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Morphological and Histochemical Study of the Duodenum in Post-Hatching and Adult (*Columba livia*)

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Abstract | The result showed difference in the growth of the duodenum between post hatching and adult of *C. Livia*. The thickness of the duodenum in post- hatching was the same as that the adult, but its width in the adult was higher than in post- hatching at the level $p<0.05$. The duodenum was consisting of four tissues layers: tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. Tunica mucosa is consisting of villi project finger- like that covered by simple columnar epithelium with goblet cells, tunica sub-mucosa is loose connective tissue which contain of vessels, collagen and elastic fibers and absent of Brunner's gland, tunica muscularis was consisted of inner circular and outer longitudinal layers, tunica serosa is a thin layer of simple epithelium cells. The importance of this study lies in providing a basic anatomical and histological database for the small intestine at two age stages (post- hatching and adult), which is important in diagnosing pathological conditions of the digestive system in pigeons.

Keywords | Post- hatching, *C. livia*, Duodenum, Adult

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INTRODUCTION

The birds (*Columba livia*) has been one of the more famous and has served as an essential model organism for the study of evolution, behavior, genetics, and physiology (Helms and Brugmann, 2007; Holt *et al.*, 2018; Al-Khafaji et al., 2024; Alkafagy et al., 2019). The digestive and the anther organs such as reproductive system are essential in birds and develop rapidly from early life to adulthood (Ahlam etal., 2024; Salim *et al.*, 2020; Qureshi *et al.*, 2017). The development of digestive organs begins immediately after hatching, and the intestine develops rapidly in comparison to the other organs of the digestive system (Dong *et al.*, 2012). The faster rate of growth after hatching has been associated with a significant increase in the number of enterocytes in the first few days after hatching, which is attributed to a massive increase in villus length (Geyra *et al.*, 2001). The duodenum in post-hatching broiler chickens (7, 14, and 21 days) is reddish-brown in color and located on the right side of the abdominal cavity. It has been U-shaped, with two limbs that descend and ascend. At the same time, the weights were 1.670±0.158 g, 2.143±0.232 g and 2.559±0.270 g, respectively, and the lengths were 160.92±2.73 mm, 250.1±2.239 mm, and 290.92±1.245 mm, respectively (Ali, 2022). The duodenum in adults is grey in color and consists of the descending and ascending parts (Aizawa *et al.*, 2013).

In the adult, the first part of the duodenum, called the descending part, has been derived from the right surface of the muscular stomach (gizzard) in the (*C. livia*). The descending part has been directed ventro-posteriorly and

then ventro-anteriorly to form the ascending part. The duodenum has been being extended from the muscular stomach to the proximal end of the ascending part. The inner lining of the duodenum has been smooth (Al-Khakani, 2013; Ali *et al*., 2024; Al-Sailawi *et al*., 2024).

The duodenum has four layer tunica mucosa has been consist of villi and lamina proper, the villus have been finger- like, not branched and cylindrical shape and the villi covered by epithelium tissue consist of absorbed cells it has single layer from columnar cells brushes border (Al-Muhammadi and Al-Taai, 2020). The tunica submucosa in the ducks (*Anas playrhynchos*) is poorly developed, almost nonexistent, and only noticeable where the Meissner's plexus and large blood vessels are present. It appears as a very thin layer of connective tissue separating the muscularis mucosa from the muscularis externa. Additionally, Brunner's glands are absent (Khaleel and Atiea, 2017). The tunica muscularis consists of smooth muscle fibers, which are divided into two layers: The inner circular layer and the outer longitudinal layer (Fatimah *et al*., 2019). The tunica serosa appears as loose connective tissue containing blood vessels and adipose tissue, and is covered by mesothelium (Khaleel and Atiea, 2017).

We conducted this study to describe and compare the morphological, histological, and histochemical differences between the post-hatching of (*C. livia*) and the adult, depending on the nature of the nutrition.

MATERIALS AND METHODS

Twenty-eight birds (*C. livia*) were used fourteen post hatching at age (4 days) and fourteen adults at age (7 month) of *C. livia* in this study, fourteen post-hatching at age 4 days and fourteen adults at age 7 months. The morphology and histochemical analysis of small intestine were examined. Samples were obtained from bird owners in local markets (Babylon Market).

