

THE HISTOLOGICAL AND HISTOCHEMICAL STRUCTURE OF ILEUM IN THE SLENDER-BILLED GULL (*Chroicocephalus genei*)

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ABSTRACT

This study was aimed to identify the histological and histochemical composition of the Ileum in the Slender-billed gull (*Chroicocephalus genei*). It was using histological stains and histological chemical techniques. The results showed that the Ileum was relatively long, so it characterized into two parts: an anterior part and a posterior part. The results showed that the mucous layer in the two parts consisted of villi that appeared conical in the anterior region and conical to a triangle in the posterior part, and the villi were more numerous and longer in the anterior part. The villi covered with a simple columnar epithelial tissue containing goblet cells in the two regions, but the goblet cells were significantly more numerous in the posterior part. The secretory units of Lieberkuhn's crypts were more widespread and numbered in the posterior part. Clusters of lymphocytes also seemed close to the lymph nodule in the anterior part. The outer muscle layer consisting of one layer of smooth circular muscles appeared in the anterior part, while it was composed of three secondary layers in the posterior part. Histochemically, the two parts of Ileum showed different responses between them for PAS, AB, TB, BP and SB techniques. The study concluded that the histological and histochemical composition of the Ileum appeared somewhat complicated to suit the nature of the food of this bird.

Keywords: ileum, histochemistry, goblet cells, Lieberkuhn's crypts.

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التركيب النسيجي والكيميائي النسيجي للفانفي في النورس مستدق الراس (*Chroicocephalus genei*)

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المستخلص

جاءت هذه الدراسة التعرف على التكوين النسيجي والكيميائي النسيجي للفانفي في النورس مستدق الراس (*Chroicocephalus genei*). باستخدام الصبغات النسيجية والتقنيات الكيميائية النسيجية. اظهرت النتائج ان الفانفي طويل نسبياً ويتميز الى جزئين امامي وخلفي. وظهرت الطبقة المخاطية في الجزئين مكونة من الزغابات المخروطية الشكل في الجزء الامامي ومخروطية الى مثلثة الشكل في الجزء الخلفي، وتكون الزغابات اكثر عدداً واطول في الجزء الامامي. تغطي الزغابات بنسيج ظهاري عمودي بسيط حاوي على خلايا كأسية في الجزئين، وتكون هذه الخلايا اكثر عدداً في الجزء الخلفي. الوحدات الفارزة لخبايا ليبركن اكثر انتشاراً في الجزء الخلفي. كما ظهرت تجمعات من الخلايا اللمفية في الجزء الامامي فقط. الطبقة العضلية الخارجية مؤلفة من طبقة دائرية في الجزء الامامي، بينما مكونة من ثلاثة طبقات ثانوية في الجزء الخلفي. من الناحية الكيميائية النسيجية، اظهرت اجزاء الفانفي استجابات مختلفة لتقنيات PAS، AB، TB، BP و SB. واستنتجت الدراسة ان التركيب النسيجي والكيميائي النسيجي للفانفي يتلاءم مع طبيعة غذاء هذا الطائر.

الكلمات المفتاحية: الفانفي، كيمياء النسيج، الخلايا الكأسية، خبايا ليبركن.

INTRODUCTION

The source of food in animals is either animal or plant, and in light of the type of food, animals divided into carnivores, herbivores and omnivores (19). The digestive tract has many functions which are necessary for organisms, such as digestion and absorption of nutrients (13). In the vertebrates, the digestive system consists of the alimentary canal and digestive glands, that are liver and pancreas (28). The histological structure of the digestive tract in birds is following feeding and dietary preferences (35). Studies have shown differences in the function, structure and morphology of the birds' digestive tract, and these may be due to evolutionary steps, as well as the type of food, the way of eating, and the environment in which the bird lives (24). The mechanism of food is an essential factor determining the success of the adaptation of birds in their environments, and the structural components of the digestive system vary according to the nature of the food and the method of feeding (18). The digestive system in birds consists mainly of the pharyngeal cavity, digestive canal and glandular attachments, the digestive system begins with the beak made of keratinized structures, which in turn surrounds the mouth. The beaks play a large role in obtaining food. The mouth also contains the tongue that differs in shape and size according to the type of bird, its way of feeding, and the nature of its food (27). Food moves from the mouth to the esophagus, which in turn connects with the glandular stomach, Proventriculus and then to the gizzard, then the small intestine or Ileum, and the large intestine or Rectum (12). In the small intestine of birds, the food components are broken down into simple molecules, such as small peptides, amino acids, free fatty acids, and monosaccharides. Often these particles are absorbed in the duodenum and jejunum and transported to other tissues. The intestinal mucosa is severely twisted and specialized to facilitate maximum absorption of nutrients. The epithelium is in villi and epithelial cells have an apical side covered by a dense microphilic covering that forms the boundaries of the brush. This increases the surface area of the small intestine for absorption by nearly 600 times, leading to an increase in the

