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Histological comparison of pancreas and duodenum in Barn Owl, Moorhen and Budgie birds

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ABSTRACT

The study aimed to describe the histological features of Pancreas and Duodenum in Common Moorhen (*Galinula choropus*), Barn Owl (*Tyto alba*) and common Budgie (*Melopsittacus undulatus*). Compare histological parameters of Pancreas and Duodenum among these birds. In the current study, Histochemistry of Pancreas showed that the use of Masson trichrome staining in Owl's pancreas shows presence of collagen fibers in the septa between lobes and also within the capsule surrounded the organ. while this stain shows positive reaction only in capsule in Moorhen and Budgie bird pancreas. Owl bird and budgerigar bird pancreas show positive reaction to Alcian blue stain. Alcian blue –PAS mixture in Owl pancreas shows the presence of mucin around the interlober ducts and in the exocrine portions. Moorhen bird pancreas has more mucin in the interlober ducts and exocrine portion than that of Owl's pancreas. Histochemistry of the duodenum by the use of Masson Trichrom staining in Budgie bird showed the presence of collagen fibers in the serosa and the core of the villi and in the submucosa, moorhen bird duodenum shows the presence of collagen fibers in submucosa as well as in serosa and within muscularis layer. While Alcian blue in Budgie bird show that presence of acidic sulphated mucin within submucosa and muscularis and serosa. Alcian blue –PAS mixture stain showed the duodenum of the three birds expresses the goblet cells positively reacted to this stain due to the presence of acid mucine within.

مقارنة نسيجية للبنكرياس والاثني عشر في طيور البومة والدجاجة المائية والبادجي نور

عاصم فرحان, احمد عبدالله حسين
كلية الطب البيطري/ جامعة ديالى/ العراق

الخلاصة

هدفت الدراسة إلى وصف السمات النسيجية للبنكرياس والاثني عشر في دجاج الماء الشائع (*Galinula choropus*)، وبومة الحظيرة (*Tyto alba*)، والببغاء الشائع (*Melopsittacus undulatus*). قورنت المعايير النسيجية للبنكرياس والاثني عشر بين هذه الطيور. في الدراسة الحالية أظهرت الكيمياء النسجية للبنكرياس أن استخدام صبغة ماسون ثلاثية الألوان في بنكرياس البومة يدل على وجود ألياف الكولاجين في الحاجز بين الفصوص وأيضا داخل المحفظة المحيطة بالعضو. بينما تظهر هذه الصبغة تفاعلًا إيجابيًا فقط في الكبسولة الموجودة في بنكرياس طائر الماء والببغاء. يُظهِر بنكرياس طائر البومة والببغاء تفاعلًا إيجابيًا مع صبغة الألسيان الزرقاء. يُظهر خليط Alcian blue –PAS الموجود في بنكرياس البومة وجود الميوسين حول القنوات البينية وفي الأجزاء الخارجية الإفراز. يحتوي بنكرياس طائر دجاج الماء على كمية أكبر من الميوسين في القنوات البينية والجزء الخارجي من تلك الموجودة في بنكرياس البومة. أظهرت الكيمياء النسيجية للاثني عشر باستخدام صبغة ماسون ثلاثية الألوان في طائر الببغاء وجود ألياف الكولاجين في المصلية ولب الزغب وفي الطبقة تحت المخاطية، وفي الاثني عشري أظهرت وجود ألياف الكولاجين في الطبقة تحت المخاطية وكذلك في المصلية. وداخل الطبقة العضلية. بينما يظهر اللون الأزرق الألسياني في طائر البادجي وجود الميوسين الكبريتي الحمضي داخل الغشاء المخاطي وتحت المخاطية والعضلية والمصلية. أظهرت صبغة خليط Alcian blue –PAS أن الاثني عشر للطيور الثلاثة يعبر عن تفاعل الخلايا الكأسية بشكل إيجابي مع هذه البقعة بسبب وجود حمض الميوسين بداخلها.

