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Some Histo-morphometric and histochemical comparison aspect of the duodenum in Collard Dove (*Frivaldszky*), Ruddy Shelduck (*Pallas*) and Owl (*Otus Scors brucei*) in south Iraq

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Abstract

The present investigation was aimed to study the histomorphological and histochemical finding of the structures wall of the duodenum of Collard, Owl and Ruddyin south Iraq. Fifteen healthy birds were collected from local suppliers from Al-Samawah city during January to May 2016. Birds were euthanized, dissected and then specimens were processed for histological and histochemical staining techniques. Histologically, the duodenum was lined by simple columnar with goblet cells the villi get long leaf-shaped villi that were arranged in a zig-zag pattern in collard. But in the owl, the villi showed blunt apical part and wide basal part, while in the ruddy it had a finger like projections. Tunica serosa was thin layer of areolar loose connective tissue. Histochemical findings showed that the columnar cells were positive reacted with the PAS stain, whereas the goblet cells were strongly reacted with this stain. On applying the combined PAS-AB (pH 0.4), the wall showed epithelial cells stained negatively with this stained while the goblet cells gave strong positive reaction (dark blue). The mucosal lining revealed no response toward the mercuric bromophenol blue staining in collard, owl and ruddy, while, the tunica muscularis was constituted by layers of were positively reacted with this stain in collard, owl and ruddy. All birds the columnar cells of small intestine gave the positive reaction with PAS, PAS-AB. In fact, the stain is an indicator for sulfated acidic mucin which are very important in digestion and absorption and subsequent body growth of the bird.

Keywords: Owl, Ruddy Shelduck, collard dove, duodenum, histochemistry, mucosa, histology

1. Introduction

There are (8948) living species of birds are assigned to (27) orders, the number of species within an order varies greatly, the largest order is the Passeriformes, which contains [5243] species.

The smallest orders are the Struthioniformes which contain one species. The Anseriformes which, contains five species, one of them, are ducks ^[1]. The Ruddy, one of the ducks species from the Anseriformes order, this birds accommodation for water living, and its flesh are very delicious from any water fowl flesh, for this reason become as a hunters target^[1]. Numerous studies discover how the dietary habits have affected the morphological features and subsequently the physiological activities of the digestiveorgans in birds ^[2].

The wall of small intestine contained three tunics viz. tunica mucosa, tunica muscularis and tunica serosa. The tunica submucosa was absent as reported earlier by Hodgesand McLelland ^[3; 4]. But the Shaymaa and Hamdy reported that the intestinal tract showed the usual consists from the four layers throughout viz, mucosa, submucosa, muscular layer and serosa, but there were marked regional differences as to their diameter and composition ^[5]. The duodenum is U-shape; it follows the greater curvature of the gizzard from its dorsal surface. It consists of pars descending, flexuraduodeni and pars ascending as well as flexuraduodeno-jejunalis. All parts are attached to the pancreatic lobes by the pancreatico- duodenal ligament ^[5].

Some reports have been published on the morphology and morphometry of the small intestine of some species of birds ^[6, 7, 8], but there is a paucity of information on the histo-morphometric and histochemical of the duodenum in the Collard Dove, Ruddy Shelduck and Striated Scope Owl. According to our knowledge there were no local studies conducted to study histomorphological and histochemical aspects of the wall of the duodenum of in Collard Dove

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(Frivaldszky), Ruddy Shelduck (Pallas) and Owl (*Otus Scors brupei*) in south Iraq.

2. Materials and Methods

A total of 15 birds (5 Collard Dove, 5 Ruddy Shelduck and 5 Owls) aged 1-2 years were used that collected from Al-Samawah city during January to May 2016 in this study. Birds were weighed before being euthanized prior to its dissection with an intravenous injection of sodium pentobarbitone (80 mg/kg for the Owl & 200 mg/kg for Collard Dove and Ruddy Shelduck) [9]. Duodenum were isolated from birds at the duodenu-gizzard junction and separated from mesenteries and pancreatic-duodenu ligaments, and gently straightened on a flat plane. Measurements such as length, diameters to the collected specimens were conducted in millimeters by using the electronic Vernier scale and the weights measured in grams by using sensitive digital balance. For histological study three caeca were cut.

The specimens were immersed in 10% neutral buffered formalin and Bouin's solution for 2 days, after well fixation the specimens is dehydrated by passing them through a series of ascending ethanol each for two hours [70%, 80%, 90%, 95% and 100%] and then specimens were cleared in xylene for one hour after that embedded in paraffin wax and then the blocks were sectioned serially at 6µm thickness and stained with either one of the following stains [10].