absorption capacity of nutrients (31). The current study aimed to identify the histological structure of the Ileum in Slender-billed gull (*Chroicocephalus genei*) from carnivores, as well as the histochemical detection of the materials secreted or grouped in the Ileum in this bird.

MATERIALS AND METHODS

Ten birds were dissected (five from each sex) after being anaesthetized with chloroform in the histology and comparative anatomy laboratory, Biology department, Education college for Pure Sciences, Mosul university. The dissection performed following the instructions of the Ethics Committee of the Canadian Animal Welfare Council (CCAC) (Certificate No. 2010-015). After the eradication of ileum, it divided into two parts: the anterior part which was close to the jejunum, and the posterior part close to the rectum. After that, were taken a suitable part for the microscopic preparation process from each region (7), and this process is done according to the method of (4). After completing the process of preparing the histological slides(8,26). They coloured with Heamatoxylin and Eosin Stain (H&E) (7,8), Mallory's Trichrome stain (TS) (1) and the Azan stain (AZ) (29), the general histological stains. As well as, Alcian Blue pH 1 and pH2.5 (AB), Toluidine blue (TB) and Periodic Acid - Schiff (PAS) technique, which used to detect carbohydrates (29), Bromophenol blue (BP) technique, which used to identify proteins (14), and Sudan black B (SB) technique, which used to detect lipids (14).

RESULTS AND DISCUSSION

Histological results: The results showed that the mucosa layer of the anterior part of the Ileum forms villi that appeared as conical extensions of a large number of approximately one shape. It characterized by being relatively long and has an average ($695.132 \pm 7.234 \mu\text{m}$) while its average thickness ($105.851 \pm 3.546 \mu\text{m}$) (fig 1a,b,c). A simple epithelial columnar tissue covers these villi based on a basement membrane. These cells are characterized by having spherical nuclei in the basal site with an average diameter ($3.902 \pm 0.917 \mu\text{m}$). The epithelial tissue also contains the goblet cells, which are unicellular secretory glands that have appeared very

widespread among the columnar cells. These cells are characterized by their large secretory vesicles. These secretory vesicles are of different sizes, which indicate that these cells are in different secretory cycles (fig 1c,d,e). The lamina propria of the mucosa layer is composed of loose connective tissue, which contains collagen fibers, blood, and lymphoid vessels, lymphocytes, and muscle fibers. The lamina propria extends inside the villi to form the structure of the villi and its supporting pillar (fig 1a,b,c,d,e). The lamina propria inside the villi distinguished by its muscle fibers and large blood vessels as well as other compounds of lamina propria. The lamina propria at the bottom of the villi contains intestinal glands or Liebrkuhn's crypts, which are branched tubular glands, whose secretory units have a spherical shape with a diameter average ($41.337 \pm 2.043 \mu\text{m}$). These units also characterized by its secretory cells that are two types of cells, the first type has columnar cells with dense cytoplasm and a central spherical nucleus, which it's a diameter average ($3.048 \pm 0.214 \mu\text{m}$). In contrast, the second type of cells has a clear cytoplasm and an oval-shaped nucleus with an average diameter ($3.657 \pm 0.344 \mu\text{m}$). The percentage of cells of the two types differs in neighbouring secretory units. Also, some secretory units contain the enteroendocrine cells, which differ from the secretory cells in their two types as being triangular or pyramid cells located in the base of the secretory units (fig a,b,c,f,g,h, i). It also found in some regions of this part of the ileum large clusters of lymphocytes that do not form a distinct lymph nodule due to not surrounding it with a particular shell, but these cells occupy a large part of the lamina propria and become the most common compound among other components of the lamina propria. The muscularis mucosa, under the lamina propria, appeared in the form of a thin layer of smooth muscle fibers, and some fibers extend to the lamina propria inside the villi (fig 1c). The submucosa layer appeared as a thin layer of loose connective tissue similar to that found in the lamina propria. In contrast, the muscularis externa layer appeared in the form of a single layer in a circular arrangement of smooth muscle fibers with an average thickness ($337.314 \pm 4.294 \mu\text{m}$) consisting of undulating

