A comparative anatomical, histological and histochemical study of small intestine in Kestrel (*Falco tunniculus*) and white eared bulbul (*Picnonotic leucotis*) according to their food type

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Summary

This study was conducted on 30 birds (15 birds for each type) divided as 10 birds for each part of study. Anatomical part revealed that the small intestine in both birds kestrel (*Falco tinniculus*) and white eared bulbul (*Picnonotic leucotis*) formed from 3 segments; duodenum, jejunum and ileum with no clear demarcation line between them. In kestrel the Meckel's diverticulum appeared as small projection to separate between jejunum and ileum. Both ratio of intestinal length to body length and of intestinal weight to body weight was higher in bulbul than those in kestrel. Histological study showed that the wall of all three parts of small intestine was composed of the same histological layers; these are mucosa, submucosa, muscularis and serosa. There was almost similarity in structure of these tunics but significant differences in several Histomorphometric measurements of each tunica. Goblet cells were more abundant in all parts of small intestine of bulbul than those in kestrel and there was a gradual increasing in the number of these cells toward the end of intestine of both birds. Histochemical part of this study appeared that in villi and crypts of all small intestinal segments of both birds the goblet cells secrete neutral mucin in nature because it showed negative reaction to Alcian blue stain and positive to PAS stain.

Keywords: **Small intestine, Anatomical, Histological, Kestrel, White eared bulbul.**

Introduction

The birds' bodies need fuel to go about their daily activities, and this is where the digestive system plays its role (1). Small intestine is the first site concerned with breakdown of enzyme, in addition to absorption of carbohydrates, fatty acid, and amino acids (2). So it may be do a substantial role in augment the digestive rate and diminishing digesta load. Morphological modulations of small intestine consider an important needful specialization for quick breakdown of ingesta and absorption of its contents (3). Kestrel is a characterized member of the falcon family, and one of the most popular birds of prey, feed on different insects types or tiny mammals such as mice, young ground squirrels and sometime feeds even small birds (4). While (pycnonotus leucotis) which commonly named White-eared Bulbul belongs to Passeriformes order, class: aves, that is the most popular garden birds .it is feed on fruits, grass and small lizards occasionally (5). Comparative anatomy helps to explain how organisms service, histological structure and some histochemical characteristics of small intestine in two different birds kestrel (*Falco tinniculus*) and white eared bulbul (*Picnonotic leucotis*) according to their food type.

Materials and Methods

Thirty (30) healthy birds (15 kestrel and 15 white eared bulbul) were obtained from birds owner in local market (Baghdad) without attention to their sex (adult male or female), and then divided into 3 parts for anatomical, histological and histochemical studies (10 birds for each study, 5 kestrel and 5 bulbul). The anatomical observations which used in this part of study are: Shape, position and relationship of small intestine, ratio of small intestine weight to body weight (IW/BW) and ratio of small intestine length to body length (IL/BL). Histological observations involved general description of each segment of intestinal wall in addition to several histological parameters were focused on, using the ocular micrometer.

The Mucosal thickness: distance from the apex of villi to the margins of submucosa, height of villus that measured from the tip of the villi to the villus crypt junction, depth of crypts (It is defined as the depth of the invagination between adjacent villi which measured from the villus-crypt axis to the base of specific crypt) and the Ratio of goblet cells number per each 100 epithelial cells in villi. Histochemical study was designed to detect the chemical properties of goblet cells secretion. Two types of stains were used to achieve this purpose: Periodic Acid Schiff (PAS) stain to detect the neutral mucin and Alcian blue (AB) stain to differentiate acidic mucins types (6).