الكلمات الافتتاحية: نسيج ، بنكرايس ، صبغة ماسون.

INTRODUCTION

Gallinula chloropus, also called Common Moorhen, Eurasian Moorhen, or Black Gallinule, is a wild aquatic bird belonging to the family Rallidae was shown to withstand harsh environmental conditions (Amininasab and Hosseini-Moosavi, 2021). Barn Owl (*Tyto alba*) commonly known as the Common Barn Owl, is from the family Tytonidae that found almost all over the world and characterized by its distinct rounded head with a heart-shaped facial disc, white to brown plumage, medium-sized. They are strictly nocturnal with auditory adaptations to help them locate prey in complete darkness. Budgies (*Melopsittacus undulatus*) is one of the smallest parrot species in the world and very easy to determine their sex. Male and female budgies can be identified by the color of their cere. The male of Budgie has blue cere while the female Budgies cere is brown in color. An exceptional case is albino and lutino budgerigars, where males have pink cere and in females it is white or cream. Budgerigars (*Melopsittacus undulatus*) are included in the class: Aves and order: Psittaciformes (Adajar *et al*., 2011).

Al-Sudany (2007) worked on the pancreas of the Turkey (*Melegris gallapavo*), Steppe Buzzard (*Buteo buteo valpinus*), and Rock Dove (*Columba livia*). The pancreas of these birds is a mixed gland, which consists of exocrine tissue, endocrine tissue. The exocrine tissue is composed of acini and ductal system. The endocrine tissue scattered throughout exocrine tissue and separated from it by a thin connective tissue capsule. The endocrine tissue is composed of three types of islets. These islets are named (A) - islet, (B) -islet, and mixed islet. The (A)-islet contains (A)- cells and a few (D)- cells, (B) islet contains (B) - cells, and a few (D)- cells. The mixed islet contains (A) - cells, (B) - cells, and (D) - cells.

The histology of duodenum is consisted from four tonicae (mucosa, sub mucosa, muscularis and serosa). The mucosa is consisted from epithelia and lamina propria, and muscularis mucosa (layer of smooth muscle fiber) is not found. The glands of duodenum (Brunner glands) are absent. The tunica sub mucosa formed thin layer of loose connective tissue between the crypts and tunica muscularis. The tunica muscularis is smooth muscle fibers formed by two sub layers. The tunica serosa is loose connective tissue, thin layer and covered by mesothelium (Al-kafagy, 2016).

MATERIALS AND METHODS

Collection of birds

A total of thirty healthy birds 10 common Moorhens (*Gallinula choropus*), 10 Barn Owls (*Tyto alba*) and 10 and Budgie (*Melopsittacus undulatus*), undetermined gender was used for current study based on the differences in their food types, to show the structure of Duodenum and Pancreas of the different stage from age of the birds.

The Owls, Moorhens and Budgies birds were obtained from local markets in Iraq. For histological study, ten indigenous Moorhen, Owl and Budgie were decapitated. Abdominal laparotomy was performed according to (Wang and Peng, 2008; Bancroft and Stevens, 1982; Luna, 1968; Collins, 2007).

Statistical analysis

One-way ANOVA followed by Dunnett's multiple comparisons test was performed using GraphPad Prism version 10.0, and the values were expressed as Mean ±SE, Significant level was set at, ($P < 0.05$) (Iji *et al*., 2001).

RESULTS AND DISCUSSION

Pancreas of Owl is surrounded by a thin fibro vascular capsule with little not fully developed septa therefore, the exocrine pancreas doesn't divide into discrete lobules (fig.1). Some adipocytes scattered among acini of the exocrine portion, some blood vessels and sinusoids surrounded them (fig.2), The pancreatic secretions collect into larger duct system that, terminally, empties into the Ascending limb of duodenum. Less developed oval shaped one islet of Langerhans per sections can be seen. The pancreas of Owl resembles other carnivores' birds. The exocrine portion is tubuloacinar (fig.3). The histometric parameters show that the mean capsule thickness, exocrine portion measurement, island of Langerhans measurement and nucleus diameter were (25.8±2.8, 22597.8±667.4, 21255.6±1839.1, 13. ±1.58µm) respectively (Tab.1).