muscle bundles interconnected with each other, between them, in some areas, blood vessels and loose connective tissue separate these bundles. The serosa layer surrounds the muscle layer from the outside and is in the form of loose connective tissue from the outside with a simple squamous epithelial tissue. Between the muscularis layer and the serosa layer, there are blood vessels and Auerbach's plexus (fig 1a,b). While the posterior part of the Ileum in this bird showed significant differences from the anterior part of it, the villi appeared conical in form, some are long, and others are short as they were branched and meet with each other which gives them a triangle shape. Their number is less than in the anterior part, as they appeared shorter in length including in the anterior part, the average length of short villi was ($218.185 \pm 4.874 \mu\text{m}$), and the length of long villi was ($504.334 \pm 7.434 \mu\text{m}$). In contrast, the average thickness in general was ($53.554 \pm 3.114 \mu\text{m}$) (fig 2a,b,c,d). These villi covered also with a simple columnar epithelial tissue with spherical nuclei with an average diameter ($4.855 \pm 1.976 \mu\text{m}$), based on a relatively thick, straight, basement membrane in most parts. While the goblet cells in this part are characterized by being more and occupying most of the epithelial tissue, and in some parts, the goblet cells are the ones that make up the almost entirely epithelial tissue. The secretory vesicles of these cells characterized by being larger and all appear to be about one size (2e,f,g,h). The lamina propria inside the villi is relatively thick and rich in blood vessels, collagen and muscle fibers. While the lamina propria at the bottom of the villi characterized by being consisted of loose connective tissue. It is rich in the secretory units of the intestinal glands, which distinguished from what appeared in the anterior part by occupying most of the lamina propria and forming three or more rows below the villi. These secretory units appear in almost one shape, which is the spherical or discoid shape has an average diameter of ($56.094 \pm 6.564 \mu\text{m}$). The secretory cells are all in the same form and are columnar cells with a relatively thick cytoplasm and an unclear nucleus. Some secretory units contained similar enteroendocrine cells as it appeared in

the interior part. While no groupings of lymphocytes that appeared in the anterior part, but there are a large number of lymphocytes between the components of the lamina propria. Muscularis mucosa appeared similar to that in the anterior part of the Ileum as well as the submucosa layer (2a,b,c,d,g,h, i). While the Muscularis layer distinguished by being composed of three secondary layers of smooth muscle, unlike the anterior part, the first layer is a relatively thin smooth circular arrangement with an average thickness ($27.467 \pm 2.435 \mu\text{m}$). In contrast, the second layer longitudinally arranged. It makes up most of the Muscularis layer as it is very thick, it the average diameter ($341.037 \pm 4.764 \mu\text{m}$). These muscles are in the form of muscle bundles tightly bound together, separating one bundle from another thin layer of loose connective tissue contain blood vessels. These bundles appear in the cross-section in the form of reciprocal triangles, while the third layer is also circular and thin, and it's the thickness is ($24.814 \pm 3.561 \mu\text{m}$). The serosa was similar to the anterior part, and it also found of the Auerbach's plexus between the muscularis and the serosa (fig 2a,b,c,d).