Mayer's Hematoxylin and Eosin routine stain for general features identification and Masson trichrome stain for the staining of the collagenous and smooth muscle fibers. To conduct the histochemical study used the Periodic acid Schiff (PAS) for the neutral mucin, PAS-AB pH 0.4 determination of the acidic mucin [11] and Mercury Bromophenol blue (MBB) for protein detection [12].

2.1 Statistical analysis

The measurement of length of each layer by ocular micrometer to histometric analysis and all numerical values were expressed as the mean ± standard error (SE). The significance level was set at $p < 0.05$.

3. Results and Discussion

3.1 Morphological aspect

The duodenum consisted of descending and ascending limbs forming U-shaped tube called duodenal loop. The pancreas observed between these limbs. The U-shape of duodenum in the current birds was commonly observed in the other avian species [1; 12].

Measurements such as organs relative weight, length and diameter of duodenum are well listed in (Table 1). There were no significant differences in the relative weight of the duodenum between male and female. The absolute weight of duodenum was higher in ruddy than both collard and owl due to the higher body weight of the ruddy. However, there were no significant differences in the relative duodenum weight to the body weight between collard, owl and ruddy in the current study.

There was no significant difference in the lengths of duodenum between males and female in the studied birds. The length was significantly longest in ruddy compared to both collard and owl and in another aspect, the owl duodenum appeared shorter than that of collard. The findings of shorter duodenum in owl and longer in ruddy and collard were in accordance with the records of Olsen *et al.*, in carnivorous and granivorous avian species, respectively [13].

3.2 Histological aspects

The organ showed microscopically the four common known layers of a tube organ: tunica mucosa, tunica submucosa, muscularis Externia and tunica serosa (Fig. 1, 2, 3). These four layers appeared in the duodenum and other parts of the small intestine in all avian species such as quail [14], Rock dove [15], African pied crow [*Corvus albus*] [16] and pigeon [*Columba livia*] [17].

3.2.1 Mucosa

The duodenal mucous membrane in the collard showed three different parts (Fig. 1), that were lining epithelium [simple columnar cells (Fig. 1), lamina propria (loose connective tissue with the presence of mucosal glands) and muscularis mucosa (two thick layers of smooth muscle arranged into inner circular and outer longitudinal bundles). Similar structures found in the ruddy but differently in owl the muscularis mucosa arranged only in one longitudinally arranged bundle.

The presence of two layers of muscularis mucosa in the duodenal mucosae of collard and ruddy was similar to the findings observed in Ostrich (*Struthio camelus*) [18]. But conversely, the muscularis mucosa in the duodenal mucosa in African pied crow (*Corvus albus*) was absent [16].

The mean of thickness of this tunica was 3510µm in collard male, whereas in collard female was higher (up to 3730µm). Same measurements in the male and female owl were 3150 µm and 3525 µm, respectively. While in ruddy, the mean thickness of this tunic in male was 1870µm, and in the female was 1694µm.

3.2.2 Duodenal Villi

They were mucosal projections, which constructed from the lamina propria, smooth muscle fibers as well as the lacteal, long leaf-shaped villi that were arranged in a zig-zag pattern in collard. However, in the owl, the villi showed blunt apical part and wide basal part, while in the ruddy it had a finger like projections. The latter was blind-ended lymphatic capillary that is lined by low columnar epithelium in studied birds.

The lining epithelium of these villi was similar to those observed previously in the same organ in Ostrich (*struthio camelus*) [19], Blue and Yellow macaws [20]. The irregularity that observed in the mucosal surface could be due to the presence of duodenal villi intervening between the bases crypts of Lieberkühn.

The means of surface area of the villi were 1091 mm² and 1002mm² in male and female collard, respectively. While in the owl lesser surface areas recorded as the mean of surface area of the villi were 817 mm² and 799 mm² in male and female, respectively. Highest means of surface area of villi measured in ruddy (821 mm² (male) and 789 mm² (female)). The differences in the surface area of the villi may reflect different absorptive capability of the duodenal epithelium and difference in the shape of villi.

3.2.3 Duodenal Crypts of Lieberkühn

These were simple tubular glands called intestinal glands that were extended from the muscularis mucosa until the bases of the villi. They were lined by a simple columnar epithelium similar to the lining epithelium of the duodenal lumen (Fig. 1, 2, 3). As mentioned by Hamida and his Colleagues in avian, the crypt covered by columnar epithelium [21].