We stop feeding the birds for 24 hours, then an anesthetic injection in the pectoral muscle. The bird was then left for about five minutes to achieve complete anesthesia. The body weight of each bird was measured using a sensitive balance, and length was measured with a tape measure. After that, the bird was putted on an anatomy board, with an opening made at the caudal end of the chest muscle, and a longitudinal incision was made on both sides at the caudal area. Then, the small intestine of birds was photographed grossly. The thickness and width of the duodenum are measured from the ascending part using a vernier digital caliper.

The samples were labeled and immersed in a 10% formalin solution for 24 hours; then, the fixation was replaced (Suvarna et al., 2018). For histological study, using Haematoxylin and Eosin (H and E) stain, Periodic acid Schiff (P.A.S), Alcian blue, and Masson's trichrome stain (M.T.S).

RESULT AND DISCUSSION

Morphologically, in the post-hatching and adult of *C. livia*, the average body weights were found to be 51.974±0.78 g and 291.71±12.24 g, respectively. The mean body length was 12.6±0.33cm and 19.871±0.31cm, respectively (Table 1).

Table 1: Weight (Mean±SE) and length (Mean±SE) in post-hatching of *C. livia* and the adult.

Parameter	Post hatching	Adult	T-test
Weight (g)	51.974±0.78	291.71±12.24	19.54
Length (g)	12.6±0.33	19.871±0.31	16.05

The numbers represent the mean± standard error. Statistically significant difference. $P < 0.05$

Table 2: Thickness (Mean±SE), width (Mean±SE) of the duodenum in the post-hatching of *C. livia* and adult.

Parameter	Post-hatching	Adult	T-test
Thickness (mm)	1.324±0.0084	1.24±0.008	7.19
Width (mm)	8.82±0.0008	10.93±0.008	182.7

The numbers represent the mean± standard error. Statistically significant difference. $P<0.05$

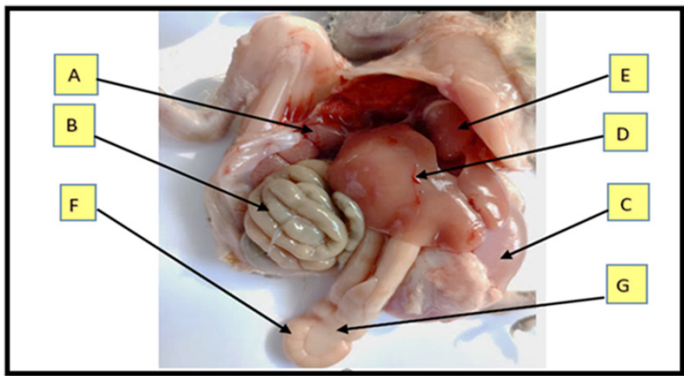

Figure 1: Post hatching of *Columba Livia* (4 days old) (A) kidney, (B) jejunum, (C) gizzard, (D) liver, (E) heart, (F) duodenum, (G) pancreas.

In the post hatching (4 days) and adult, the duodenum was first part of small intestine which located in right side of abdomen cavity started from pyloric orifices of stomach to the jejunum, it is tube U- shape consisted of descending and ascending tubes called duodenal loop connected by tight fold from the mesenteric membrane and the pancreas located between these limbs (Figures 1, 2) this result concur with those reported previously (Ali, 2022; Al-Khakany, 2013; Aizawa, 2013). It had a reddish–brown color (Figures 1, 2). The external and inner surfaces of the duodenum were smooth. The thickness of the duodenum in post-hatching and adult specimens was 1.324±0.0084 mm and 1.24±0.008mm, respectively. The width was

8.82±0.008 mm and 10.93±0.008mm (Table 2). The interpretation of the increased thickness of duodenal wall in the post-hatching reverse adult is that it depends on the milk crop in its food, which is a liquid rich in proteins and fat. This result aligns with that of Gillespie *et al.* (2013). The reason for the increased width of the duodenum in adult reverse post is that adult *C. livia* are fed solid foods such as grains, which leads to the expansion of the lumen to absorb a large amount of undigested food. This result aligns with Uni *et al.* (2003).

Histologically, the structure of the duodenum in *C. livia* at the post-hatching and adult stages was composed of four tunics: mucosa, submucosa, muscularis, and serosa. This finding agrees with Al-Kafagy et al. (2022) in the northern bobwhite quail (*Colinus virginianus*).

The tunica mucosa of the duodenum in post-hatching and adult stages consisted of villi, lamina propria, and muscular mucosa. The current study concurred with Al-Samawy et al. (2017) in the mallard (*Anas platyhynchos*).