Histochemical results

The histochemical results showed a variation in the response of the Ileum tissues between the anterior part and the posterior part on the one hand, and the histochemical techniques used on the other hand. Table (1) shows the response of epithelial tissue, goblet cells and secretory units of the intestinal glands in the anterior part of the Ileum to the histochemical techniques used. The table shows a positive response that ranges from medium to strong for PAS technique, as the response was strong for epithelial tissue while the reaction was a medium positive for the secretory units of the intestinal glands, and this indicates the presence of secretions of neutral mucous substances and polysaccharides in the composition and secretions of these tissues (fig1e,g). Table (1) also indicates a medium positive response to the TB technique in the epithelial tissue as well as the secretory units. That suggests the presence of medium concentrations of mucosal acid polysaccharides, as the secretory vesicles of the goblet cells (fig1i). Also, Table (1) shows a

strong positive response in the epithelial tissue and a medium positive for the secretory units of AB pH1, pH2.5 techniques. Which indicates the presence of sulfated mucous substances that are highly acidic and mucous substances with weak acidity for the two techniques respectively (Fig 1a,d,f). While Table (1) shows a negative response to epithelial tissue and a medium positive in some secretory units of BP technique, which indicates the absence of protein secretions in the epithelial tissue, while some secretory units have the presence of protein substances in their secretions (fig 1h). Also, the epithelial tissue and intestinal glands showed a negative response to the SB technique as indicated in Table (1), which leads to the absence of secretions or aggregation of fatty substances inside these structures. While Table (2) indicates the response of epithelial tissue, goblet cells and intestinal glands in the posterior part of the Ileum in this bird to the histochemical techniques used. The epithelial tissue and secretory units of the intestinal glands showed a positive response to the PAS technique as indicated in Table (2), which means the epithelial tissue had a similar reaction to that seen in the anterior part. In contrast, the secretory units with a positive response were stronger than they appeared in the anterior part, which indicates the presence of neutral mucosal substances in the secretions of the epithelial tissue and intestinal glands (fig 2f,h, i). While the response of these tissues to the TB technique was stronger than in the anterior part of the Ileum in this bird, and this indicates the presence of acid mucosal polysaccharides as shown in Table (2) (fig 2c). Also, (Table 2) shows that there is a stronger positive response than it appeared in the anterior part of AB pH 1, pH 2.5 techniques for both epithelial tissue and secretory units. That shows the presence of higher concentrations of sulfated mucous substances with strong acidic and weak acidic in the secretions (fig 2a,e). While epithelial tissue and secretory units showed a negative response to BP technique, as Table (2), which suggests the absence of protein secretions in the secretions of these glands. While the response of these structures was similar to

their reaction in the anterior part regarding the SB technique.

Histological results

The results showed that the mucosa layer consisted of villi that work in this bird and other birds to increase the surface area of the ileum and thus increase the absorption surface. The villi appeared conical in the anterior part and conical to triangular in the posterior part, this is similar to what appeared in Japanese quail (34), Indigenous Ducks (20), starling, zebra finch and Pin-tailed sandgrouse (3,5). Whereas it appeared in small leaf-shaped arranged in a zig-zag pattern in Mallard (6). The villi were longer and more numerous in the anterior part. In contrast, Ileal villi had a more blunt appearance and were shorter than

those of the broiler chicken's jejunum (9). There is an increase of the villi thickness in Japanese quail (34), Mallard (6), while in the cattle egret, the villi are straight, short and less numerous the cattle egret (17), whereas, in pheasant, the villi shorter and thicker (25). The villi covered with a simple columnar epithelial tissue containing goblet cells, which is evident in all studied birds (25,34). The goblet cells were more numerous in the posterior part, and this observation was similar to some studies that reported that goblet cells more in number as the gut traced caudally (23). The goblet cells play a role in the formation of the mucous barrier, and this barrier in turn acts as the first line of defense against food pathogens as well as other luminal antigens (11).

