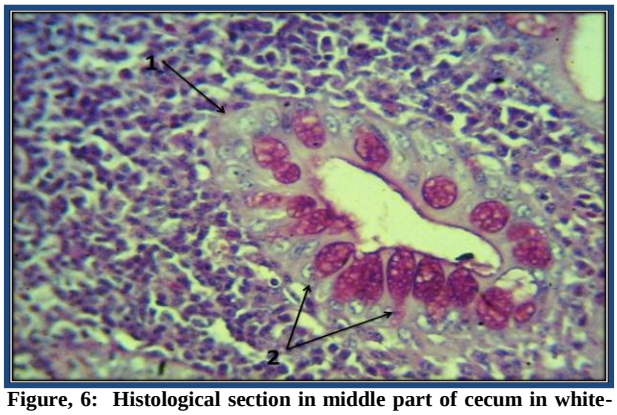
Figure, 3: Histological section in middle part of cecum in common kestrel, 1.Folds 2.Crypt of lieberkuhn 3.lamina propria, H and E X400.



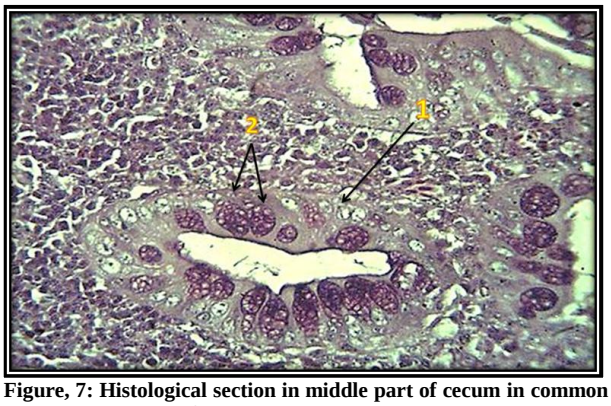
Figure, 4: Histological section in middle part of cecum in white-eared bulbul, 1.Narrow lumen, 2.Folds of mucosa, H and E X400.



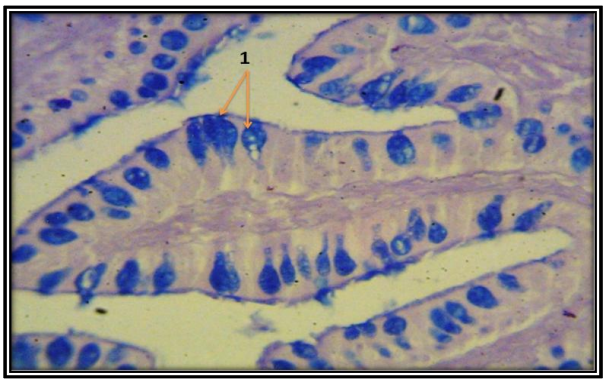
Figure, 5: Histological section in middle part of cecum in common kestrel, 1.Crypt of lieberkhun, 2.Lamina propria with diffuse lymphatic tissue, 3.Muscularis mucosa, H and E X100.



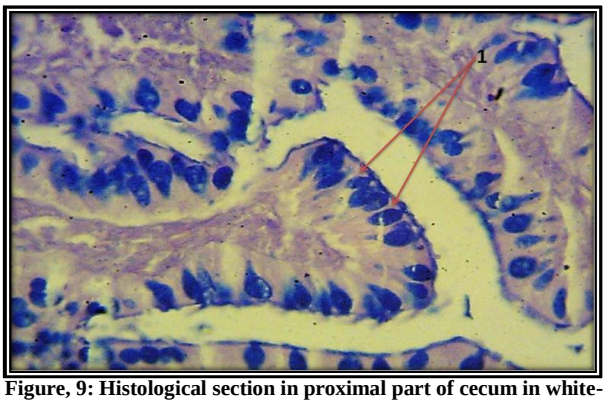
Figure, 6: Histological section in middle part of cecum in white-eared bulbul, 1.goblet cells, PASX400.