3.2.4 Submucosa

It was formed irregular dense connective tissue situated, beneath the muscularis mucosa, and the layer composed of large blood, lymphatic vessels (Fig. 1, 2, 3). The mean thickness of this tunica was higher in the collard female 591 μm compared to the male 460 μm , whereas in owl the mean thickness of this tunic in male was 240 μm , while in the female was 230 μm . In ruddy, the mean thickness of this tunica in male was 570 μm , while, in the female was 530 μm . Absence of Brunner glands that found in submucosa in mammals concert in chicken when Aitken mentioned that the Brunner's glands are apparently lacking in submucosal layer [22].

3.2.5 Muscularis Externa

Underneath submucosa the muscular coat consists of the smooth muscles fibers arranged into two layers, the inner longitudinal and the outer circular layers in the studied birds (Fig. 1, 2, 3). Evenly the outer layer was thicker than the longitudinal layer over all parts of the duodenum. This finding was agreed with Zaher and his Colleagues in common quail (*Coturnix coturnix*) [14] and Rodrigues *et al.*, in digestive tract of Blue and Yellow macaws that stated this tunica formed two layers [20].

The mean of thickness of this in collard was thinner in the male (890 μm) compared to that of the female (994 μm) and also in owl the mean of thickness of this tunic was 720 μm and 510 μm in male and female, respectively. While in ruddy, the mean thickness of this tunica in male was 1310 μm , and in female was 1120 μm .

3.2.6 Serosa

The layer appeared thin in thickness constructed by loose connective tissue covered by a layer of mesothelial cells (Fig. 1, 2, 3). The serosa lined externally the muscularis and mean of the thickness of the serosa in male and female collard were 160 μm and 190 μm and in the male and female owls were 140 μm , and 160 μm whereas in male and female ruddy were 200 μm and 170 μm , respectively. These findings were similarly recorded in other avian species such as African pied crow [*Corvus albus*] [16] and pigeon (*Columba livia*) [17].

3.3 Histochemical aspects

The duodenum was well studied histochemically by applying three stains: PAS, PAS-AB [pH 0.4] MBB stain. These staining techniques were conducted to view the presence or absence of neutral mucins, acidic mucins and total protein respectively.

The histochemical examination of the wall of the duodenum showed that the mucosal layer as well as the villi possessed two types of cells that were the columnar cells and goblet cells. The columnar cells gave the negative reaction with the

PAS stain in the duodenum of the collard, owl and ruddy. Whereas the goblet cells were strongly reacted with this stain in studied birds (Fig. 1, 2, 3). These findings were in a good agreement with the recent records of Hamida *et al.*, in the duodenal surface lining and the crypts of Lieberkühn of theof the black-winged kite (*Elanus caeruleus*), which is one of the carnivorous avian species [21].

The connective tissue in the lamina propria, submucosa and serosa give mild reaction with PAS in collard and ruddy, whereas, in owl negatively reacted. Additionally, the current findings revealed that the smooth muscle fibers which were constitutes the muscularis mucosa as well as tunica muscularis gave rise fair reaction with PAS in collard and ruddy but it was negative in the owl (Fig. 1, 2, 3).

On applying the combined PAS-AB (pH 0.4), the goblet cells present in the epithelium showed a strong reaction for acidic mucopolysaccharides but the columnar epithelium showed poor reaction with this stain in collard, owl and ruddy (Fig. 1, 2, 3). In addition to that, the connective tissue of the submucosa gave positive reaction for PAS, but negative toward AB part of the stain in case of owl and ruddy, whereas, in collard gave negative reaction for the stain as a whole (Fig. 1, 2, 3). The smooth muscle fibers present in the tunica muscularis showed modest reaction with this staining technique in studied birds.

Histochemically, the mucosal lining revealed no response toward the mercuric bromophenol blue staining in collard, owl and ruddy (Fig. 1, 2, 3). While, the submucosal connective tissue revealed positive reaction for this technique in all studied birds. The tunica muscularis was constituted layers of smooth muscle fibers which were positively reacted with this stain in collard, owl and ruddy.

Current findings were inconsistency with those found in the black-winged kite, because Bromophenol blue stain reacts positively with the absorptive columnar cells of the mucosal folds and the lamina propria structures of the duodenum, but a weak reaction was observed in the goblet cells [21].