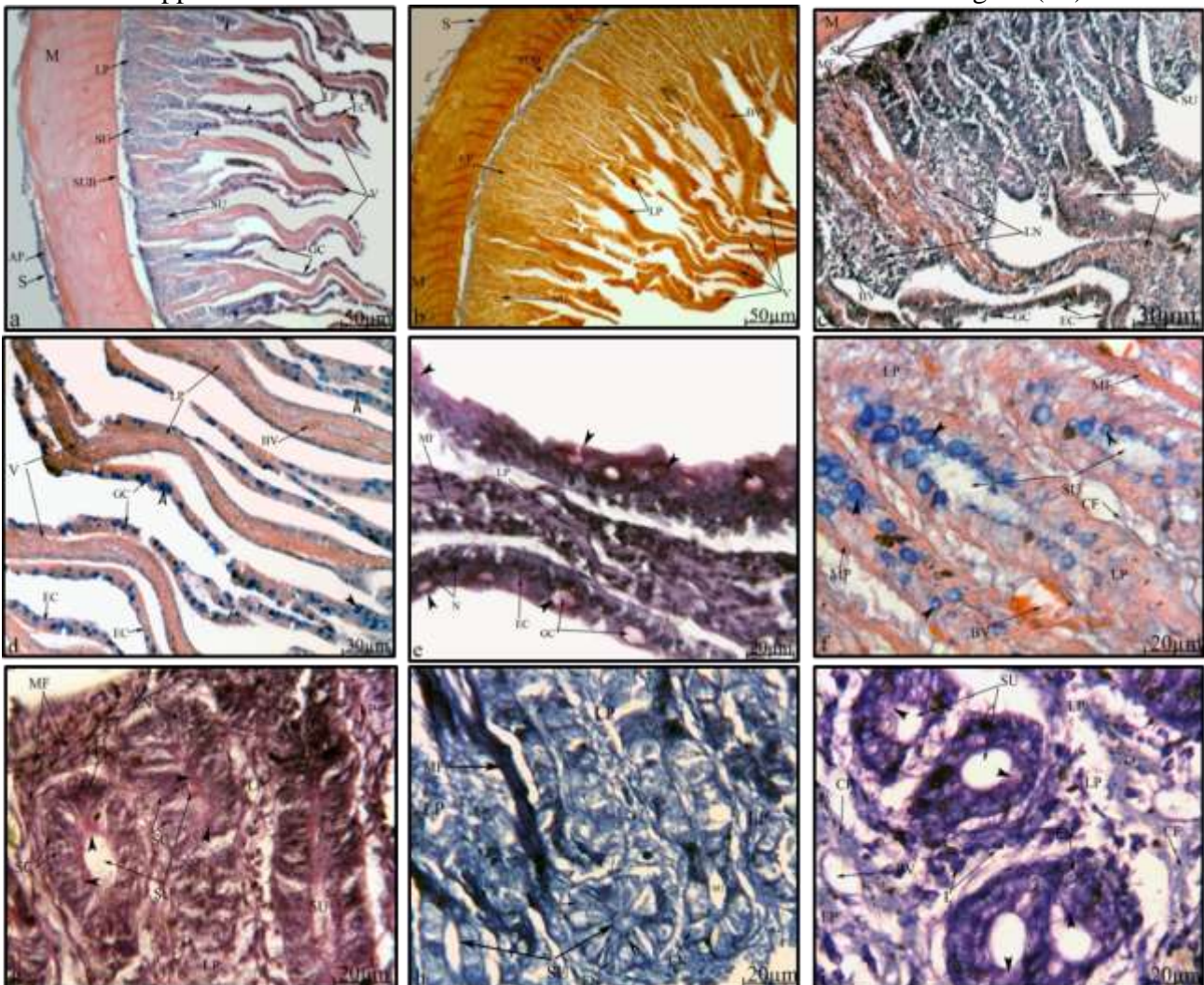


Figure 1. Ileum in Slender-billed gull (anterior part): (a) AB pH 1 technique; (b) AZ stain ; (c) H&E stain; (d) AB pH 2.5 technique; (e) PAS technique; (f) AB pH 1 technique. (g) PAS technique; (h) BP technique; (i) TB technique. (abbreviations: M= Muscularis externa; SUB= Submucosa; S= Serosa; AP= Auerbach's plexus; SU= Secretory units; V= Villi ; EC= epithelial cells; GC= Goblet cells; N= Nucleus; LP= Lamina propria; MF= Muscles fibers; EN= Enteroendocrine cells; BV= Blood vessels; CF=Collagen fibers; LN=lymphoid nodules; SC= Secretory cells; L= lymphocytes; arrowhead= positive response).