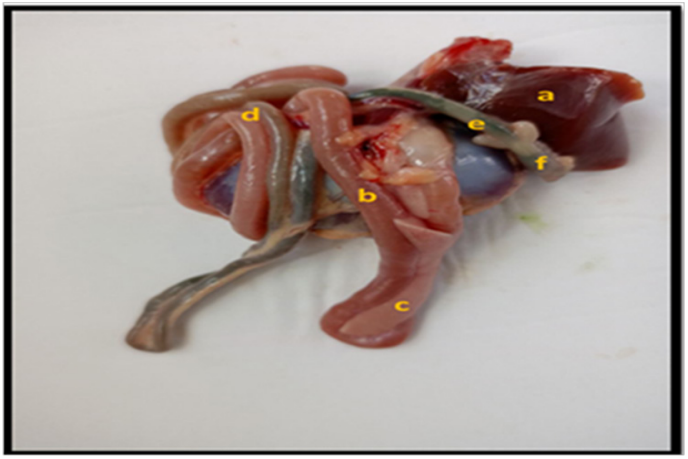

Figure 2: The adult of *Columba Livia:* a-liver, b- duodenum, c-pancreas, d-jejunum, e-ilium, f- rectum.

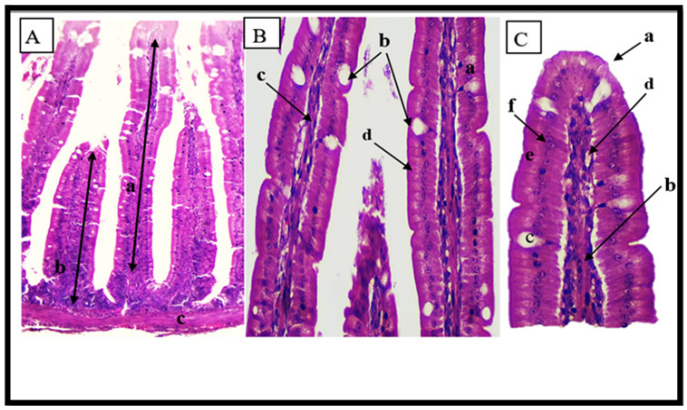

Figure 3: The duodenum in post (A) a-villi, b-tunica mucosa, c-tunica musculares. (B) a-villi, b-goblet cells, c-core, d-columnar epithelia. (C) a-villi, b-core, c-goblet cells, d-lacteal vessels, e- simple columnar epithelia, f-oval nucieus. HandE (A) 20x (B) 40x (C) 100x.

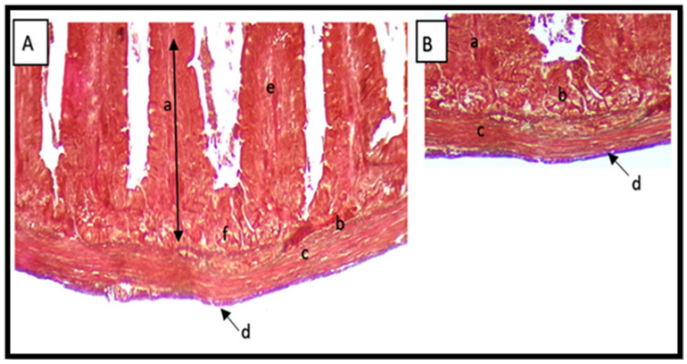

Figure 4: The duodenum in post hatching (A) a-tunica mucosa, b-tunica sub-mucosa, c-tunica muscularis, d-tunica serosa, e-villi, f-crypt of Lieberkühn, (B) a- villi, b- crypt of lieberkuhn , c-tunica muscularis consist of two layer inner circular and outer longitudinal layers, d- tunica serosa. Masson Trichrome (A) 20x (B) 40x.

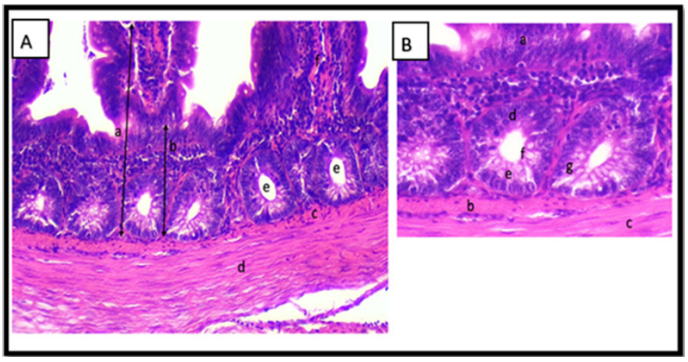

Figure 5: Transvers section of duodenum in adult (A) a-mucosa, b-lamina propria, c- sub-mucosa, d-tunica muscularis, e-crypt of Lieberkühn, f- villi, (B) a- villi, b-sub-mucosa, c-tunica muscularis, d-crypt of Lieberkühn, e-paneth cells, f-goblet cells, g-enteroendocrine cell. H and E (A) 40X (B)100X.

