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A Histochemical Study Of The Small Intestine Of Adult Male Turkey (*Meleagris Gallopavo*)

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Abstract:

This study was designed to investigate histological and histochemical structures of the intestinal tract of adult (1-3years) male turkey (*Meleagris gallopavo*). Ten bird were collected from local suppliers in Diyala province. Birds were weighed and euthanized by Ketamine (100 mg /Kg) intravenous and xylazine (10mg/Kg)I\M injection, the intestinal wall had four tunicae; tunica mucosa, submucosa, muscularis, and serosa. The mucosa showed different shape, size and dimensions. The long finger or leaf-like villi in the duodenum, whereas, shorten and wider villi were seen in other segments of small and large intestine There was a significant difference in the microscopic measurements (height and width of villi, crypts depth and in the thickness of some tunicae of the small intestine wall. Histochemically, the goblet cells in the villi and crypts had mucin reaction and the type of mucin was both neutral and acidic mucin.

Keywords : *Meleagris gallopavo,* histological and histochemical structures, intestinal tract

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Introduction

small intestinal in local adult male turkey (Meleagris gallopavo). The turkey bird is breed in many parts in the world, and industrialized breeding of turkey has made its meat poorly [1,2]. These birds are fast-growing, it ability to converted the feed stuffs to high-quality meat. The male is larger and much more colorful than the female these birds are fast-growing, it ability to converted the Feed stuffs to high-quality meat [3]. The bird's requirements of feed are mostly determining by the anatomy and capability of its digestive system, these determine what food types can be ingested into the nutrients required to sustain the body and the form in which these nutrients will be delivered to the circulation for distribution to the body cells [4] .As in mammals the birds digestive system appeared as a double-ended opened tube, starts at the beak and terminate at the vent, in sequential orders [5] . Generally, the small intestine is simple, short, high efficient and slightly coiled in meat eating birds, while longer and highly coiled in herbivores and omnivoris birds [6,7]. The small intestine of some bird is long, coil mass that started from the pyloric end of the gizzard to the ileocecal colic junction, it is divided into three main parts; duodenum, jejunum and ileum [8]

Material and methods

Ten adult male turkey were weighed and euthanized by Ketamin (100 mg /Kg) intravenous and xylazine(10mg/Kg) intramuscular injection [9] .Used for histological and histochemical studies after removal of intestinal segments they were emptied from its contents and washed by normal saline

The aims of this Study to determine the histological and histochemical structure of solution (0.9 %). The specimens were collected from the middle part of each of (duodenum, jejunum, ileum, ceca and rectum), then kept in a labeled clean plastic container, half of the specimens were fixed with 10% neutral buffered formalin then where they kept for 48 hours, washed with tap water and dehydrated by using a series of graded ethanol alcohol. These specimens were cleared by xylen. Infiltrated in melted paraffin wax). thickness was prepared by using a rotary microtome (Series MRS3500, Histo-Line laboratories Ltd, Italy). Then the sections mounted on a glass slides and stained with Mayer's Hematoxylin and Eosin and special stains (AB (PH 2.5),PAS ,combined AB (PH 2.5)-PAS)to detect the general histological structures of the studied tissues [10].

Result:

The microscopic findings of the three small intestine segments (Dudenum, Jejunum and Ileum) of the male turkey showed four classic tunicae; Mucosa, Submucosa, Muscularis and Serosa (Fig.1 A).

Duodenum: The mucosal tunica of the small intestine composed of traditional three laminae include epithelium, lamina propria and the lamina muscularis (Fig.1A1). The doudenal mucosa in turkey was observed length of villi as leaf shaped villi with blunt apex lie by simple columnar epithelium, narrow tall columnar cells resting on thin basement membrane (Fig 1 B1). They are variable and size long and width. between the villi are small opening of the simple tubular branched gland called intestinal gland, these continuous with that epithelium

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of villi (Fig.1B2).The epithelium of the duodenum villus was simple columnar cells composed of the four types of the cells include