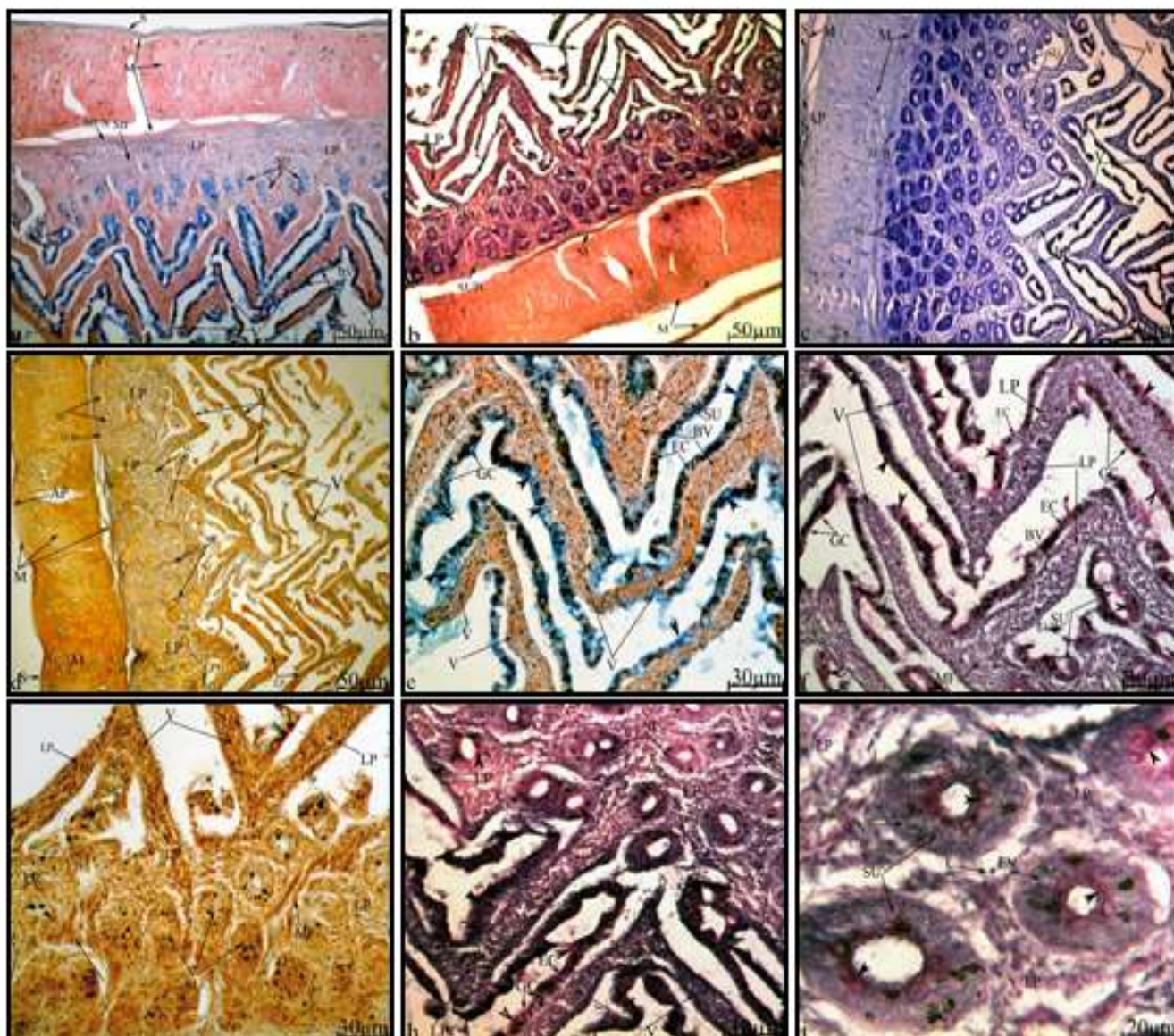


Figure 2. Ileum in Slender-billed gull (posterior part): (a) AB pH 1 technique; (b) H&E stain ; (c) TB technique; (d) TS stain; (e) AB pH 2.5 technique; (f) PAS technique; (g) AZ stain; (h) PAS technique; (i) PAS technique. (abbreviations: M= Muscularis externa; SUB= Submucosa; S= Serosa; AP= Auerbach's plexus; SU= Secretory units; V= Villi ; EC= epithelial cells; GC= Goblet cells; N= Nucleus; LP= Lamina propria; MF= Muscles fibers; EN= Enteroendocrine cells; BV= Blood vessels; CF=Collagen fibers; LN=lymphoid nodules; SC= Secretory cells; L= lymphocytes; arrowhead= positive response).