Results and Discussion

Anatomical part (Shape and position): The present results revealed that the small intestine in both birds (kestrel and bulbul) was relatively short and formed from many loops that occupied the most caudal area of abdomen, exactly to the right side of abdominal cavity and right to the proventriculus and gizzard. It composed of three segments that named; duodenum, jejunum and ileum with no clear anatomical demarcation line between them (Fig. 1, 2, 3 and 4). The duodenum of kestrel formed the first loop of small intestine, like comma or incomplete U-shape, pink in color; arise immediately after ventricle-duodenal junction and extend caudally right to the gizzard (Fig. 1 and 3). While the duodenum of bulbul is light pink color organ, closely attached to the right abdominal wall and to the gizzard at very distinguished ventriculo-duodenal junction, covered partially by the right lobe of liver. It is U-shaped part shows descending or ventral limb and ascending or dorsal limb that bind together by small peritoneal fold which holdout the single lobed pancreas (Fig. 2 and 4).

The present results were in agreement with the findings of (7 and 8) but incompatible with what reported by (3) in African Pied Crow (*Corus albus*) that the duodenum is situated in the caudal part of the left side in abdominal cavity. The jejunum in kestrel is dark pink part organized in sequential narrow U-shaped loops, directed caudally. Meckels diverticulum appeared like blind segment or small pouch about 1 cm in length or less, (Fig. 3). The ileum lies on and attached to floor of abdomen, directed caudally to end at its junction with the large intestine (Fig. 1 and 3). In bulbul there are no demarcation lines that separate anatomically between jejunum and ileum. They appear as single loop, left and closely attached to the duodenum (Fig. 2 and 4). These findings in both birds intersected with what reported by (9) in pigeons and (3) the jejunum and ileum in indigenous ducks and his findings were similar to present found (10). The intestine of kestrel comprised several loops especially in jejunum that give it spiral like appearance and that may be because acclimation of this bird type to employment largest space in abdomen, as well as to exposed the food to large surface area of absorption that aid in acceleration and increasing digestive rate (3) so the digesta load will be minimized and that help the birds to manoeuvrability and takeoff through flying. The cost of flight is increased with large load of digesta and the manoeuvrability and takeoff could be diminished with heavier masses (11).

Anatomical morphometric measurements: The mean ratio of (IL/BL) in bulbul was (65.1%) which is larger and differs significantly (P<0.01) from that of kestrel (48.4%) although the mean length of intestine of bulbul (12.2± 0.1 cm) was shorter than that in kestrel (17± 0.5 cm) (Table, 1). This finding was harmonious with findings of (12) who suggested that in carnivores' birds the intestine be shorter than it in herbivores or frugivorous. Otherwise the mean ratio of (IW/BW) in kestrel was (3.2%) which is significantly lesser than this ratio in bulbul (6.4%) regardless of the mean weight of intestine of kestrel (4.7 ± 0.3 gm) is higher in value than the mean of same ratio in bulbul (1.9 ± 0.1 gm). Finding in (Table, 2) was supported by (13 and 14) whom noticed that the insectivores and carnivores birds have shorter and lighter intestine than granivores and herbivores species because the extremely digestible nature of their food which composed few fibers amount.

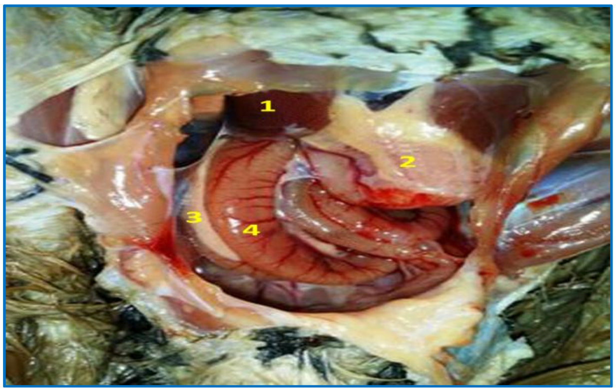
Table, 1: the body length (cm), small intestine length (cm) and the ratio of (IL/BL) in kestrel and bulbul.

Parameter / Bird	Body length Mean± SE	Intestine length Mean ± SE	Ratio of intestinal length / body length %
Kestrel	35.2± 0.7*	17 ± 0.5*	48.43%*
Bulbul	18.7 ± 0.2	12.2 ± 0.1	65.10%

Table, 2: the body weight (gm), small intestine weight (gm) and the ratio of (IW/BW) in kestrel and bulbul.