Columnar cells: absorptive cells were large cells characterized the apical border, it has plentiful micro villi are present at the luminal surface, the cells have center, large, oval nuclei have clear cytoplasm is acidophilic (Fig .1B1). Goblet cells: mucine cells are readily seen in micrograph were they large cells with vary stage scattered among the columnar cell, it varies in size according maturation. The nuclei closed to the basal with clear cytoplasm but the apical of cell without the striated border like that columinar cells, (Fig.1B 1) cells. increase Argentaffine cells: distally of small intestine they were pyramidal cells occasionally they found in, villi and crypt, they are bottle to spindle in shape cells lying among the epithelial cells. 1B1). Lymphocyte mostly provides the defense to gut against the organism invaded the intestine. There is dark purple have large nucleus (basophilic) there are vary in size (small, medium and large) (Fig.1 B1)

All epithelium cells in villi are lies on the basement membrane and which separated the epithelium from lamina propria (Fig.1B1). The crypts of leiberkuhn, display as a simple tubular glands, which lined by similar columnar epithelium that covered the villi and few goblet cells, situated between the bases of the villi and extended till the layer formed by a bundles of longitudinal arrangement of smooth muscle fibers which represent the muscularis mucosa (Fig.1B 2,C). The core of the villi was occupied by loosely connective tissue highly vascularized and containing many free cells increased in number at base of villi and continuous with the connective tissue of the lamina propria (Fig.1 B2,C)

The mean measurements heights of the epithelial cells, height of villi, width of the villi, depth of crypts, the ratio of height villi to crypts depth and the mean thickness of all tunica mucosa (Table 1). Duodenal submucosa was very thin layer and, not easily distinguished its situated between the muscularis mucosa and tunica muscularis, it forms of loose connective tissue occupied Tunica muscularis was formed of two layers of the smooth muscle fibers; thin outer longitudinal and inner thick circular layers of smooth muscle fibers, within and between these layers there were fine narrow connective tissue layer contains myentric plexus (Auerbach's) collagen fibers, lymphatic and blood vessels (Fig. C).

Entire segments of the small intestine have same structure of tunica serosa, this coated layer is composed from loose connective tissue rich with collagen fibers as a bundles, in addition to the large blood vessels, its covered by a single layer of squamous cells (mesothelial cells) (Fig.C).

PAS –technique: The duodenal villi possessed two types of cells that were the columnar cells and goblet cells. used the PAS method to determined most the complex carbohydrate involve the mucin, Whereas the goblet cells were strongly reacted to PAS stains give magenta color (Fig.1B1)

Alcian blue technique : Application of the Alcine blue, stain AB (ph.2.5) which revealed the strong reaction of the goblet cell and density in villus epithelium and crypt of

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Lieberkühn, these strong reaction gave indicate found acid mucine produce by the goblet cells and secreted to lumen (Fig.1D1) .

Regarding the combined PAS-AB (PH-2.5) procedure, the duodenal mucosal goblet cells were positively reacted (red color and dark blue) indicating the presence of neutral mucin in some goblet cells and acidic mucin in others respectively, while the other epithelial cells were negatively reacted with the same stain (Fig.1 D2)

Jejunum

The Jejunum was the middle portion of the small intestine, histologically was similar to that observed in the duodenum in that constructed of the tubular organ layers (Fig.2 A), with some differences in the mucosa such as decrease of mucosal height, wider and shorter villi and increase in density of goblet cells.(Fig.1 A B C D E)

The epithelium of the mucosa covering villi and lining the jejunal crypts (intestinal glands) was simple columnar cells with typical goblet cells. The latter increase in its density more than that in duodenum. The lamina propria formed by connective tissue contains numerous intestinal glands which appeared as a simple tubular glands observed among the bases of the villi and occupied the space between the villi bases and muscularis mucosa. Thin smooth muscle fibers extended between the intestinal glands, collagen fibers, many lymphocytes.

The muscularis mucosa of jejunum was easily distinguish Its made up of longitudinal bundles of smooth muscle fibers beneath the crypt of Lieberkühn (Fig.2 B). The mean measurements heights of the epithelial cells, height of villi, width of the villi, depth of crypts, the ratio of height villi to crypts depth and the mean thickness of all tunica mucosa (Table 1).

Jejunual submucosa was thin layer it consists, of collagenous fibers connective tissue و contains blood and lymphatic vessels in addition to the submucosal plexus Meissner plexus with some of collagen (Fig.2 B).

