



## Comparative histological and histochemical study of the ileum in two different birds

A.M. Taha 

Department of Biology, College of Education for Pure Science, University of Mosul, Mosul, Iraq

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#### Correspondence:

A.M. Taha  
[amhamdany@uomosul.edu.iq](mailto:amhamdany@uomosul.edu.iq)

### Abstract

This study aimed to know the comparative histological structure and histochemistry of the Ileum in two birds that are the Turkey (*Meleagris gallopavo*) and the Cockatiel (*Nymphicus hollandicus*) using histological stains and histochemical techniques. The results showed that the Ileum wall in the two birds is composed of the histological layers that make up the rest of the gut wall, with many differences between them. The villi appeared in Turkey in various shapes, longer and more numerous than in the Cockatiel. The villi lined with a simple epithelial tissue in the two birds, containing the goblet cells. Lieberkuhn's crypts under the villi appeared in the two birds and had spherical secretory, and some secretory units contained the enteroendocrine cells. The Muscularis Externa appeared distinctively in Turkey than in the Cockatiel, and it was of three sublayers while in the Cockatiel from two sublayers. Histochemical results showed medium to a strong positive response to the epithelial tissue to AB pH 1, AB pH 2.5, PAS techniques while a weak positive in the intestinal glands to these techniques, with varying degrees between the two birds. While the response was positive for BP technique in the epithelial tissue and negative in the glands, as well as the same response appeared for the SB technique. The current study concluded that the Ileum structure in the two birds is identical with histological and histochemical differences that are appropriate to the nature of each bird's food.

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### Introduction

The Class of Avian places second in the number of species among vertebrates. There are approximately 8,600 species of birds in the world. All birds adapted to their different environments for food sources, the seashore, ponds, small rivers, fields, or mountains. Reflecting their different lifestyles, birds have different feeding habits, with corresponding differences in the structures of their digestive canal (1). The avian digestive canal has undergone a physiological structure inapposite to other animals to accommodate physical and chemical features of a wide variety of food types, and requirements for flight (2). The structure of the avian intestine varies from one species to another. It thought that those differences based on changing diets in different species. In carnivorous birds like the

buzzard, the small intestine develops fully, but the cecum does not grow as well as the small intestine (3-4). The small intestine in the birds located between the gizzard and ceca and consists of three undifferentiated sections in the avian gut (the duodenum, jejunum and Ileum). The functions of the Avian's small intestine are similar to those in mammals and include processing of food using enzymes and bile excreted from the pancreas and liver, as well as nutrient absorption (5). The vertebrate digestive tract also is an important endocrine organ because it contains an array of endocrine cells, which produce a range of regulatory peptides such as cholecystokinin, somatostatin, neurotensin, gastrin and serotonin. The secreted regulatory peptides control functions of the gastrointestinal tract that include motility, digestion and secretion (6).

This study came to complete the previous studies that dealt with the digestive tract in birds. It aimed to identify the histological and histochemical structure of the Ileum in two different types of birds which are Turkey (*Meleagris gallopavo*) and Cockatiel (*Nymphicus hollandicus*).

**Materials and methods**

Five adult birds (5 of each type of bird 3 male and 2 female) dissected in the laboratories of the Department of Biology / College of Education for Pure Sciences, University of Mosul, and conditions for animal welfare and euthanasia observed during the dissection process (7,8). After the ileum extraction and samples were taken from a different of regions, the histological preparation steps performed according to the method (9,10). Then the staining process was performed using the following stains, Heamatoxylin and Eosin Stain (H&E) (11,12), Mallory's Trichrome stain (TS) (9), Azan stain (AZ) (13), Alcian Blue (AB) pH 1 and pH 2.5 techniques (13), and Acid - Schiff (PAS) technique, which used to detect carbohydrates and mucous substances (13), 5-Bromophenol blue (BP) technique, which used to identify proteins (14), Sudan black B (SB) technique, which used to detect lipids (14).