Parameter / Bird	Body weight Mean ± SE	Intestine weight Mean ± SE	Ratio of intestinal weight/body weight%
Kestrel	149.3 ± 2.1*	4.7 ± 0.3*	3.2%*
Bulbul	30.5 ± 1.8	1.9 ± 0.1	6.4%

The numbers represent the mean ± standard error.
***= There was a statistically significant difference between the two bird (P = <0.01).**



Figure, 1: Shape and position of small intestine of kestrel (1) liver right lobe ,(2) gizzard, (3) pancreas, (4)intestine.

Figure, 2: Shape and position of small intestine of whited eared bulbul (1) liver right lobe, (2) gizzard, (3)small intestine.

Figure, 3: parts of small intestine in kestrel (1) Descending limb,(2) Ascending limb, (3) pancreas, (4) duodenum, (5) jejunum, (6) Meckel's diverticulum.

Figure, 4: parts of small intestine in white eared bulbul (1) proventriculus, (2) gizzard, (3) ventriculo-duodenal junction, (4) duodenum, (5) jejunum, (6) ileum, (7) pancreas.

The remarkable increasing of length and weight of small intestine in bulbul may be due to the high contents of fibers in their diet which need more time and largest area to digest in contrast to easy digestible diet of kestrel which comprised lesser amount of fibers. The longer digestive tracts observed in herbivorous vertebrates are thought to increase the volume of food that can be ingested per feeding bout and lead to lengthier retention times of refractory compounds in the alimentary canal, thereby increasing exposure of ingesta to the battery of digestive processes in the gut (15 and 16). Such an increase in exposure can increase the efficiency by which an herbivorous diet can be digested (16).

Histologically each segment of small intestine was involved four tunica; mucosa, submucosa, muscularis and serosa. In both birds the duodenal mucosa formed firstly from epithelia (simple columnar epithelial cells) (Fig. 5) that arranged in finger projection like villi which were shorter in each segment of small intestine of bulbul (Table, 3) as well as more crowded and more branched (Fig. 6) and secondly the lamina propria that occupied by crypt of lieberkhun which were deeper in bulbul and arranged in about 3-5 layers with no muscularis mucosa (Fig. 6). Different number of goblet cells was invaded epithelium (Fig. 5). These cells were higher in number inside each villus for all intestinal segments of bulbul and increased gradually toward the end of intestine (Table, 4). The tunica submucosa was reduced into thin layer of loose connective tissue which indented between the epithelial crypts and tunica muscularis of both birds. The tunica muscularis was thinner in bulbul and has composed of double layers of smooth muscle fibers that very thick inner circular layer and thine well vascular outer layer which showed auerbach plexuses. The tunica serosa of both birds was thin layer of loose connective tissue covered by mesothelium (Fig. 6 and 7). The high percentage of goblet cells that found in duodenum of white eared bulbul could be because the nature of its food components which comprised high level of fibers that is difficult in digestion unless presence of goblet cells. Jejunum and Ileum in both birds (3) were histologically composed of the same layers to that of duodenum (Fig. 8 and 9) and the differences were restricted on the histo-morphometric parameters (Table, 5 and 6).

Table, 3: Mucosal thickness, villus height, depth of Crypt of duodenum in kestrel and bulbul.

Birds	Mucosal thickness (µm) Mean ± SE	Villus height (µm) Mean ± SE	Depth of Crypt (µm) Mean ± SE
Kestrel	1099.6 ± 54.9*	660 ± 13.2*	128.5 ± 8.06*
Bulbul	1481 ± 70	885 ± 19.9	522.1± 14.6

Table, 4: Ratio of goblet cells number pear 100 epithelial cells in villus in duodenum, jejunum, ileum of kestrel and bulbul.