Table 1- Histometric parameters of pancreas

Bird type	Capsule thickness (um)	Islet of Langerhans diameter(um)	Exocrine diameter (um)	Nucleus diameter
Owl bird	25.8±2.8 SE	22597.8±667.4 SE	21255.6±1839.1 SE	13.±1.58 SE
Moorhen bird	20.6±2.9 SE	40515.4±558.7 SE	30177.8±4234.8 SE	22.4±1.98 SE
Budgie bird	11.8±0.91 SE	81207.6±1535.4 SE	15057.2±614.09 SE	18.6±1.46 SE

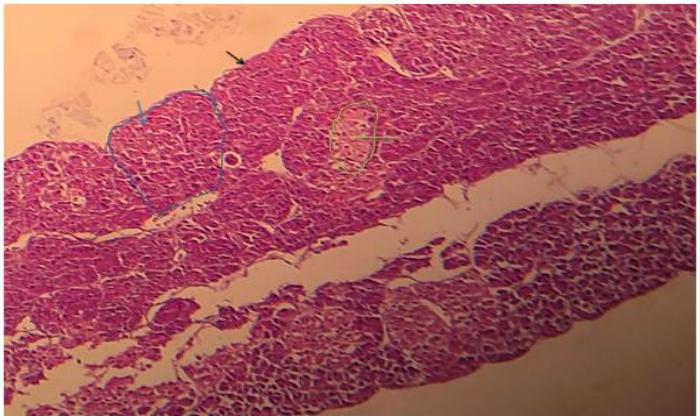

Fig.1. Histological section of Owl's Pancreas shows capsule (black arrow), exocrine portion (Blue encircle), endocrine portions (green encircle) H&E 10X

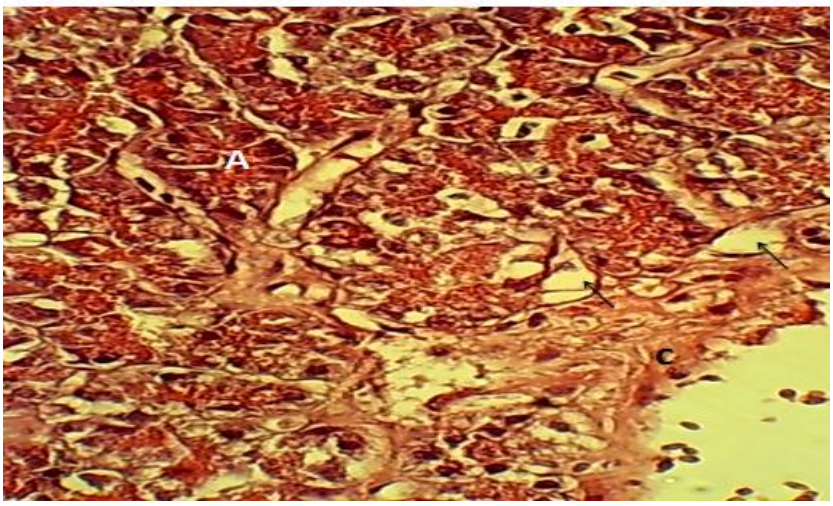

Fig.2. Histological section of the Owl's Pancreas shows capsule (C), exocrine portion (A), endocrine portions (A) fat drops (A).H&E 40X.