**Results**

**Histological results**

The results showed that the mucosa layer of the Ileum in both birds consisted of villi, which are extensions towards the lumen of the Ileum. In the turkey, the villi had multiple shapes, different lengths, and uneven distribution because it's too long. Some villi had conical shape and this predominant shape in addition to the triangular, spindle, fungal, filamentous and rectangular villi. Some of them also appeared long, extending to the middle of the ileum lumen, reaching length to  $585.213 \pm 7.547 \mu\text{m}$ . In contrast, others were short and falling between long villi and their average length  $196.533 \pm 6.032 \mu\text{m}$ , as the thickness of these villi differed, as some of them were very thick, it's average thickness was  $88.321 \pm 4.214 \mu\text{m}$ , and some other is thin and has an average width  $37.854 \pm 2.845 \mu\text{m}$ . It also appeared in some areas with a large number and the other regions with fewer numbers, but in general, it was a large number (Figures 1-3). While the villi in the Cockatiel were shorter in length and less in number than the Turkey villi, but almost all in one form, which is a conical shape, some of which also appeared branched. Some villi showed long, and others were short, the average length of long villi  $360.055 \pm 5.931 \mu\text{m}$  and the average of short villi  $130.536 \pm 3.554 \mu\text{m}$ , while its average thickness was  $45.101 \pm 1.217 \mu\text{m}$  (Figures 4-6).

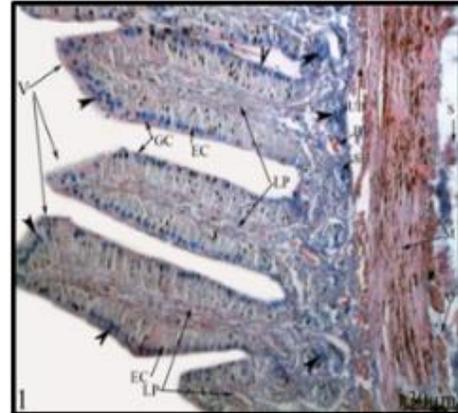


Figure 1: cross-section in the Ileal villi in Turkey. AB pH 2.5 technique.

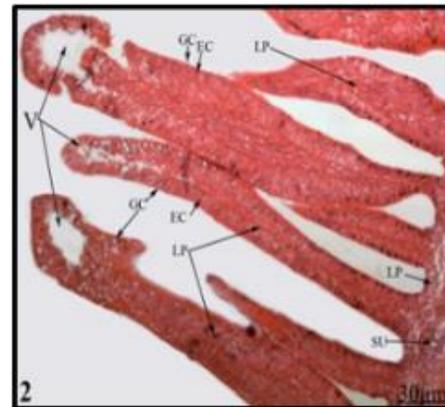


Figure 2: cross-section in the Ileal villi in Turkey. HandE stain.

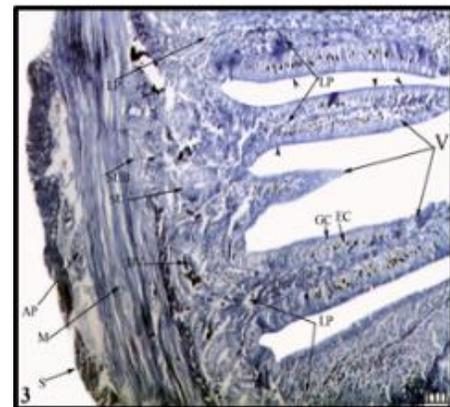


Figure 3: cross-section in the Ileal villi in Turkey. BP technique.

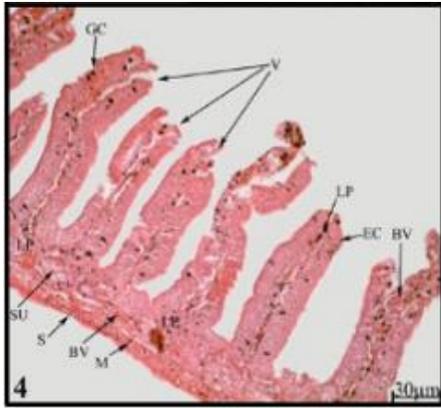


Figure 4: cross-section in the Ileal villi in Cockatiel. HandE stain.

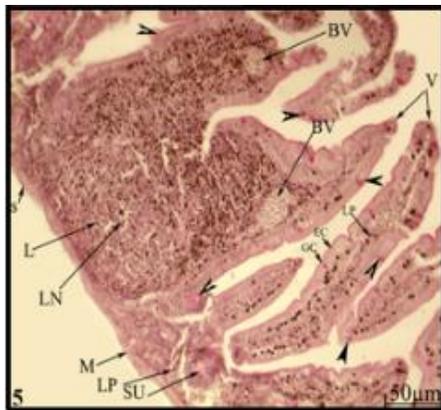


Figure 5: cross-section in the Ileal villi in Cockatiel. PAS technique.