	Birds	
Intestinal segment	Bulbul %	Kestrel %
Duodenum	24%	28%
Jejunum	28%	41%
Ileum	47%	50%

The numbers represent the mean ± standard error.
***= There was a statistically significant difference between the two bird (P = <0.01).**

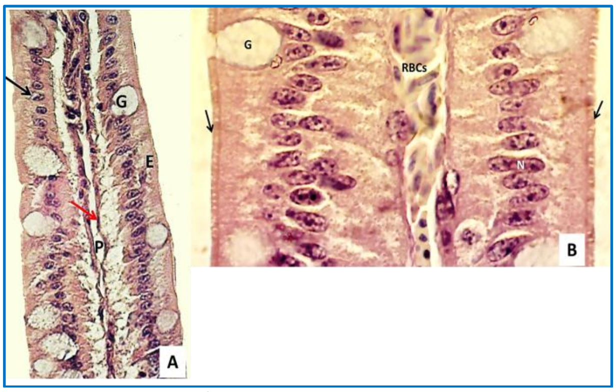
Table, 5: Mucosal thickness, villus height, depth of Crypt of jejunum in kestrel and bulbul.

Birds	Mucosal thickness (µm)	Villus height (µm)	Depth of Crypt (µm)
Kestrel	945.2 ±16.4*	705 ± 22.1*	83.6 ± 2.4*
Bulbul	894.03 ± 9.8	456 ± 34.1	409.3± 12.6

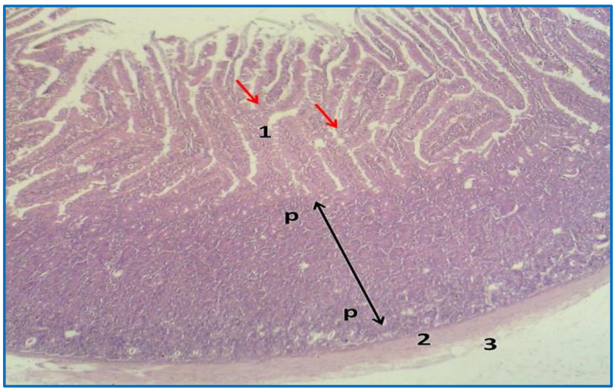
Table, 6: Mucosal thickness, villus height, depth of Crypt of ileum in kestrel and bulbul.

Birds	Mucosal thickness (µm) Mean ± SE	Villus height (µm) Mean ± SE	Depth of Crypt (µm) Mean ± SE
Kestrel	956.7±9.4*	874±13.2*	87±2.9*
Bulbul	695.1±14	372±1.2	300.1±16.4

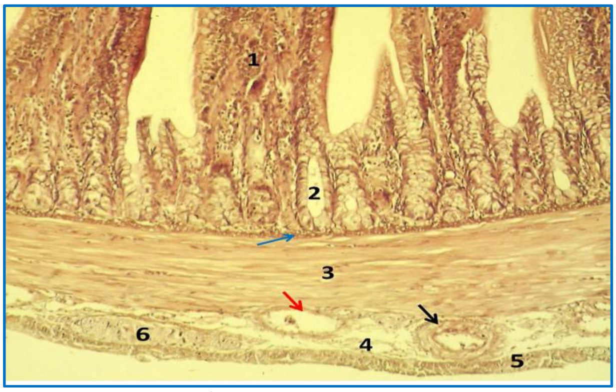
The villi of ileum in both birds (sparrow hawk and dove rock) were covered with columnar cells have oval nuclei infiltrated with goblet cells which gradually increased as cranio-caudal direction (17). This result was agreed with present study in both birds. In present work, Crypt of lieberkhun in lamina properia of ileum in bulbul appeared deeper 3.5 times more than that in kestrel that may be because the type of food of bulbul which contained high ratio of fibers that make it consider as rough diet. (18) Reported that good amount of fibers in diet of chicken prefer height of duodenal villi in addition to depth of ileal crypts.