Table 1. Response of anterior part of the Ileum to the histochemical techniques

Tissue	Technique					
	PAS	TB	AB pH 1	AB pH 2.5	BP	SB
The epithelial tissue	+++	++	++	++	.	.
Intestinal glands	++	++	++	++	++	.

Table 2. Response of posterior part of the Ileum to the histochemical techniques

Tissue	Technique					
	PAS	TB	AB pH 1	AB pH 2.5	BP	SB
The epithelial tissue	+++	+++	+++	+++	.	.
Intestinal glands	+++	++/+++	+++	+++	.	.

The results also showed that the lamina propria is formed of loose connective tissue of similar composition in the two parts of the ileum. These also appeared in the common pigeon (30), the cattle egret (17), and Indigenous Ducks (19). Liebrkuhn's crypts appeared in the anterior and posterior parts. Still, they are more numerous and more widespread in the posterior part, which is what appeared in other birds as in cattle egret (17), common pigeon (30), and Japanese quail (34). The secretory units in the front part were distinguished by having two types of secretory cells. In contrast, the secretory units in the posterior part contained a kind of cells, which also appeared in the zebra finch (3). Also, some secretory units contained enteroendocrine cells in the anterior and posterior parts, which also appeared in Indigenous Ducks (20), starling, the zebra finch (3). The lamina propria in the anterior part was also distinguished by having large clusters of lymphoid cells, these lymphoid cells also appeared in others birds (2). The muscularis layer composed of one layer of muscle, and it formed from three secondary layers at the posterior region. These three secondary layers appeared in goose (21), also appeared in the common pigeon. Still, the middle layer was circular and thicker (30), whereas, it composed from two layers in most birds (25).

Histochemical results

The histochemical results showed that the epithelial tissue and the goblet cells showed a strong positive response to the PAS technique in the anterior and posterior parts of the ileum. At the same time, the reaction of the intestinal glands to this technique was also positive. It is stronger in the posterior part, and this indicates the presence of neutral mucous substances and polysaccharides. The same results appeared in starling, zebra finch and Pin-tailed sandgrouse (3,25). The goblet cells' positive reaction of the PAS well recorded in the broiler's ileum indicating a significant role in facilitating the movement of the ingesta and lubricating the tract (31). The neutral mucous in the mallard of crypts of Lieberkühn cells have appeared, and previously, similar findings recorded in the black-winged kite's ileum (16). When goblet cells secreted Mucins, It provide an area in which growth factors, cytokines, and

chemokines are sequestered and create an effective physical barrier (22). While the response of the anterior and posterior parts was positive for TB technique, it was stronger in the posterior part than in the anterior part and for both tissues, and this indicates the presence of medium concentrations of mucosal acid polysaccharides. These results also founded in starling and zebra (3). As for the techniques of AB pH1 and pH 2.5, the epithelial tissue and secretory units showed positive for the technique in both parts, but they were stronger in the posterior part. The same results appeared in starling, zebra finch, Pin-tailed sandgrouse and Indigenous Ducks (20,25). The mucous layer plays an essential role in protecting the epithelial cells of the small intestinal, lubrication and nutrient transport between the lumen and the brush border (32). While some secretory units of the intestinal glands of the anterior part showed an average positive response to BP technique, while the rest of the tissues showed a negative reaction in both regions, while the mucosa in Mallard and duck gave a weak response to the same technique (15). While the reaction was negative for SB technique and all tissues and both the anterior and posterior parts, and this what appeared in other birds starling, zebra finch and Pin-tailed sandgrouse (3,5).

Conclusions

We can conclude from the current study that the ileum in this bird was complementary to the rest of the small intestine and is somewhat complicated to fit in with it being the last part of the digestion process and to fit in with the nature of the food of this bird and this demonstrated by the type of secretions secreted by the tissues of this organ, which was mostly mucous, is suitable for the location of this organ and its function in digestion and absorption, as well as defending against organisms and pathogens that may found in the food intake.

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