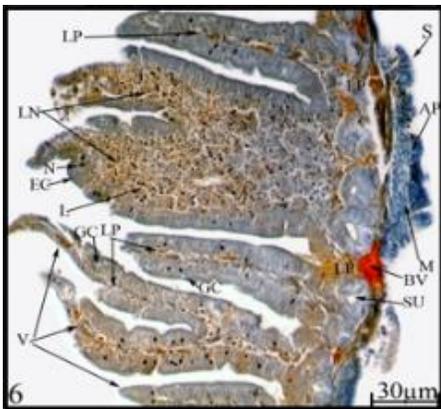


Figure 6: cross-section in the Ileal villi in Cockatiel. TS stain. (V) villi; (GC) Goblet cells; (EC) Epithelial cells; (LP) Lamina propria; (SUB) Submucosa; (BV) Blood vessels; (SU) Secretory units; (S) Serosa; (M) Muscularis; (AP) Auerbach's plexus; (LN) Lymph nodules; (L) Lymphocytes; (arrowhead) positive response.

These villi covered in both birds with a simple, non-ciliated columnar epithelial tissue. Its average length  $26.705 \pm 1.815 \mu\text{m}$  and width  $5.976 \pm 0.447 \mu\text{m}$  in Turkey, and these cells had oval nuclei located at the bottom of the cells with an average diameter  $3.886 \pm 0.112 \mu\text{m}$ . These cells were based on a square basement membrane, whereas the apical surface had microvilli that form with each other the brush border. The average length of these microvilli had reached  $3.294 \pm 0.747 \mu\text{m}$ . These cells were also characterized by the abundance of cellular organelles inside these cells, which indicates the activity of these cells (Figures 7 and 8). While the average length of cells of epithelial tissue in the Cockatiel  $15.119 \pm 2.537 \mu\text{m}$  and width  $4.96 \pm 0.331 \mu\text{m}$ , these cells were more pronounced than in Turkey and were based on a less noticeable basement membrane than what appeared in Turkey. The nuclei of these cells were spherical with an average diameter  $4.268 \pm 0.187 \mu\text{m}$ , and the nuclei of these cells were more distinct from them in Turkey. These nuclei appeared more evident than in Turkey, and some nuclei may contain two nuclei. The microvilli of the apical surface also composed the brush border, and the average length of microvilli was  $2.803 \pm 0.007 \mu\text{m}$  (Figures 9-11).

The epithelial tissue of both birds contains goblet cells that differ in numbers in the ileum regions of the turkey. Its appeared in large number in some areas and a medium in number in other areas, but in general, it was a large number, especially at the top of villi (Figures 1-3, 7 and 8). While it appeared in the Cockatiel less significantly than in Turkey, as it seemed to spread among epithelial cells at distances far from each other and the secretory vesicles were smaller than their Turkey (Figures 4-6, 9-11).

The lamina propria was composed of a loose connective tissue containing blood vessels, collagen fibres, lymphocytes and other cells in both birds. The lamina propria extended inside the villi to form the supporting structure for it. All villi contained the components of the lamina propria, and the distribution and density of the elements of the lamina propria vary from villi to another. In Turkey, there were some large blood vessels in some, while there were other muscle fibres that were prevalent, as lymphocytes spread within the components of the lamina propria within villi (Figures 1-3, 7 and 8). While the lamina propria inside the villi in the Cockatiel was less thick than what appeared in Turkey and also characterized by its containment of blood vessels, muscle fibres, many lymphocytes, and some large blood vessels and occupies most of the lamina propria inside the villi (Figures 4-6, 9-11).