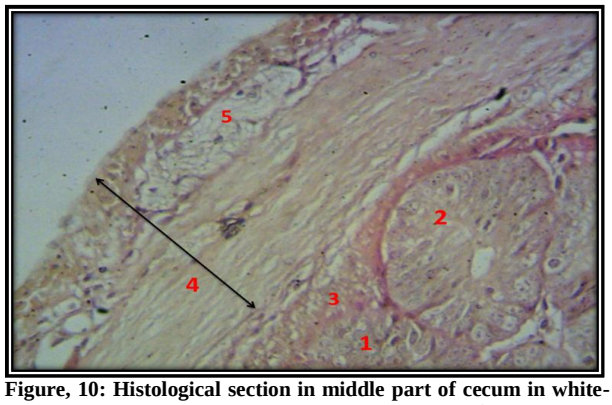
Figure, 7: Histological section in middle part of cecum in common kestrel, 1.Crypts of lieberkuhn 2. Goblet cells give positive reaction with PAS stain, PAS X400.



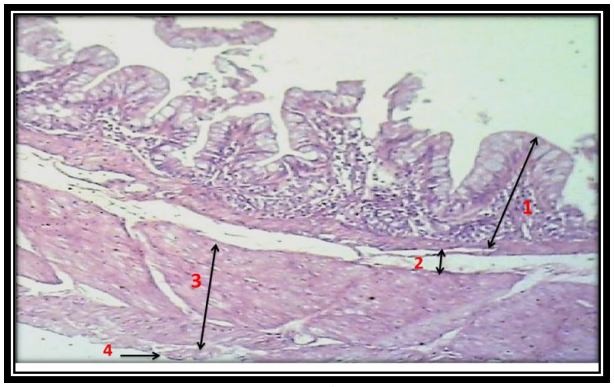
Figure, 8: Histological section of cecum in the proximal part of ceca in common kestrel, 1.Goblet cells, AB-PAS X400.



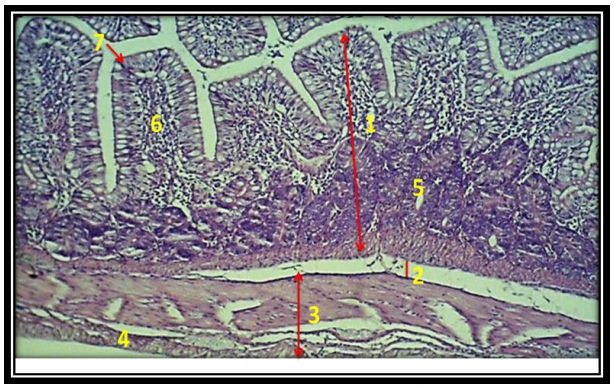
Figure, 9: Histological section in proximal part of cecum in white-eared bulbul, 1.Goblet cells, AB-PAS X400.



Figure, 10: Histological section in middle part of cecum in white-eared bulbul, 1.Lamina ppopria, 2.Crypts of lieberkuhn, 3.Muscularis mucosa, 4.Tunica muscularis 5.Auerbach's plexus, Van Geison X400.

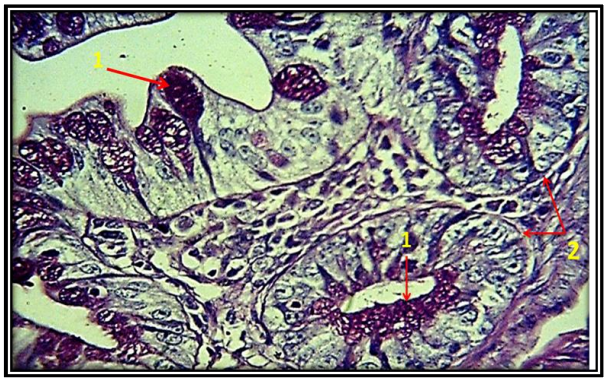


Figure, 11: Histological section of rectum in common kestrel, 1.Tunica Mucosa 2.Submucosa 3.Tunica musculars externa, 4.Serosa, H and E X400.