Underneath the submucosa was thick layer arranged in a thick circular inner layer and thin longitudinal outer layer of smooth muscle bundles, between their two layers a narrow sparse connective tissue layer contains blood vessels and myentric plexus tunica serosa similar in duodenum (Fig. 2 c**)**

Ileum

Histologically, the ileum was similar to the previous segments in both duodenum and jejunum, it was from four main layers, mucosa, submucosa, muscularis and serosa (Fig.3 A).

the ileal mucosa was modified into different shape and size of villi, it appeared as wider and shorter mucosal projection, display leaf like shape projections less than the previous segments, some villi showed leaf like shape and others formed as tongue like, it was short, wide villi and shorter and broader than those of jejunum (Fig.3 A,B). These villi were covered by the simple columnar epithelium, similar to those of duodenum and jejunum, however the goblet cells increase distally (Fig. 3 B). The core of villi was constituted from many cells, the core extended with lamina propria the core of villi, lamina propria which composed of loose connective tissue scattered around the crypts of Lieberkühn. (Fig,3 B). The muscularis mucosa in ileum is composed layers of

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smooth muscle bundles fibers and fine collagen fibers lies immediately beneath the base of the crypts (Fig.3 B). made up of smooth vibes

168.0 ± 3.18µm, mean depth of crypts was 142.40 ± 2.38 µm, the mean ratio of villus height to crypts depth was 4.60 ± 0.28 µm. The mean thickness of tunica mucosa was 768.0 ± 3.85 µm (Table 1).Tunica submucosa similar in the duodenum and jejunum, this tunica was thin layer to the mean thickness was 10.60 ± 0.051 µm (Table 1). The tunica muscular is and serosa were same as found in previous segments, the muscular is tunica was made up of two layers separated by fine bundles of collagen fibers, blood vessels. Between the two layers, there are nerve plexus (Fig. 3.C). The mean thickness of this tunica was 595.20 ± 4.26µm (Table 1) The mean thickness of this tunica was 55.20 ± 1.46 µm (Table 1).

Histochemical assessment of jejunum and ileum

By applying the PAS technique, the goblet cells covered the villi and lining the crypts were reacted positively toward the PAS giving rise to magenta color, while the simple columnar cells showed a negative reaction with the same procedure (Fig.2 D1)(Fig.3B).

Alcian blue technique: Alcian blue technique was used in jejunum and ileum to reveal the strong reaction of acid mucin that produce by the goblet cells in villi and crypt in mucosa of these segments gave dark blue color (Fig.2D2,)(Fig. 3 D).By applying the AB-PAS (PH-2.5), the cellular cytoplasm of the surface lining columnar epithelial cells of the mucosal villi and crypts showed negative reaction, while the goblet cells gave strong positive reaction (blue coloration) for acidic mucin in some

The mean heights of the epithelial cells were 50.58 ± 0.22 µm, the mean height of villi 695.0 ± 3.49 µm, mean width of the villi was

cells and magenta coloration for neutral mucin in others in the ileum mucosa (Fig.2 E)(Fig.3 E).

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Table 1: The microscopic measurements of Height of epithelium, Height and width of villi, Crypts depth, Ratio of Height of Villi to crypts depth, and the mean thickness of the four tunicae of each part of small intestine (Mean± SE) in Adult male Turkey (*Meleagris gallopavo*)

Measurements (µm)	Duodenum	Jejunum	Ileum
	Mean ± SE	Mean ± SE	Mean ± SE
Height of Epithelium	A 51.22 ± 0.30	A 51.18 ± 0.24	A 50.58 ± 0.22
Height of Villi	A 1810.40 ± 3.3	B 1182.0 ± 5.41	C 695.0 ± 3.49
Width of Villi	B 153.0 ± 2.24	A 163.0 ± 2.55	A 168.0 ± 3.18
Crypts depth	A 180.0 ± 4.60	B 156.0 ± 2.0	C 142.40 ± 2.38
Height of Villi Crypts depth	A 10.08 ± 0.27	B 7.53 ± 0.06	C 4.60 ± 0.28
Thickness of Tunica Mucosa	A 2104.80 ± 1.50	B 1255.20 ± 4.81	C 768.0 ± 3.85
Thickness of Tunica Submucasa	A 11.80 ± 0.37	A 10.0 ± 0.45	A 10.60 ± 0.051
Thickness of Tunica Musclaris	A 1436.40 ± 3.23	B 840.40 ± 3.23	C 595.20 ± 4.26
Thickness of Tunica Serosa	A 54.60 ± 1.66	A 56.80 ± 1.39	A 55.20 ± 1.46
The different letters in the horizontal comparison between the groups referred to a significant difference (P < 0.05), while the similar letters referred to a non-significant difference (P > 0.05)			