While the lamina propria under the villi contained the secretion units of the intestinal glands or Lieberkuhn's crypts, which appear in the form of compound tubular glands in the two birds. These units in Turkey were spherical with an average diameter  $46.215 \pm 3.417 \mu\text{m}$  and the secretion cells inside these units were sizeable columnar size with an average length was  $11.949 \pm 2.047 \mu\text{m}$  and their width  $4.756 \pm 1.017 \mu\text{m}$ . Most of these cells had cytoplasm

containing a spherical and basal nucleus its average diameter  $3.719 \pm 0.027 \mu\text{m}$ . Some of these cells appeared to have two nuclei, and the cavity of most of these units is relatively small (Figures 1-3,12-14). Whereas the secretion units of the intestinal glands in the Cockatiel were also spherical, the average diameter  $38.655 \pm 5.915 \mu\text{m}$ , it was in the form of one row in some regions and two rows in the other areas under the villi. The cells of the secretion units were similar to those that appeared in Turkey, except these cells were less transparent than they appeared in Turkey. The average length of these cells  $14.51 \pm 1.584 \mu\text{m}$  and their width  $5.976 \pm 1.021 \mu\text{m}$  as their spherical nuclei were of an average diameter  $3.657 \pm 0.051 \mu\text{m}$ , and they are also less pronounced than in Turkey (Figures 4-6, 15-17). Some secretion units in the two birds also contained enteroendocrine cells that appeared pyramidal with a transparent cytoplasm and a central nucleus. The average nucleus diameter was  $3.048 \pm 0.045 \mu\text{m}$  in Turkey and the Cockatiel  $3.902 \pm 0.557 \mu\text{m}$  (Figures 12-14 and 16).

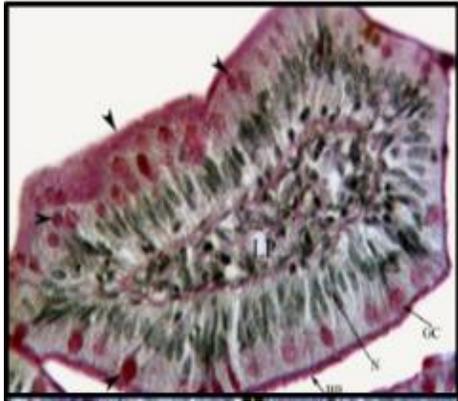


Figure 7: cross-section in the villi of Ileum in Turkey. PAS technique.

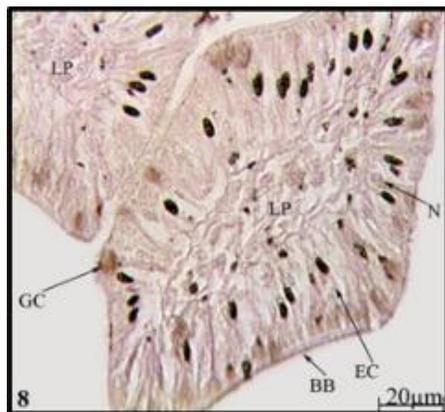


Figure 8: cross-section in the villi of Ileum in Turkey. B technique.

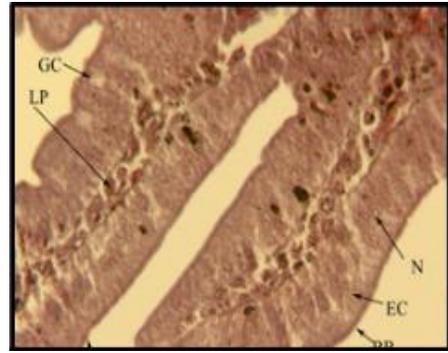


Figure 9: cross-section in the villi (V) of Ileum in Cockatiel. AZ stain.

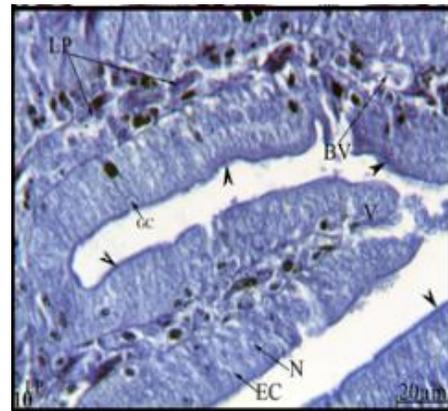


Figure 10: cross-section in the villi (V) of Ileum in Cockatiel.



Figure 11: cross-section in the villi (V) of Ileum in Cockatiel. PAS technique.