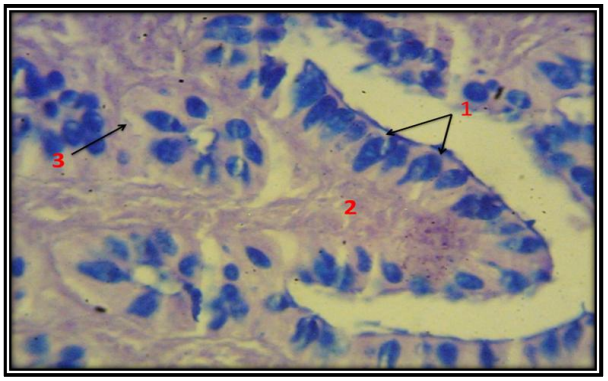


Figure, 12: Histological section in Rectum of white-eared bulbul, 1.Tunica mucosa, 2.Tunica submucosa, 3.Tunica muscularis externa, 4.Tunica serosa, 5.crypt of lieberkuhn, 6.Lamina propria, 7.Simple columnar epithelial cell, H and E X100.

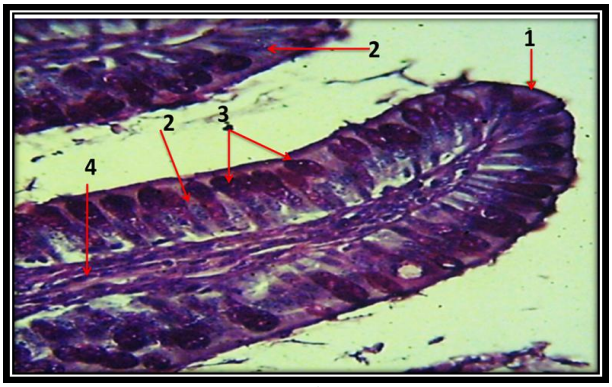
Figure, 13: Histological section of rectum in common kestrel, 1.Simple columnar epithelial cell, 2.Goblet cells, 3.brush border, 4.Lamina propria, H and E X400.



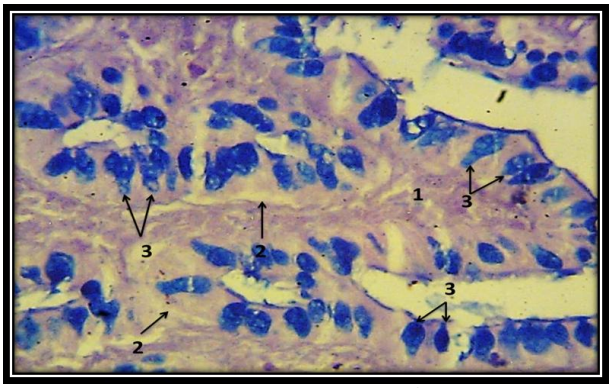
Figure, 14: Histological section in rectum of common kestrel, 1.goblet cells, 2.Crypt of lieberkuhun, PAS X400.



Figure, 15: Histological section in rectum of common kestrel, 1.Goblet cell, 2.Core of villi, 3.Crypts of lieberkuhn, AB-PAS X400.



Figure, 16: Histological section of rectum in white-eared bulbul, 1.Villi, 2.Simple columnar cells, 3.Goblet cells, 4.Smooth muscle fibers, 4.Core of lamina propria, PAS X400.



Figure, 17: Histological section in rectum of white-eared bulbul, 1.Core of villi, 2.Crypts of lieberkuhn, 3.Goblet cells, AB-PAS X400.

Figure, 18: Histological section in rectum in common kestrel, 1.Tunica Muscularis, 2.Inner circular layer, 3.Outer longitudinal layer, 4.Aurebach's plexus, 5.serosa, VG- stain X400.

Table, 1: The thickness of the four tunics of the cecum wall (micrometer) in the two studied birds numbers represents Mean±SE.

Birds species	Tunica Mucosa				Tunica Submucosa Mean±SE	Tunica Muscularis externa Mean±SE	Tunica serosa Mean±SE
	Height of epithelium Mean±SE	Thickness of lamina propera Mean±SE	Thickness of muscularis mucosa Mean±SE	Height of villi Mean±SE			
F. tinnuculus	26.37±0.55 µ	497.25±2.92 µ	8.53±0.16 µ	b 100.25±0.85 µ	–	37.15±0.07 µ	8.15±0.06 µ
P. leucotis	26.12±0.65 µ	500.25±1.25 µ	8.75±0.06 µ	a 152.75±1.10 µ	–	38.02±0.25 µ	7.80±0.18 µ

Deferent small letter vertically denote there was a significant differences at P<0.05 between the two studied of birds.