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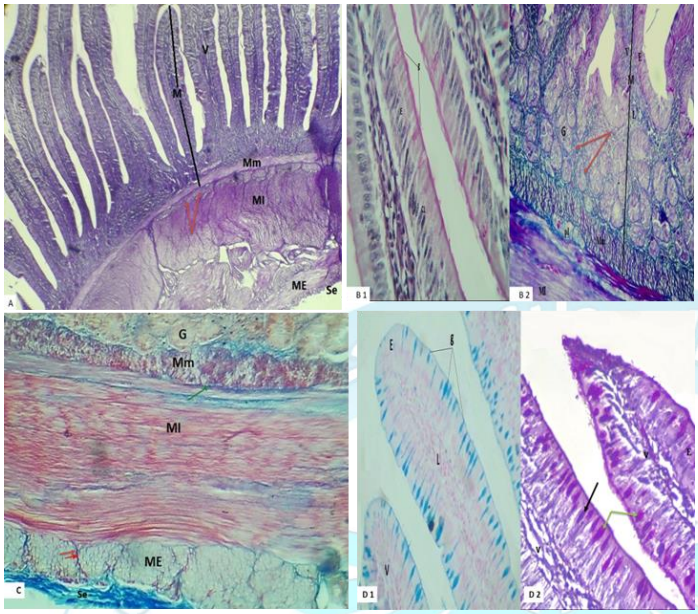

Fig.1:Histological Cross Section of the Duodenum Mucosa(M), Epithelium(E), Sub mucosa (black arrow), Core of villi (v), Intestinal glands(G) ,Lamina propria (L), Collagen Fibers (black arrows) Muscularis mucosa (Mm), Muscularis MI), : Inner layer (MI), Outer layer (ME) and Serosa (Se) H&E Stain X40, Submucosal plexus (orange aarrow), C: Masson's Trichrom Stain X100, B: H&E Stain X200. D 1: Combined stain, D 2 :AB Stain X400.

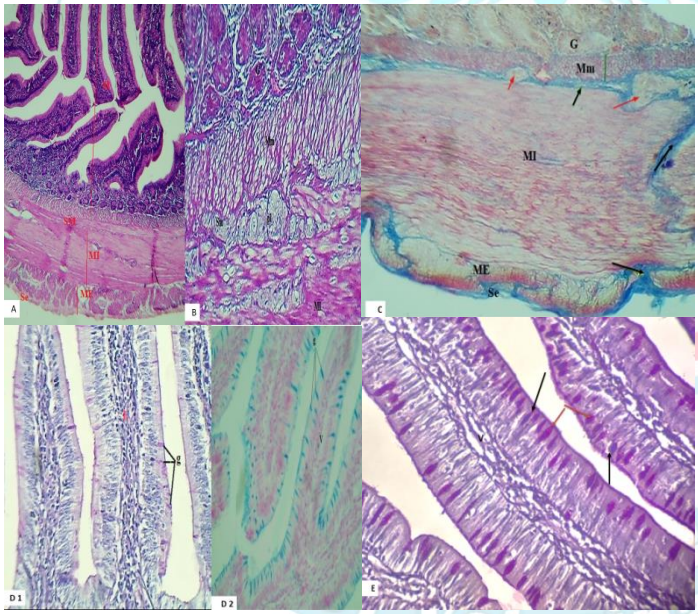

Fig. 2: Histological Section of the Jejunum in adult male turkey shows: Simple Columnar Epithelium (E), Villi (V), and goblet cells (black arrows), lamina propria (L), Muscularis mucosa (Mm), intestinal glands (G),nerve plexus (Pl, red arrow) Collagen Fibers (Black arrows), Muscularis; Inner layer (MI) A: X40 H&E , B: X200 PAS Stain, D: Masson's Trichrom Stain X200. ,D;PAS stain X20 0 D 2:AB stain x200.,E; Combined AB-PAS Stain X 200