Table 3: Height and width of villi, depth of crypt, height of epithelium, and thickness of tunica mucosa of duodenum in post and adult of *C. Livia* (Mean±E).

Measurement(µm)	Post	Adult	T-test
Height of villi	0.139±0.011	0.239±0.014	5.474
Width of villi	0.0312±0.014	0.0327±0.021	0.58
Depth of crypt	0.0428±0.0029	0.0597±0.0037	3.631
Height of epithelium	0.0362±0.0029	0.253±0.027	2.73
Lamina propria	0.0021±0.0165	0.00213±0.0195	0.985
Thick of mucosa	0.1812±0.1011	0.2973±0.1475	6.491

The number represents the mean± standard error. Statistically significant difference. P<0.05.

The villi appeared as finger- like projections, cylindrical in shape, long, and not branched (Figure 3, 5). The height of villi in post and adult stage was 0.139±0.011 µm and 0.239±0.014 µm, respectively. The width of villi in post and adult stages was 0.0312±0.014 µm and 0.0327±0.021 µm, respectively (Table 3). This result agrees with Al-

Khakany (2013) in *C. livia*. Each villus was covered by a single layer of epithelial tissue containing of absorbed cells (columnar cells), called simple columnar epithelium, which has an oval nucleus located at the middle of the cells (Figures 4, 5). The height of the epithelium in post and adult was 0.0362±0.0029 µm and 0.0253±0.027 µm (Table 3). The thickness of tunica mucosa in post and adult was 0.1812±0.1011 µm and 0.2973±0.1475 µm, respectively (Table 3). The goblet cells are present inner the columnar absorptive cells of the intestinal epithelium, characterized by pale cytoplasm, and appear long and cup-shaped (Figures 7, 9) due to the production of mucus; their nucleus is located near their base (Figure 3). This study agrees with Al-Muhammedi and Al-Tai (2020) in the *C. livia*. The number of goblet cells in post and adult was 17.10±2.34 µm and 21.43±2.54 µm (Table 4). The crypt of lieberkuhn also called intestinal gland were located in the basal of each villus lined by simple tubular epithelial cells with oval nucleus (Figure 3, 5) of these cells' depth of crypts in post and adult were 0.0428±0.0029 µm and 0.0585±0.0036 µm, respectively (Table 3). This result is in harmony with (Fatimah *et al*., 2019) in the guinea fowl and *Numidia meleagris*. In the glands are present the goblet cells, paneth cells, and enteroendocrine cells which secreted digestive enzymes and produce new cells. The lamina propria was consisted of connective tissue, fibers and blood vascular which supporting the mucosal membrane (Figure 6). The thickness of lamina propera in post-hatching and adult was 0.0021±0.0165 µm and 0.00213±0.0195 µm (Table 3). This finding aligns with Nasrin *et al*. (2012) in broilers. The tunica submucosa in adult and post-hatching of *C. livia* was a thin layer of connective tissue containing vessels, and collagen (Figures 4, 6). This result aligns with Al-Jeraisy's (2017) findings. It was absent of Brunner's gland, and the thickness of sub-mucosa in post and adult was 0.0074±0.00101µm and 0.0078±0.0082 µm (Table 4). Tunica muscularis in post and adult was consisted of two layers: Inner circular layer and outer longitudinal layer (Figures 4, 6) The thickness tunica musclaris in post-hatching and adult was 0.0392±0.01531µm and 0.0386±0.00291µm (Table 4). In the post-hatching and adult, the tunica serosa was thin layer of loose connective tissue that contained blood vessels, nerve, adipose tissue, and collagen fibers (Figure 8). The results of this study align with those of Al-Jebori *et al*. (2020). The thickness in post-hatching and adult was 0.0171±0.0090 µm and 0.0077±0.00081 µm (Table 4).