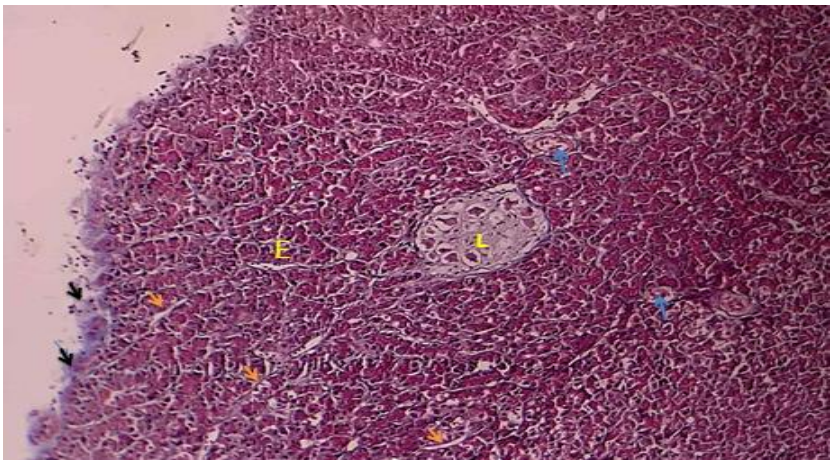

Fig.3. histological section of Owl's Pancreas shows capsule (black arrows), trabecula (orange arrows) exocrine portion (E), endocrine portions(L)blood vessels (blue arrows).H&E 10X.

Pancreas in Moorhen was covered with a thin capsule associated in some area with the subcapsular adipocytes. The capsule sent a small septae divided the parenchyma in to small lobules, a mesh network of collagen and reticular fibers were observed in the interacinar and/or interlobular area, in addition to some elastic fibers were encountered in the interlobular tissues. The endocrine part comprised of one large and irregular shape and sizes of islet among the exocrine portion (Fig. 4). The exocrine portion was composed of a compound tubulo-acinar gland with duct system and adipocytes. This region was occupied a larger part of the pancreas and appeared organized in form of serous tubuloacinar glands. The secretory acini had a several number of pyramidal cells with either triangular or tall columnar shape, and it had also various apical acidophilic zymogen granules and basal basophilic stain (Fig. 5). The mean capsule thickness, exocrine portion measurement, island of Langerhans measurement and nucleus diameter were (20.6 ± 2.9, 40515.4 ± 558.7, 30177.8 ± 4234.8, 22.4 ± 1.98µm) respectively (Tab.1). While Pancreas of Budgie bird is a thin capsule was covering the pancreas of this bird. The parenchyma was divided into many lobules each one composed of exocrine portion surrounded the endocrine portion. The network of collagen and reticular fibers

were observed in the interacinar and/or interlobular area, in addition to some elastic fibers were encountered in the interlobular tissues (Fig.6).

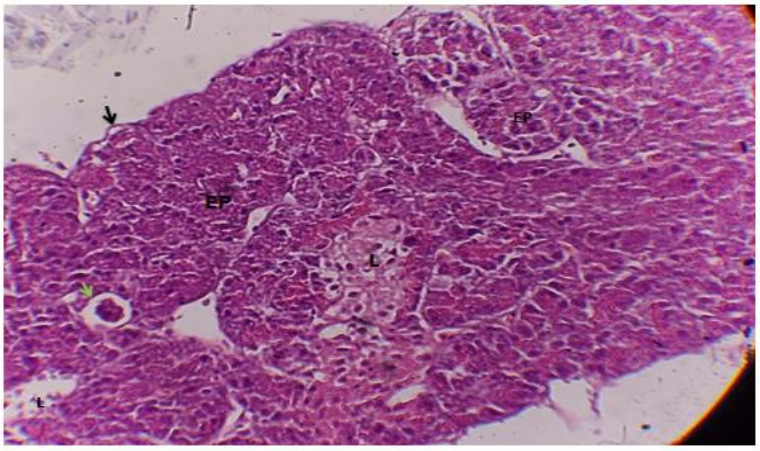

Fig.4. histological section of the Moorhen Pancreas shows capsule (black arrow), exocrine portion (EP), endocrine portions(L)blood vessels (green arrows).H&E 10X.