4. Conclusion

Because the bird is omnivores, carnivorous and granovoruos species, the columnar cells of small intestine gave the positive reaction with PAS, PAS-AB (pH 0.4) whereas, the goblet cells were strongly positive reacted. In fact, the latter stain is an indicator for sulfated acidic mucin substances which are very important in digestion and absorption and subsequent body growth of the bird.

5. Acknowledgement

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Table 1: Gross measurements (Mean \pm SE) of the duodenum in three studied birds.

Gross Measurements	Sex	Collard Mean \pm SE	Owl Mean \pm SE	Ruddy Mean \pm SE
Weight [gm]	Male	2.21 \pm 0.012	2.42 \pm 0.027	7.7 \pm 0.42
	female	3.41 \pm 0.016	2.29 \pm 0.019	6.1 \pm 0.031
Length [mm]	Male	101.62 \pm 0.071	91.2 \pm 0.019	211.12 \pm 0.041
	female	100.64 \pm 0.068	90.31 \pm 0.017	209.15 \pm 0.032
Diameter [mm]	Male	4.2 \pm 0.061	3.31 \pm 0.041	4.1 \pm 0.059
	female	4.1 \pm 0.039	3.49 \pm 0.048	3.6 \pm 0.038
Organ weight/ Body weight	Male	6.83 \pm 0.013	7.12 \pm 0.34	94.8 \pm 2.13
	female	10.6 \pm 0.051	6.57 \pm 0.28	71.4 \pm 1.99
Body weight [gm]	Male	309.1 \pm 16.08	294.5 \pm 15.04	1232 \pm 42
	female	311.2 \pm 18.01	286.9 \pm 16.01	1171 \pm 36

*Different small letters mean significant differences ($P \leq 0.01$) between different birds.

Table 2: Microscopic thicknesses (means) of the four layers of the duodenum walls measured in the Collard, Ruddy and Owl.

Birds	Sex	Layers				
		T. Mucosa	T. submucosa	T. muscularis	T. serosa	Total
Collard	Male	3510 ± 81	591 ± 21	890 ± 60	160 ± 15	5151
	Female	3730 ± 82	460 ± 19	994 ± 63	190 ± 16	4880
Owl	Male	3150 ± 79	240 ± 18	720 ± 61	140 ± 13	4250
	Female	3525 ± 80	230 ± 16	610 ± 55	160 ± 16	4425
Ruddy	Male	1870 ± 36	570 ± 20	1310 ± 66	200 ± 16	3950
	Female	1694 ± 34	530 ± 17	1120 ± 65	180 ± 17	3520

* Different small letters mean significant differences ($P \leq 0.05$) between different layers.

Table 3: Means of surface area (μm^2) of the villi measured in the duodenum, of Collard, Ruddy and Owl.

Birds	Sex	Surface area (μm^2) of villi
Collard	Male	1091 ± 78.1
	Female	1002 ± 74.4
Owl	Male	817 ± 68.2
	Female	799 ± 67.4
Ruddy	Male	821 ± 68.3
	Female	789 ± 63.1

Note: The measurements were conducted at X40 magnification

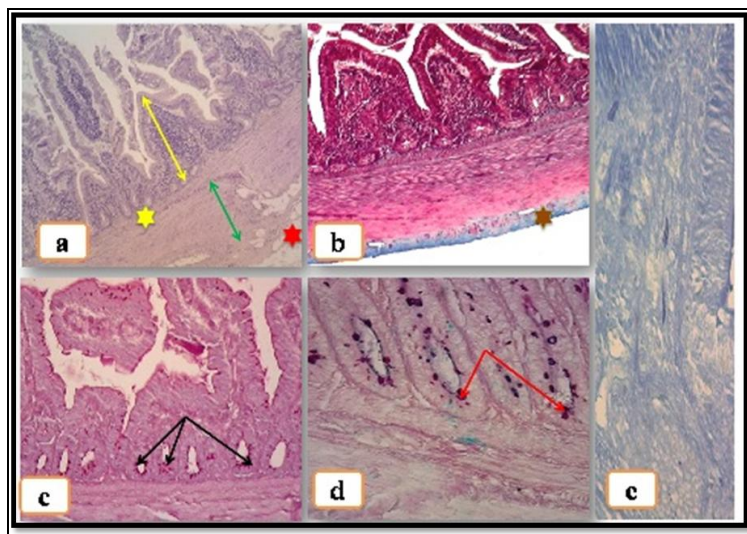


Fig 1: Cross section in the duodenum wall of Collard showed mucosa (yellow double head arrow), submucosa (yellow star), muscularis externa (green double head arrow), serosa (red and brown star), intestinal glands with neutral mucin (black one head arrow), goblet cells with acidic sulfated mucin (red one head arrow). (a) H&E X100, (b) Masson Trichrom stain X200, (c) PAS X 100, (d) PAS + AB = pH (0.4) X 400 and (e) MBB X 100.