Histochemically, in the post use PAS to coloring the (basement membrane) and muco protein and carbohydrates and PAS technique was used for identification of neutral mucin in duodenum epithelial and goblet cells, it gave a strong positive reaction and Alcian blue use to using determination of acid mucin (Figures 7, 8).

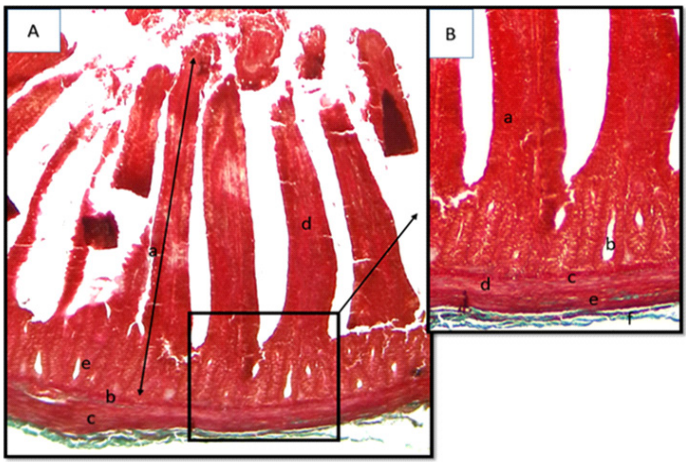

Figure 6: Transvers section of duodenum in adult (A) a-tunica mucosa, b-tunica sub-mucosa, c-tunica nuscularis, d-villi, e-crypt of Lieberkühn (B) a-villi, b-crypt of Lieberkühn, c-tunica sub-mucosa, d- inner circular, e-outer longitudinal, f- tunica serosa. Masson Trichrome (A)20x (B)40x.

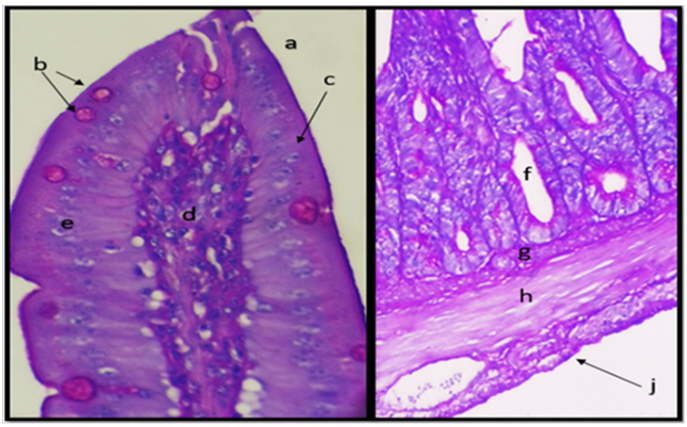

Figure 7: duodenum in post hatching a-villi, b-goblet cells, c-simple columnar epithelium, d-core, e-oval nucleus, f-crypt of Lieberkühn, g-tunica sub-mucosa, h- tunica muscularis, j-tunica serosa. PAS 40x.

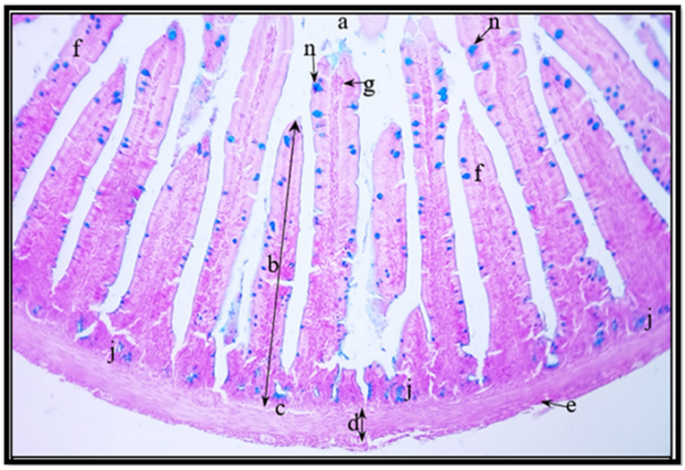

Figure 8: Transvers section of duodenum in post a-intestinal lumen, b-tunica mucosa, c-tunica sub-mucosa, d-tunica muscularis, e-tunica serosa, f- villi, g-core, j-crypt of Lieberkühn, n-goblet cells. Alcian blue 20x.

Table 4: Thickness of tunica sub-mucosa, tunica muscularis, tunica serosa, and number of goblet cells of the duodenum in post and *C. Livia* (Mean±SE).