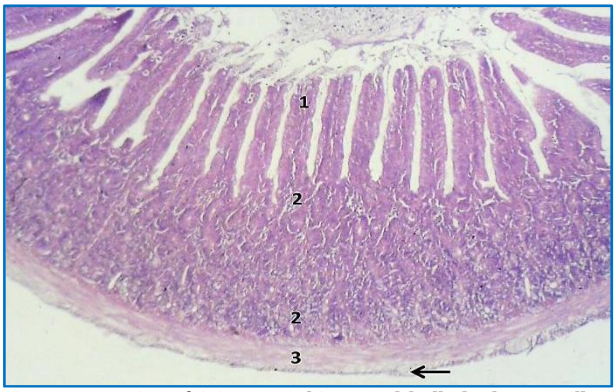
Figure, 5: Section of villus (kestrel). Micrograph (A) shows: Goblet cells (G), lamina propria (P), Nucleus of epithelial cells (Black arrow), and nuclus of fibrocyte (Red arrow). H&E stain, 400X. Micrograph (B) shows: Goblet cells (G), Red blood cells (RBCs), Nuclus (N), and brush border (arrows). H and E stain. 1000X.



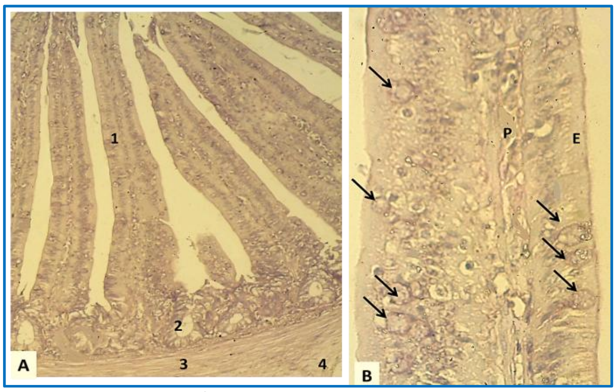
Figure, 6: Section of duodenum (white eared bulbul) shows: Villus (1), tunica muscularis (2), tunica serosa (3) lamina propria (P) and branches of villi (Red arrows). H and E stain 100X.



Figure, 7: Section of duodenum (Kestrel) shows: Villus (1), epithelial crypts (2), tunica muscularis inner layer (3), tunica muscularis outer layer (4), tunica serosa (5), aurbach plexuses (6), submucosa (blue arrow), small artery (Black arrow) and small vein (Red arrow). H and E stain, 400X

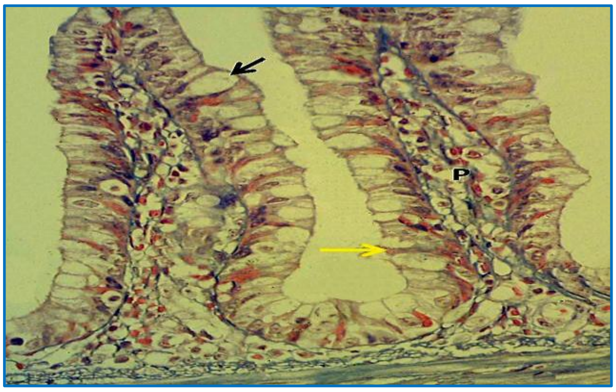


Figure, 8: section of jejunum (white eared bulbul) shows: Villus (1), lamina propria (2), tunica muscularis (3), tunica serosa (Arrow). H and E stain, 100X.



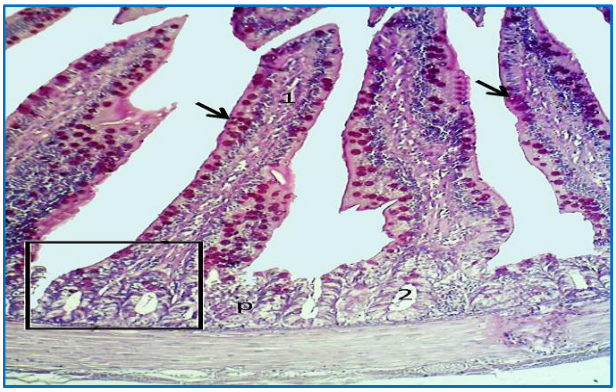
Figure, 9: Section of ileum (Kestrel), (A) Micrograph shows: Villus (1), crypt (2), inner circular layer of tunica muscularis (3) and outer longitudinal layer of tunica muscularis (4). H and E stain, 400X. (B) Micrograph shows: Epithelium (E), Lamina propria (P) and much of goblet cells (Arrows) 1000X.