Table, 2: The thickness of the four tunics of the Rectum wall (micrometer) in the two studies birds. The numbers represents (Mean±SE).

Bird species	Tunica Mucosa				Tunica Submucosa Mean±SE	Tunica Muscularis externa Mean±SE	Tunica Serosa Mean±SE
	Height of Epithelium Mean±SE	Thickness of lamina properia Mean±SE	Thickness of muscularis mucosa Mean±SE	Height of villi Mean±SE			
F. tinnuculus	27.0±0.20 µ	262.75±1.10 µ	22.45±0.10 µ	205.26±1.25 µ	60.25±0.85 µ	a 299.50±2.10 µ	25.05±0.41 µ
P. leucotis	27.1±0.14 µ	264.5±1.70 µ	22.57±0.23 µ	208.5±0.64 µ	61.85±0.48 µ	b 211.25±4.26 µ	25.12±0.31 µ

Deferent small letter vertically denote there was a significant differences at P<0.05 between the two studied birds.

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دراسة نسجية مقارنة للأعورين والمستقيم لطائر العوسق (*Falco tinnunculus*) والبلبل أبيض الأذن (*Pycnonotus leucotis*) حسب نوع الغذاء

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الخلاصة

صُممت الدراسة الحالية للتعرف على التركيب النسجي للأمعاء الغليظة لنوعين من الطيور العراقية وهما طائر العوسق وهو من آكلات اللحوم والبلبل أبيض الأذن وهو من القوارت. استعملت 10 طيور (5 طائر العوسق و5 بلبل) لغرض الدراسة النسجية لكل من الأعورين والمستقيم في كلا النوعين من الطيور وقد شملت الدراسة قياس معدل ارتفاع الخلايا الظهارية المبطنة لهما، ومعدل ارتفاع الزغابات وقياس سمك الطبقات المكونة لجدرانها. استعملت خمسة أنواع من الصبغات وهي صبغة الهيماتوكسلين والأيوسين وبيرودك أسد شيف وفان-جيسون وزرقة الألسيان-بيرودك أسد شيف. أظهرت نتائج الدراسة النسجية ان الأعور في الطيور المفحوصة يحتوي على النسيج اللمفي. وأشارت النتائج إلى أنّ جدار الأعورين يتكون من ثلاث طبقات وهي الطبقة المخاطية والعضلية الخارجية والمصلية وعدم وجود الطبقة تحت المخاطية . كما أظهر مخاطية الأعورين بأنها تتكون من زغابات طويلة وأشكال مختلفه في الجزء الداني للأعورين أما المنطقة الوسطية فلم تلاحظ زغابات نموذجية إنما ظهرت المخاطية بشكل طيات تشبه التلال والتي أصبحت شكل مخاطية مسطحة عند الجزء القاصي للأعورين ولكلا الطائرين المدروسين كما ظهرت مخاطية الأعورين بأنها مبطنة بخلايا عمودية بسيطة مكونة من الخلايا الظهارية العمودية المزودة بحافة الفرشاة وكذلك الخلايا الكأسية وامتدت هذه الظهارة لتبطن خبايا ليبركن الواقعة عند قواعد الزغابات وقواعد الطيات. ظهر المستقيم بشكل أنبوب عضلي مكونة من الطبقات الأربعة المخاطية وتحت المخاطية والعضلية والمصلية ولوحظ بأن المخاطية مغطاة بظهارة عمودية بسيطة مكونة من الخلايا العمودية المزودة بحافة الفرشاة والخلايا الكأسية التي ظهرت بشكل غزيرفي مستقيم كلا النوعين. امتدت الظهارة العمودية البسيطة لتبطن خبايا ليبركن الواقعه عند قواعد الزغابات التي اشغلتا معظم الصفيحة اللبادية. لم تختلف أشكال الزغابات المكونة لمخاطية المستقيم بين الطيرين وظهرت بأشكال مختلفة حيث ظهرت معظمها بشكل زوائد سميكة وعريضة ذات نهايات مسطحة كما ظهرت بشكل زوائد أصبعية وأخرى بشكل أوراق الشجرة .

الكلمات المفتاحية: الامعاء الغليظة، أعور طائر العوسق والبلبل أبيض الأذن، مستقيم طائر العوسق والبلبل أبيض الأذن.