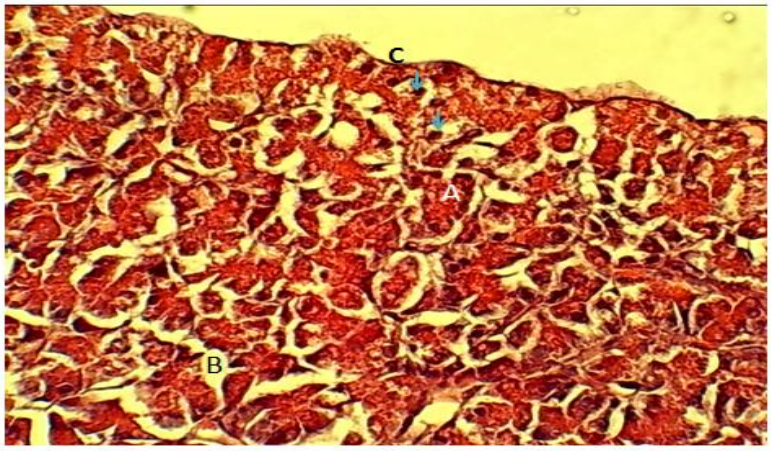

Fig.5.histological section of Moorhen Pancreas show capsule(C), Pancreatic cells (blue arrows) exocrine portion(A), blood vessels(B).H&E 40X.

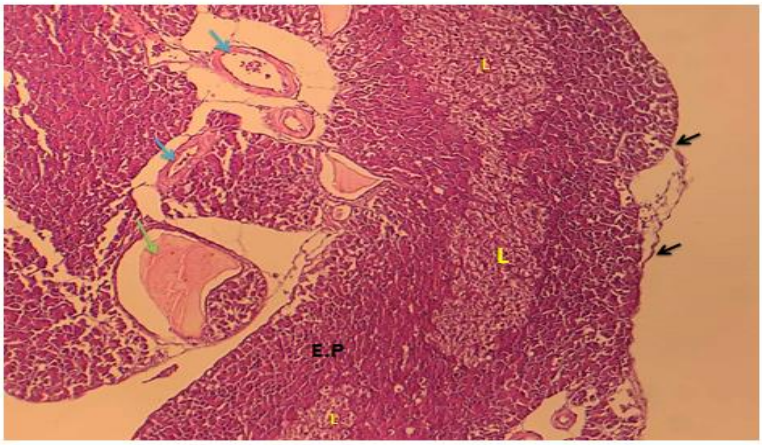

Fig.8.histological section of Budgie Pancreas show capsule (black arrows), exocrine portion (EP), endocrine portions(L)blood vessels (blue arrows) pancreatic ducts (green arrows).H&E 10X.

The exocrine portion was composed of a compound tubulo-acinar gland, ductal system as well as some adipocytes within. This region was occupied a larger part of the pancreas and appeared organized in form of serous tubuloacinar glands (fig.7). The secretory acini had a several number of pyramidal cells with either triangular or tall columnar shape, and it had also various apical acidophilic zymogen granules and basal basophilic stain (Fig.6). Two or more large, oval lobulated and well-developed islets of Langerhans were seen in one field (Fig.8). The mean capsule thickness, exocrine portion measurement, island of Langerhans measurement and nucleus diameter were (11.8 ± 0.91, 81207.6 ±1535.4, 15057.2 ± 614.09, 18.6 ± 1.46) respectively (Tab.1). The histological statistical analysis of the capsule thickness, exocrine portion measurement, island of Langerhans measurement and nucleus diameter in the three birds in this study showed that the capsule was in owl bird than that of the other two birds, islets of Langerhans in Budgie bird pancreas was higher than of the other birds, exocrine portion diameter and nucleus diameter in Moorhen higher than that in other two birds (diagram 4,5,6,and 7).