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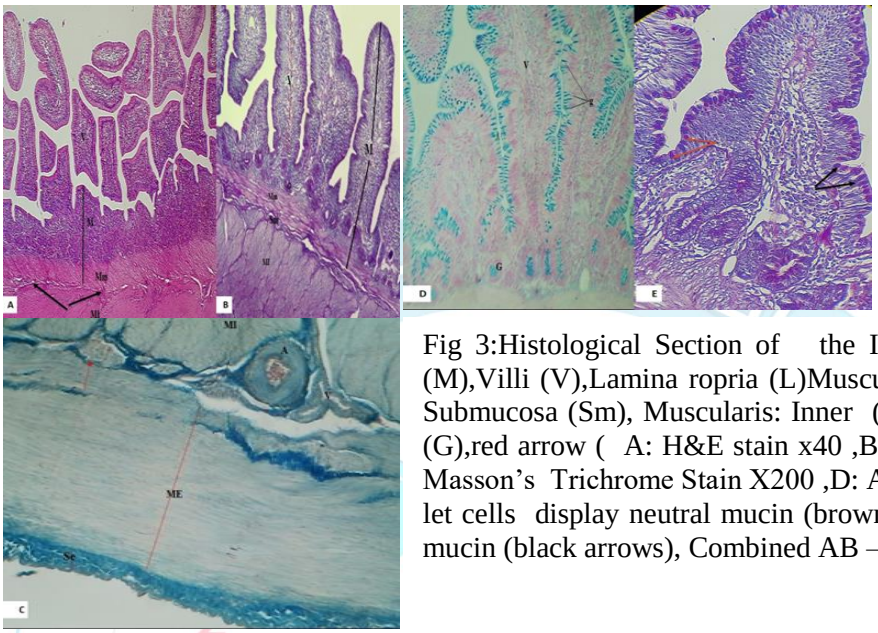

Fig 3:Histological Section of the Ileum shows: Mucosa (M),Villi (V),Lamina ropria (L)Muscularius mucosa (Mm), Submucosa (Sm), Muscularis: Inner (MI), intestinal glands (G),red arrow (A: H&E stain x40 ,B:PAS Stain X100 , C: Masson's Trichrome Stain X200 ,D: AB stain x100, E: goblet cells display neutral mucin (brown arrows) and acidic mucin (black arrows), Combined AB –PAS Stain X 200.

Discussion:

In the present study the duodenal mucosa composed of three different laminae including the epithelium, lamina propria, and muscularis mucosa, this result was similar to that found in pigeon (*Columba livia*) in African pied crow (*Corvus albus*) by[11] , mallard by [12] , in goose by [13], in Japanese quail by [14].The surface of epithelium covering and lining the villi and the intestinal glands respectively was simple columnar epithelium containing. Absorptive cell (columnar) cells with straight border this similar found in most species of bird epithelium in avian [8] min Africa pied crow (*corvus albus*) [15].The goblet cells: it is second cells scattered among columnar epithelium same as researches [16,17]. The distinct structure of the duodenal mucosa was different shape and size of well-developed long villi which its core were constructed from a connective tissue of the lamina propria that appeared very developed and contains longitudinal bundles of smooth muscle fibers, collagen fibers and blood vessels, same as reported by Kalita *et al.* (2012) in kadaknath fowls, [8] in black-winged kite *Elanus caeruleus*, by [18] in cattle egret. This result observed that the long villi was in the duodenum, it suggests that the long villi act to increase the surface area and greater absorption[19]

These results were in parallel with previous studies in it he yellows and blue macaws by [20] ,in Uttara fowl by [21] and [22], The goblet cells of the duodenal epithelium appeared few, scattered among the columnar cells in both villi and crypts, similar characteristic feature to that observed by [23] in pigeon and similar to that reported in

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African pied crow by [11] in which is observed the goblet cells are little also.