The histochemical results of the three parts of small intestine in kestrel and white eared bulbul have been showed that the mucous secreting goblet cells within epithelium of villi and the epithelial crypts were negatively reacted to Alcian blue stain (Fig.10), that mean these cells have been secreted not acidic mucuopolysaachrides.



Figure, 10: Jejunum of Kestrel shows: negative Alcian blue reaction of goblet cells (Black arrow), epithelial cells (yellow arrow) and lamina propria (P), 1000X.

Whereas the results of PAS stain of goblet cells in the epithelium of villi at these three parts of both birds have been given positive reaction and appeared red in color owing to the existence of neutral mucins, while the goblet cells of epithelial crypts were showed variable reactions (Fig. 11). The epithelial cells of the intestine were rich in mucopolysaccharide, although which were a weaker intensity. These finding were differ from what mentioned by (10) on small intestine of indigenous duck in which (10) reported that the secretion of goblet cell had positive reaction to PAS stain (red color) and positive reaction to AB stain (blue color) at both (1.0) and (2.5) pH.



Figure, 11: duodenum of bulbul shows the goblet cells within villi showed positive reaction to PAS stain (arrows) and the negative reaction within crypts (2), lamina propria (p). 400X.

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دراسة تشريحية، نسجية وكيمياء نسجية مقارنة للأمعاء الدقيقة في طائر العوسق (*Falco tunniculus*) وطائر البلبل أبيض الأذن (*Picnonotic leucotis*) اعتماداً على نوع الغذاء

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الخلاصة

صممت هذه الدراسة لإظهار الاختلافات في الشكل التشريحي والتركيب النسجي وخصائص الكيمياء النسجّية بين نوعين من الطيور هما طائر العوسق وهو أحد أنواع الصقور، وطائر البلبل أبيض الأذن وهو من فصيلة البلابل اعتماداً على إختلافها في نوع الغذاء. لتحقيق أهداف هذه الدراسة إستُعمل 30 طائراً (15 طائر لكل نوع) مقسّمة إلى 10 طيور لكل جزء من الأجزاء الثلاثة للدراسة. بيّنت الدراسة التشريحية أن الأمعاء الدقيقة في كلا الطائرين (طائر الوسق و طائر البلبل أبيض الأذن) مكونة من ثلاث قطع هي العفج والصائم واللفائفي مع عدم وجود خطوط تشريحية واضحة للفصل بين هذه الأجزاء. في العوسق ظهر رتج ميكل كبروز صغير يفصل بين الصائم واللفائفي. في البلبل كانت كلا النسبتين (نسبة طول الأمعاء إلى طول الجسم ونسبة وزن الأمعاء الى وزن الجسم) ذات قيمة أعلى مما هي في العوسق. أوضحت الدراسة النسيجية إن جدار الأجزاء الثلاثة للأمعاء في كلا الطائرين متشابهة ويتكون من الغلالة المخاطية وتحت المخاطية والعضلية والغلاله المصلية. كما أوضحت الدراسة إن هنالك تشابهاً كبيراً في تركيب هذه الغلالات ولكنها تختلف معنويا في القياسات النسيجية الخاصة بكل منها. أظهرت الخلايا الكاسية كثافة عالية في أجزاء الأمعاء الدقيقة للبلبل مقارنة بالعوسق وإن أعداد هذه الخلايا يزداد تدريجياً بإتجاه نهاية الأمعاء في الطائرين كليهما. في جانب الكيمياء النسجي، أظهرت النتائج أنّ الخلايا الكأسية في زغابات وخبايا الأجزاء الثلاثة للأمعاء الدقيقة في الطائرين كليهما تفرز مخاطا متعادلا نتيجة إظهار تفاعل نسجية الأليشان الأزرق في حين أظهر هذا المخاط رد فعل إيجابي عند معاملته بملون شف.

الكلمات المفتاحية: الأمعاء الدقيقة، تشريحية، نسجية، العوسق، بلبل أبيض الأذن.