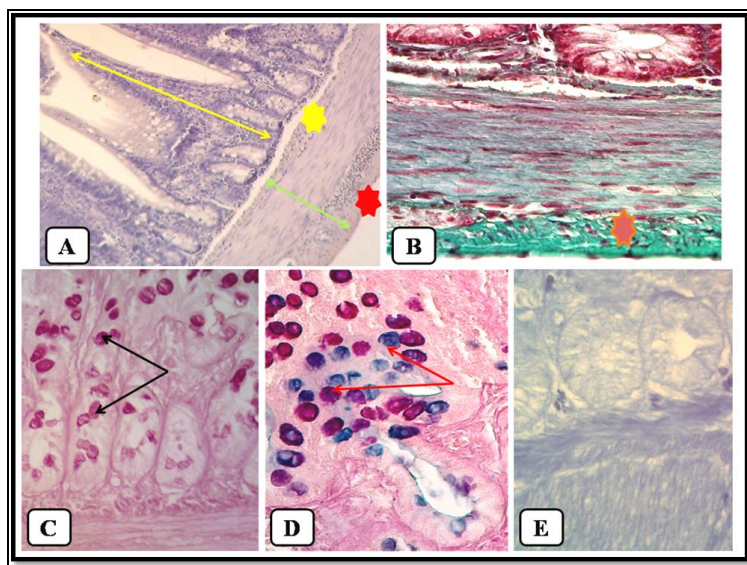


Fig 2: Cross section in the duodenum wall of Owl showed mucosa (yellow double head arrow), submucosa (yellow star), muscularis externa (green double head arrow), serosa (red and brown star), intestinal glands with neutral mucin (black one head arrow), goblet cells with acidic sulfated mucin (red one head arrow). (A) H&E, X100, (B) Masson Trichrom stain X400, (C) PAS X 200, (D) PAS + AB = pH (0.4) X 400 and (E) MBB X 200.

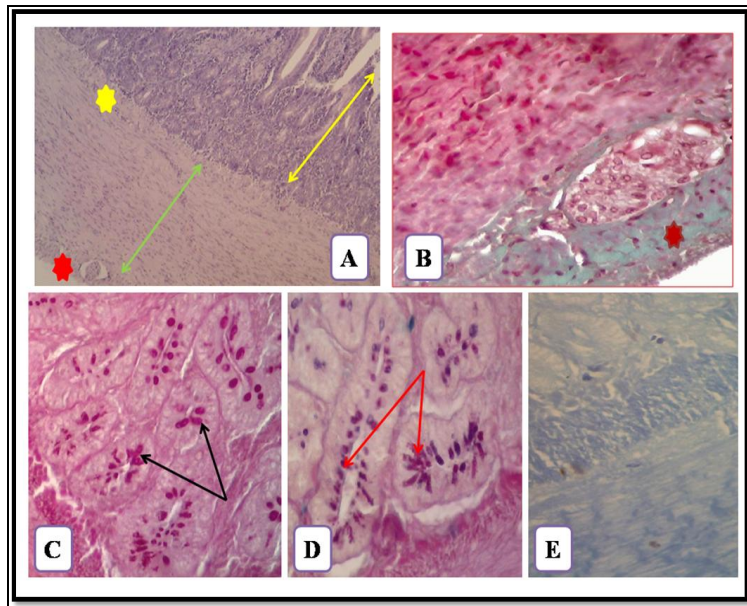


Fig 3: Cross section in the duodenum wall of Ruddy showed mucosa (yellow double head arrow), submucosa (yellow star), muscularis externa (green double head arrow), serosa (red and brown star), intestinal glands with neutral mucin (black one head arrow), goblet cells with acidic sulfated mucin (red one head arrow). (A) H&E, X40, (B) Masson Trichrom stain X400, (C) PAS X 200, (D)PAS + AB = pH (0.4) X 400 and (E) MBB X 100.

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