The Ileum of the two birds distinguished by the presences of lymph nodules or payer's patches, which appeared in Turkey in the last part of the Ileum, which was very large, as it occupied about five villi and extended their length from the submucosa layer to the top of these villi, and the average range of this node was  $445.002 \pm 5.592 \mu\text{m}$ . This nodule distinguished by being entirely occupied by lymphocytes and other lymph node contents from blood and lymph vessels. In

contrast, the rest of the lamina propria contents disappeared utterly, as the secretory units and muscle fibres did not notice, on the other hand, these nodules contained clusters of lymphocytes were spherically separated from the rest of the nodule by a row of cells representing the cortex and separated from each other. These clusters randomly distributed (Figure 18). While Peyer's patches in the Cockatiel were smaller in size, but they were more pronounced than they appeared in Turkey, as they occupied two adjacent villi and surrounded them from the top and sides of the epithelial tissue. At the same time, they bordered by the submucosa and had reached a height almost  $293.576 \pm 4.583 \mu\text{m}$  and width  $187.303 \pm 3.125 \mu\text{m}$  as well they were characterized by the large number of large blood vessels contained in them, as well as lymphocyte agglomerations (Figures 5 and 6).

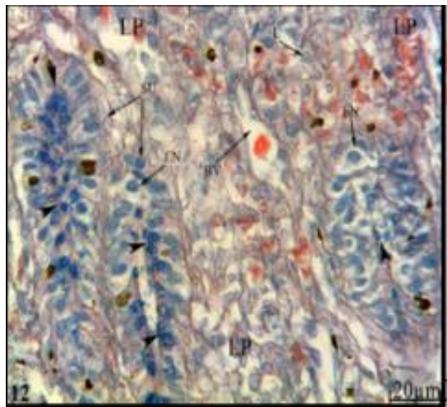


Figure 12: cross-section in the Lamina propria of Ileum in Turkey. AB pH 1 technique. (GC) Goblet cells; (EC) Epithelial cells; (LP) Lamina propria; (BB) Brush border; (N) Nucleus; (BV) Blood vessels; (SU) Secretory units; (L) Lymphocytes; (EN) Enteroendocrine cells; (arrowhead) positive response.

The muscularis mucosa layer in the two birds was in the form of smooth muscle fibres that extended in random directions within the lamina propria and inside the villi. The submucosa layer was composed of loose connective tissue, and its components overlap with the lamina propria components in both birds.

The muscularis externa layer appeared somewhat distinctively in the Turkey Ileum, as it consisted of three secondary layers. Still, the arrangement of these layers differed from the anterior part of the Ileum from the posterior part of it, as it was in the anterior part consisting of a sheet of smooth longitudinal arrangement located below submucosa and its average thickness was  $19.408 \pm 1.547 \mu\text{m}$ . The second layer was circular smooth muscles, and it's the thicker layers. Its average diameter was  $104.332 \pm 4.745 \mu\text{m}$ . It consisted of enormous muscle bundles, which were very slowly compact in some regions and disjointed in others.

While the third layer longitudinally arranged and had an average thickness  $22.324 \pm 2.458 \mu\text{m}$  (Figures 1 and 3). While it appeared in the posterior part of different arrangement, as the first layer seemed to be a circular arrangement, its average thickness  $30.577 \pm 3.543 \mu\text{m}$  and the second layer had a longitudinal arrangement, which was the thickness of the layers and its average thickness  $186.822 \pm 7.856 \mu\text{m}$ , appeared in bundles in the cross-section of the square shape, separated from each other by a connective tissue containing blood vessels. In contrast, the third layer was circular, with an average thickness  $37.359 \pm 4.521 \mu\text{m}$  (Figure 14). Whereas, the muscularis externa in Cockatiel appeared in a single order along the Ileum, as it appeared consisting of two sub-layers, the first internal circular in the form of large bundles with a thick thickness rate  $48.332 \pm 5.047 \mu\text{m}$ . At the same time, the outer muscle layer was longitudinal with an average thickness  $26.148 \pm 1.852 \mu\text{m}$  (Figures 4-6, 15-17). The serous was composed of loose connective tissue surrounded on the outside by a simple squamous epithelial tissue in the two birds, and there were blood vessels and Auerbach's plexus between the muscularis and serous layers (Figures 1, 3, 6 and 17).