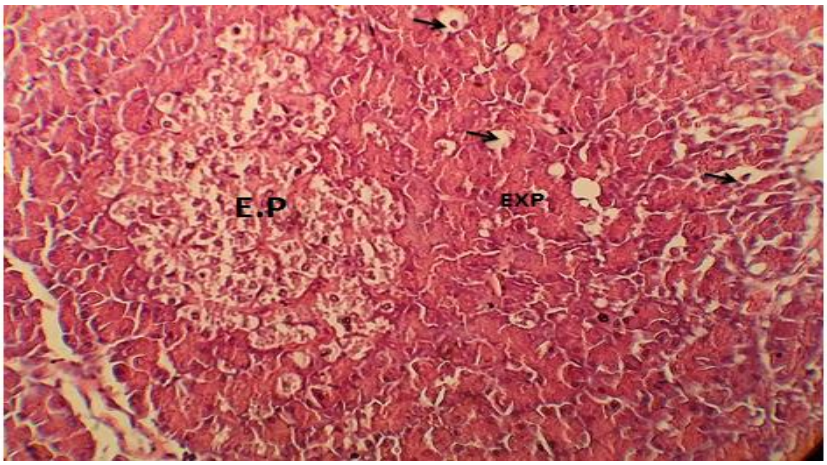

Fig.6.Histological section of Budgie Pancreas show fat drops (black arrows), exocrine portion (EXP), endocrine portions (EP).H&E 10X.

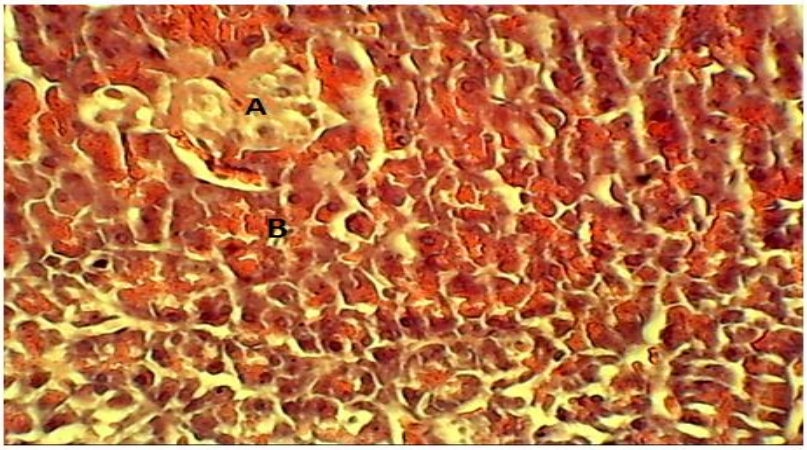

Fig.7.Histological section of the Budgie Pancreas show exocrine portion(B), endocrine portions(A).H&E 40X .

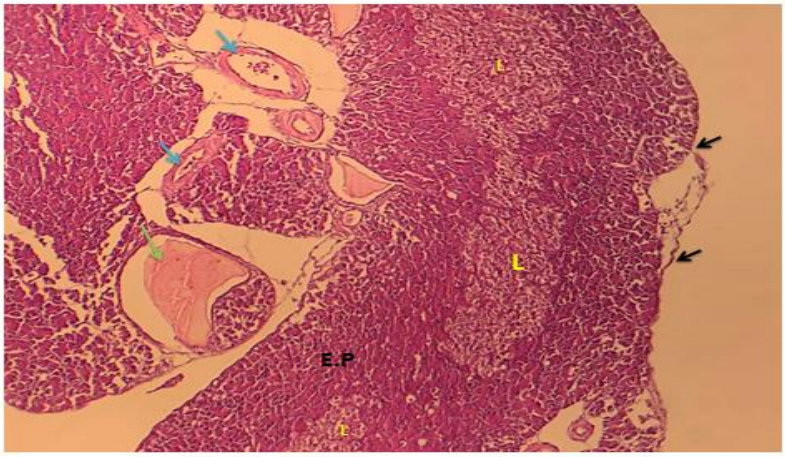

Fig.8.histological section of Budgie Pancreas show capsule (black arrows), exocrine portion (EP), endocrine portions(L)blood vessels (blue arrows) pancreatic ducts (green arrows).H&E 10X.