The crypts of leiberkuhn lined by the same epithelium covered the villi found between the bases of the villi and extended till the musculris mucosa, similar observations were recorded by, [24] in Owl (*Otus scors brucei)*. These studies showed that the duodenal mucosal layer was invaginated in the bases of the villi into straight tubular glands (crypts of Lieberkühn) which were continuous with the epithelial lining the villi, these glands were embedded in the connective tissue of the lamina propria and represent a further increase of the mucosal surface area. The crypts of Leiberkühn have been described in other avian species in Blue and Yellow macaws [20] in avian [8] .

The third elements of the mucosa was the muscularis mucosa which constructed by longitudinal smooth muscle fibers, similar structure was observed in mallard by (khaleel and Atiea, 2017), and in owl by [24] in which the muscularis mucosa arranged in longitudinal layer of smooth muscle fibers, but differ from that observed in ostrich *(Struthio camelus*) by [16] and by [25] in goose in which it composed of two layers of smooth muscle fibers while in African pied crow in which the muscularis mucosa was absent as reported by [11].

There were no significant differences in the height of columnar cells between the three segments of the small intestine, due to had no considerable difference in nutrients absorption capacity of enterocyte in the all segments of small intestine. In the present study, the villi height, mean crypts depth and the ratio of height of villi to crypts depth were higher in the duodenum than those of jejunum and ileum, as found by [26]. The intestinal epithelial cells change constantly, they compensate villi cells losses through the proliferation and maturation inside intestinal crypts then migration upward. The crypts depth is related with the intestinal cells turnover rate and increase in the crypts depth indicates to need of absorptive cells replacement and higher tissue turnover as reported in broilers [27]. The crypts depth may be an important factor which determines the ability of the crypts to sustain increase in the villus height and to maintain villi structure[28] . Ratio of villi height to crypt depth appeared higher in the duodenum than in other two segments. The increase of villi height to crypts depth ratio associated with the better nutrients absorption and faster growth [29].AS observed in most species, the present showed that the mean villi width was less significantly in the duodenum than those of two other segments. The mean thickness of mucosa was lesser than that recorded by [24] in pigeon, these differences may be attributed to species variation and diet habits. This study showed that mean thickness of mucosa was higher in the duodenum than those of two other parts of the small intestine, this may be due to the correlation between the height of villi with the thickness of mucosa.

In this study the submucosa appeared as a very thin layer of loose connective tissue that observed mostly around the nerve plexus and blood vessels, similar observation was reported by, [24] in pigeon. In current study, the absence of Brunner's glands in the duodenal submucosa was agreement with the previous study by [30] in chicken, and [21] in kadaknth fowls, in Mal-

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lard and [31] in guinea fowls, the authors stated that the lacking of Brunner's glands and their mucus secretion role was replaced by a many goblet cells and enteroendocrine cells present in the villi epithelium and intestinal glands, the I- entero endocrine cells effect on the pancreas exocrine part for secrete the sodium bicarbonate to neutralize the gastric acidity, not show significantly respectively[12] .

Thickness of muscular layer appeared to be higher significantly in the duodenum than those of the jejunum and ileum as noticed in [31] in guinea fowls, they observed an increase in thickness of tunica muscularis in the duodenum towards the ileum, this may be attributed to that the duodenum needs more muscle contractile activity to emptied its contents towards the ileum. This layer is responsible for peristalsis which aids propelling the [32].

This layer was the outer most layer formed from a thin layer of loose connective tissue covered by mesothelium, same finding found in owl and mallard by [24]. There were no significant differences in the thickness of this layer between duodenum, jejunum and ileum.

Generally, the histological structure of jejunal wall in turkey same as the duodenum. Which form four layers, this similar observation most avian species as in poultry turkey by[33] . In current study, the mucosa of the jejunum was arranged into two fundamental structures; villi and crypts, and it was thrown into large numerous leaf-shaped villi , resembling to that observed in broiler chicken [33], in Mallard *Anas platyrhynchos* by [12], in canary by [34] , In current results, some jejunal sections showed long leaf-shaped villi .it is arranged in branched to secondary and tertiary similar results were reported [24] in mallard and [31] in guinea fowls, the authors reported that the mucous membrane is thrown into numerous leaf-shaped villi that arranged in a zig-zag pattern in the pigeon, while in owl and mallard appear as blunt apical portion and wide basal part in the former and finger-like projections in the latter. Nutrients absorption is more efficient when villi are organized like this arrangement than if they are in parallel or randomly positioned. This would occur because the passage of food through the alimentary canal would take longer in the increase the contact between the nutrients and the villi [32] .

differences between the duodenum, jejunum and ileum.