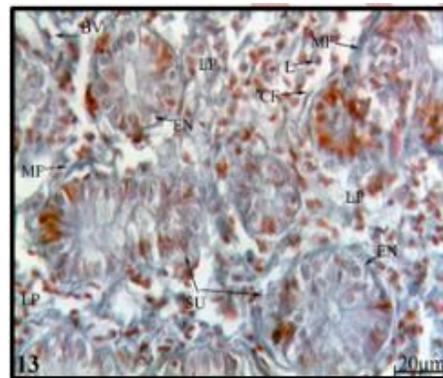


Figure 13: cross-section in the Lamina propria of Ileum in Turkey. AZ stain.

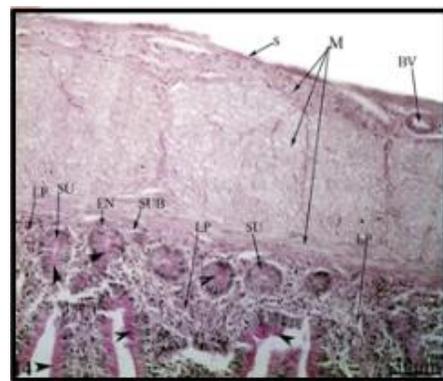


Figure 14: cross-section in the Ileum in Turkey. PAS technique.

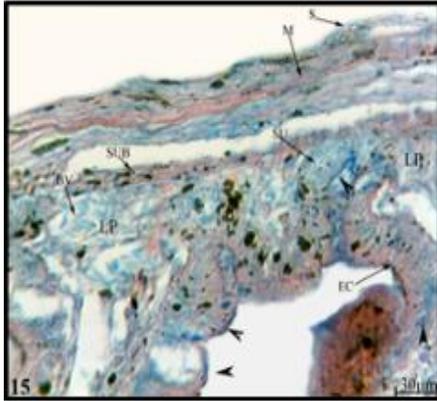


Figure 15: cross-section in the Ileum in Cockatiel. AB pH 2.5 technique.

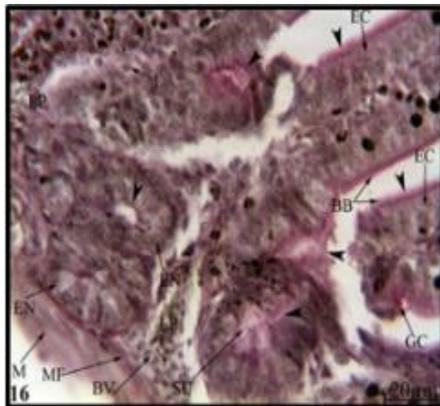


Figure 16: cross-section in the Ileum in Cockatiel. PAS technique.

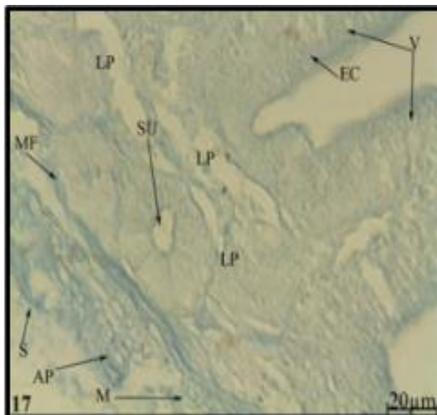


Figure 17: cross-section in the Ileum in Cockatiel. SB technique.

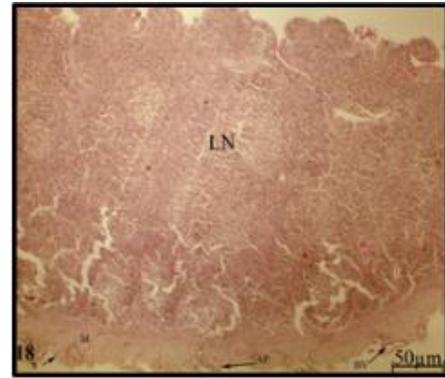


Figure 18: cross-section in the lymph node (LN) in the Ileum of Turkey. PAS technique. (BV) Blood vessels; (SU) Secretory units; (L) Lymphocytes; (EN) Enteroendocrine cells; (CF) Collagen fibers; (MF) Muscles fibers; (LP) Lamina propria; (SUB) Submucosa; (S) Serosa; (M) Muscularis; (EC) Epithelial cells; (GC) Goblet cells; (BB) Brush border; (V) Villi; (AP) Auerbach's plexus; (arrowhead) positive response.