Pancreas of Owl in the current study is surrounded by a thin fibro vascular capsule with little not fully developed septa therefore the exocrine pancreas doesn't divide into discrete lobules. In Moorhen, it was covered with a thin capsule associated in some area with the subcapsular adipocytes. The capsule sent a small septae divided the parenchyma in to small lobules, a mesh network of collagen and reticular fibers were observed in the interacinar and/or interlobular area, in addition to some elastic fibers were encountered in the interlobular tissues. In Budgerigar, "the parenchyma were divided into many lobules each one composed of exocrine portion surrounded the endocrine portion. The network of collagen and reticular fibers were observed in the interacinar and/or interlobular area, in addition to some elastic fibers were encountered in the interlobular tissues".

Al-Haaik (2019) found that "The pancreas of adult Kestrel (*Falco tinnunculus*) was covered very thin layer capsule which consists of connective tissue with mesothelial cells, the capsule sent septa of connective tissue into parenchyma which dividing it into many lobules" also Mahmood *et al.* (2022) referred that he pancreas of native ducks (*Anas Platyrhynchos*) is covered loose connective tissue capsule and mesothelial; septa extend from the capsule into parenchyma dividing it into many lobules. Some adipocytes scattered among by the exocrine portion, some blood vessels and sinusoids were seen. The pancreatic secretions collect into larger duct system that, terminally, empties into the Ascending limb of duodenum. less developed one oval shape islet of Langerhans per sections can be seen.

Mahmood *et al.*, (2022) mentioned that the pancreatic lobules in native duct are consisted of exocrine acini exocrine component consisting of round or oval acini with zymogen granules. Alpha islets are formed of alpha cells and a few delta cells, beta islets are composed of peripherally located beta and delta cells and mixed islets are composed of alpha, beta, and delta cells. The pancreas of the owl resembles other carnivores' birds. The exocrine portion is tubuloacinar duct system and adipocytes. In budgerigar, the exocrine portion was composed of a compound tubuloacinar gland. This region was occupied a larger part of the pancreas and appeared organized in form of serous tubuloacinar glands. Mahmood *et al.* (2022) also found that the connective tissue found around the blood vessels and in within capsule of native duck pancreas.

Hussein & Bargooth (2022) mentioned that in the ducks and turkeys the pancreas surrounded by capsule and consisted of exocrine and endocrine parts. The exocrine portion in both birds consisted of the acini and ductal system in different diameters. Also, (Mobini, 2013; Motta *et al.*, 1997) referred that the exocrine part in bird was composed of tubuloacinar gland. Goblet cells and glands were not present in duct system. The endocrine pant was composed of large alpha and small beta islets but mixed islets were not observed.

The wall of the owl's duodenum consists of four layers: tunica mucosa, submucosa, muscularis and serosa. Tunica mucosa was composed of the lamina mucosa lined by simple columnar epithelia, lamina propria and lamina muscularis (Fig. 9). The tunica mucosa is divided in to fingerlike projections (villi) which is folded and of different shape, size which were lined with simple columnar epithelium having more goblet cells in proximal part in contrast

middle and distal part (Fig.9). The goblet cells are distributed between enterocytes and secrete mucus that covers the mucosa. The apical part of villi of proximal duodenum were slightly sharp, pointed while the basal and middle parts of villi were thicker than middle and apical duodenum. The lamina propria had loose connective tissue with few lymphoid cells in proximal duodenum in contrast the middle and distal duodenum. The crypt of leberkuhen is located between the bases of two villi and invaginated extend deeply as intestinal gland. lamina muscularis mucosa is various in thickness in all parts of duodenum and was made up of smooth muscle fibers. Tunica submucosa in duodenum of owl is consist of loose connective tissue rich with lymphoid fibers and duodenal glands. The lamina muscularis is consists of two smooth muscle layer outer longitudinal layer and a thick circular muscle layer that allow mixing and propulsion of the digestion through the intestinal tract (Fig .10). The serosa is outer layer of flattened simple squamous epithelium.