In current study this muscular coat was made up of two layers of smooth muscle bundles, this was in line with [20]. In other avian species as chicken, blue and yellow macaw, common quail pigeon, duck and owl

In this study underneath the lamina propria was the muscularis mucosa, which observed as single layers of longitudinal smooth muscle fibers, similarly as documented in pigeon by Al-Samawy (2015). where they stated that in broiler the muscularis mucosa made up from longitudinally oriented smooth muscle fibers, but it was differently documented by [24] in owl and [35] in *Accipite Linnaeus*, they found that the muscularis mucosa was absent.

The submucosa layer was very reduced and poorly developed. similar organization of the jejunal submucosa was ob-

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served in pigeon [36], [35] in grainivorous birds and [25] in middle and heavy goose breeds.

Tunica muscularis: As observed in this study for the duodenum, the muscularis layer display as a thick inner circular and a thin outer longitudinal layers of smooth muscle bundles. were found within connective tissue between these two layers, the histological organization was mentioned in some avian species by; [37] in duck(mallard) , **Tunica serosa**: Similar structure of serosal layer of the duodenum was present in the jejunum same findings were observed in most avian species as mallard Khaleel and Atiea (2017), guinea fowls [31]

Ileum

The histological structure of ileum wall was similar to the previous two segments in consisting of four layers, similarly to that observed in chicken by, Dang (2009), in gosling white Roman by [38] and [39].Tunica mucosa :In this study the ileum mucosa showed various shape and size of villi which appeared as shorter, broader observed previously in the ileum of African pied crow *Corvus albus* by [11] and Mallard *Anas platyrhynchos* by [24], by [20], in mallard (*Anas platyrhynchos*) [12]. The small intestine of birds was known to have high absorptive rates to water and electrolytes .

Underneath the mucosal epithelium, the connective tissue formed lamina propria and occupied by the crypts (intestinal glands) which found at the bases of villi and opened between them, they formed as simple branches invaginations of epithelium, these finding were in compatible with that reported in mallard by [24] . The third element of mucosa was the muscularis mucosa which formed by a single layers of smooth muscle fibers, this finding was differ from that observed previously by [24] in both owl and pigeon in which this layer was absent, whereas in ostrich, the muscularis mucosa was constructed from three layers of smooth muscle fibers [16],but agree with that observed in guinea fowl by [31], responsible for the rhythmic movement of the villi which are important for absorption.

Tunica Submucosa: this layer appeared as a very thin. were found, as observed in Racing Pigeon *Columba livia domestica* by [36],in indigenous mallard, [37].Tunica Muscularis: in present study, the tunica muscularis was represented by two layers of smooth muscle fibers this observation was in line with that found [41], in Green winged teal and common quail. The thickness of this layer was less than that recorded in previous two segments as reported in some avian species by [42]in CARI Shyama chicken and [40] in Blue and Yellow Macaws

In present finding showed that the small intestine (duodenum, jejunum and ileum) contain the villi and crypt gland the goblet cell in both villi and gland which give positive reaction when used PAS stain this result means the present of polysaccharides substance those reported by [24]in both owl and pigeon, [8] in black Winged Kite[43] , the mucosa layer product by goblet cells were play a vital role in protecting the small intestine .Alcian blue stain reaction .when using AB(PH 2.5) stain the goblet cells in both villi and crypt give strong reaction those same finding was reported in [8] in black Winged Kite, [37] in mallard .combined AB-PAS stains reactions :when applying the combined PAS-AB (PH 2.5).the goblet cells gave positive reaction for combined AB(PH

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2.5) –PAS .these result indicated to presence of both neutral and acidic mucopolysaccharid and total protein similar result was reported in black wing kite by [8] the mucin contain neutral and acidic mucopolysaccharids .

of Lieberkühn, the villi varied in their shapes, heights, and width according to the part of intestine.

Conclusion:

Generally, the characteristic feature of the intestinal tract were the villi and crypts

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