### Histochemical results

Table 1 shows the response of the epithelial tissue and the goblet cells in it to the histochemical techniques used. It indicates a medium positive reaction to the AB pH 1 technique in Turkey and a weak positive in the Cockatiel, which suggests the presence of moderate quantities in the first bird and few in the second bird of sulfurous mucous substances with high acidity in the cells of this tissue (Figure 12). While table 1 indicates that there was a strong positive response in Turkey and a weak positive in the Cockatiel of AB pH 2.5 technique, which suggests the presence of large quantities of sulfurous mucous substances with weak acidity in Turkey, while the amounts were less in the Cockatiel (Figures 1 and 15). While the epithelial tissue response was very positive for the PAS technique in Turkey and this was clearly shown in the vesicles of the goblet cells and the apical surface of the epithelial cells, while the response was a medium positive in the Cockatiel, and this indicates the presence of neutral mucous substances and polysaccharides in the secretions of these cells, but in varying quantities between the two birds as well table 1 indicates (Figures 7 and 11). While the epithelial tissue response to the two birds was positive for the BP technique, but it was medium in Cockatiel and weak in Turkey, as in Table 1, which indicates the presence of medium to few section of protein substances in the secretions of this tissue (Figures 3 and 10). At the same time, the result was negative for the SB technique in both birds, which indicates the absence of fatty substances in the cells of the two birds (figures 8 and 17).

While table 2 indicates the response of secretory cells in secretory units of intestinal glands to histochemical techniques. table 2 shows a medium positive and weak

positive response in both birds for AB pH 1 and pH 2.5 techniques of respectively, which indicates the presence of medium to weak secretions of sulfurous mucous substances with high acidity and weak acidity, respectively (Figures 1, 12 and 15). Whereas table 2 shows a weak positive response in both birds of PAS technique, this indicates a few secretions of neutral mucous substances and polysaccharides in these cells (Figures 5, 14 and 16). In contrast, the negative response appeared in both birds for BP and SB techniques, which indicates the absence of protein or fatty substances, respectively, in the secretions of these cells (Table 2) (Figures 3 and 17).

Table 1: The response of the epithelial tissue of Ileum to the histochemical techniques

Technique	Turkey	Cockatiel
AB pH 1	++	+
AB pH 2.5	+++	+
PAS	+++	++
BP	+	++
SB	-	-

Table 2: The response of the Intestinal glands of Ileum to the histochemical techniques

Technique	Turkey	Cockatiel
AB pH 1	++	+
AB pH 2.5	++	+
PAS	+	+
BP	-	-
SB	-	-

## Discussion

The results showed that the mucosa layer in both birds was composed of villi that appeared in polymorphic in Turkey and have one shape in the Cockatiel, which was the conical shape. It's also appeared in finger-like shape in other birds as in European Starlings, African pied crow (15). It also appeared more in number and length in Turkey than the Cockatiel, and this may be due to the difference in the feeding of the two birds, which affected the number and length of the villi. These also recorded in some birds as a starling, zebra finch, and Pin-tailed sandgrouse (16), while the cattle Egret, the villi are straight, short and less numerous (17).

These villi covered with simple columnar epithelium interspersed with goblet cells, this is what found in all studied birds (4,15). It believed that the columnar cells absorb the fatty substances, amino acids, and carbohydrates, while the goblet cells specialized in forming mucus (18). The number of goblet cells is very large in Turkey, while it is very few in Cockatiel, this may be because the Cockatiel depends on one type of food. At the same time, Turkey is multi-

nourished, and therefore needs to produce large quantities of mucus secreted from the goblet cells to deal with different types of food. The goblet cells also appeared in large number in the Cattle Egret (17) and the Striated Scope Owls (19), while the goblet cells decreased towards the apex of the villi in Duck and Domestic pigeon (20). Goblet cells are responsible for the secretion of mucin that used for the mucinous lining of the intestinal epithelium. Thus, a higher density of goblet cells may increase the flow of mucin. Changes in mucin content or the composition of the mucosal surface may decrease nutrient absorption or increase the energy requirement for gut maintenance.

The secretory units of the intestinal glands appeared under the villi, which seemed almost similarly in the two birds, and it is a common condition in birds (15,17). These glands probably secrete a large amount of various digestive enzymes necessary for breakdown and absorption of the digested (21). The enteroendocrine cells also appeared in some secretory units in the two birds. These cells also founded in some birds as in the chicken (22), Starlings and zebra finch, it also reported in other studies. The Enteroendocrine cells found scattered throughout the epithelium of the gastrointestinal tract from the stomach to the rectum. The Enteroendocrine cells release gut hormones in response to meal-related stimuli and thereby exert actions ranging from the local control of gut motility and secretion to the regulation of insulin release and food intake.