Parameter (µm)	Post	Adult	T-test
Thickness of sub-mucosa	0.0074±0.00101	0.0078±0.0082	0.319
Thickness of muscularis	0.0392±0.01531	0.0386±0.00291	0.40
Thickness of serosa	0.0171±0.0090	0.0077±0.00081	4.536
Number of goblet cells per 100 lining epithelia	17.10±2.34	21.43±2.54	1.249

The number represents the mean ± standard error. Statistically significant difference. P<0.05

In the adult, the periodic acid-schiff's positive reaction with carbohydrates is an oxidative process where some polysaccharides react with the periodic acid, producing an oxidized compound, an aldehyde. The red or pink coloration reveals the presence of aldehydes. The stain appears pink, which indicates the persistence of the intra- or extracellular mucins; it gave a weak reaction. Using the AB stain, of epithelial tissue and the goblet cells exhibit a distinct staining pattern (Figures 9, 10). This result agrees with Taha (2021).

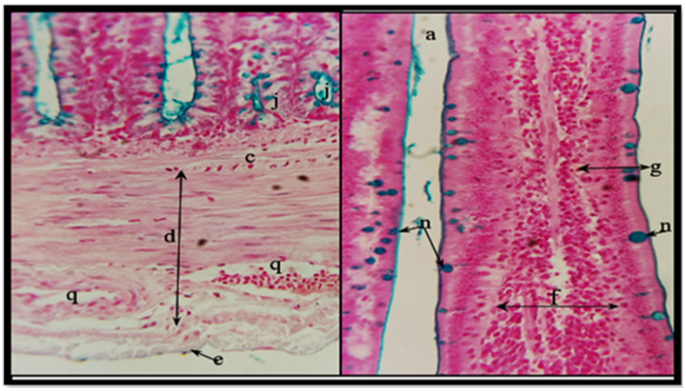

Figure 9: Transvers section of the duodenum in adult Columba Livia. Indicator: a-Intestinal lumen c-Submucosa. d-Muscularis. e-Serosa. g-core. f-Simple columnar Epi. j-crypt of lieberkuhn. n-Goblet cell. q-blood vessels. Alcian blue stain. 40X.

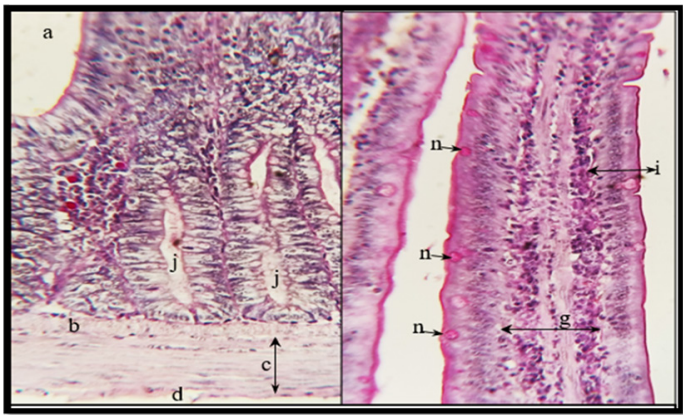

Figure 10: Transvers section of the duodenum in adult Columba Livia. Indicator: a-Intestinal lumen b-Submucosa. c-Muscularis externa. d-Serosa. g-core. i-Simple columnar Epi. j-crypt of Lieberkühn. n-Goblet cell. PAS stain. 100X.

CONCLUSIONS AND RECOMMENDATIONS

The ratio of weight between of small intestine to body in post hatching was high than adult, the weight of small intestine in post and adult does not change but the only the increase is in body weight. Thickness of tunica mucosa increase in adult more than post because the adult was feeding on grains and fibers that cause mechanical friction with the intestinal wall. Good reaction goblet cell in villi and crypt Lieberkühn of jejunum in adult for staining PAS and Alcian blue and ilium in post and adult for staining PAS and Alcian blue. Study the small intestine by electron microscopic and relationship development villi and depth of crypt with bird's growth.

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NOVELTY STATEMENT

This study introduces a novel approach through morphological and histochemical comparison of small intestine. The results highlight the changes that occur in the small intestine and bird's body.

AUTHOR'S CONTRIBUTION

Aya Wael Tarteeb and Salim Salih Ali Al-Khakani conceptualized, designed the experiments, carried out the experimental work and analyzed the data.

Generative AI or AI-assisted Technology Statement

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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