The lamina propria in the two birds also distinguished by contained lymph nodules or payer's patches, and it was more abundant in Turkey, but it was more organized in Cockatiel. These nodules also appeared in buzzard, African pied crow, starling, zebra finch, and Pin-tailed sandgrouse, (15). The gut-associated lymphoid tissue plays a vital role in the immune system by protecting the mucosa against the harmful antigens that enter the body through food and air (23).

The muscularis layer in Turkey composed from three-sublayers; its arrangement differs from the anterior part to the posterior region. At the same time, in the Cockatiel formed from two sublayers, these three sublayers were also appeared in goose and common pigeon (16), whereas, it composed from two-layer in most birds (24). The muscularis layer was most probably involved in the peristaltic activities that propel the intestinal content towards the large intestine (21).

Histochemically, the results showed a positive response in the epithelial tissue and intestinal glands to the AB pH1 and AB pH2.5 techniques in the two birds in different degrees of response. Also, the results showed a positive response in the epithelial tissue and intestinal glands to PAS technique in the two birds in various degrees. These results appeared in starling, zebra finch, and Pin-tailed sandgrouse (15), and others birds (25).

While the response was positive in the epithelial tissue for BP technique, while the reaction was negative in the

intestinal glands for this technique, the same response appeared in Mallard and duck gave a weak response to the same technique (24-25). While the reaction was negative for SB technique in the two birds, and this what appeared in other birds starling, zebra finch and Pin-tailed sandgrouse.

## Conclusions

It concluded from this study that the ileum structure in the two birds is similar in terms of the basic structure. Still, they differ in some structures in a way that is appropriate to the nature of these birds' food and environment and the function of the ileum in transferring the digested material to the last part of the gut. The secretions of these tissues also differ with the nature of the food intake.

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## Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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المعي، إلا أن هناك العديد من الاختلافات بينهما. حيث كانت الزغابات في الدجاج الرومي متعددة الأشكال، أطول وأكثر عدداً منها في طائر الكوكاتيل. تبطنت الزغابات في الطائر بنسيج ظهاري عمودي بسيط يحوي على خلايا كأسية. بينما وقعت خبايا ليبركن أسفل الزغابات في الطائرين وكانت وحداتها الإفرازية كروية الشكل في معظمها واحتوت بعضها على خلايا معوية صميه. الطبقة العضلية الخارجية كانت مميزة في الدجاج الرومي عنها في طائر الكوكاتيل، إذ تألفت من ثلاث طبقات ثانوية في الدجاج الرومي بينما تكونت من طبقتين في طائر الكوكاتيل. أوضحت النتائج استجابة متوسطة إلى قوية في النسيج الظهاري لتقنيات الاليشان الأزرق عند اس ها ١ و ٢,٥ وتقنية حامض البريوديك - شيف بينما كانت الاستجابة ضعيفة لهذه التقنيات في الغدد المعوية. بينما كانت الاستجابة موجبة لتقنية ازرق البروموفينول BP في النسيج الظهاري للفاثي وسالبة في الغدد المعوية، وظهرت نفس الاستجابة لتقنية اسود سودان. استنتجت الدراسة الحالية أن نسيج اللفاففي في الطائرين يحوي اختلافات نسجية وكيميائية نسجية بينهما لكي يلائم تركيب اللفاففي مع طبيعة غذاء كل طائر.

## دراسة نسجية وكيميائية نسجية مقارنة للفاثي في نوعين مختلفين من الطيور

أمير محمود طه

قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة الموصل، الموصل، العراق

### الخلاصة

هدفت هذه الدراسة للتعرف على التركيب النسجي وكيمياء النسيج للفاثي في نوعين من الطيور هما الدجاج الرومي (*Meleagris gallopavo*) وطائر الكوكاتيل (*Nymphicus hollandicus*) باستخدام الملونات النسجية والتقنيات الكيميائية النسجية. بينت النتائج أن جدار اللفاففي في الطائرين يتكون من الطبقات النسجية التي يتكون منها جدار