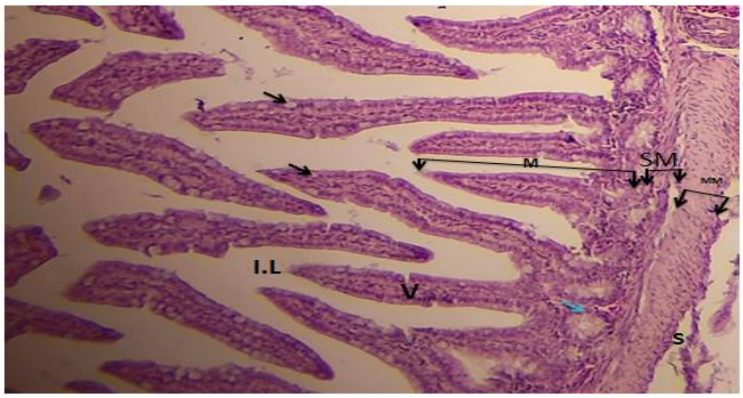

Fig.9. Histological section of Owl's duodenum shows mucosa(M), submucosa (SM), muscularis (MM), serosa(S), goblet cells (black arrows), intestinal crypts (blue arrow), villi(V). intestinal lumen (IL).H&E 10X.

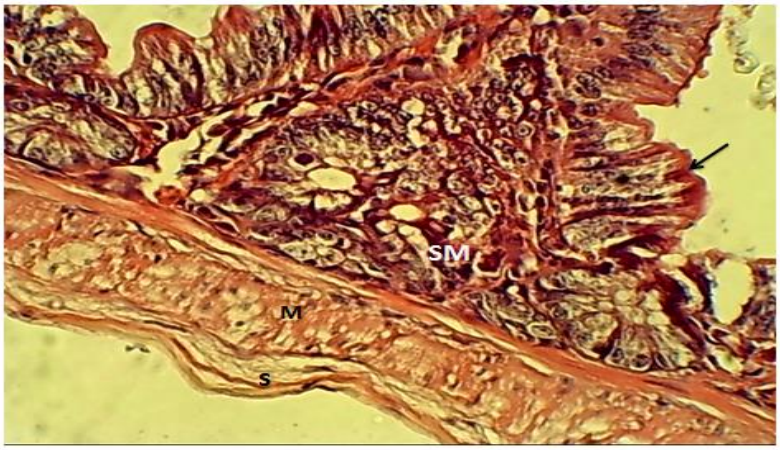

Fig.10. Histological section of Owl's duodenum show mucosal cells (black arrow), sub mucosa (SM), muscularis(M), serosa (S).H&E 40X

The duodenum of Moorhen was composed of 4 layers enumerated from inner to outer as: The tunica mucosa was containing many finger-like villi with different height. Each one was covered by an epithelium surrounding the connective tissue in the center of each villus. The tunica mucosae of all components of the duodenum were simple columnar epithelium. A lot of goblet cells scattered among the simple columnar cells were present (Fig.11). Goblet cells were very prominent in Moorhen duodenum. Due to the nature of Moorhen nutrition which contain protein,

therefore a lot of goblet cells can be seen as a compared with herbivores birds. Some Columnar cells developed to special type of crypts among the villi. The connective tissue of the tunica submucosa and lamina propria mucosae of the duodenum was laden with many intestinal glands and blood vessels. Tunica muscularis: The tunica muscularis was made up of an inner circularly arranged layer of smooth muscle fibers, and an outer longitudinally arranged layer of smooth muscles. The Blood vessels and nerve plexuses were present within the muscularis. The tunica serosa was a thin layer of connective tissue whose external surface was lined by mesothelium, a simple squamous epithelium (fig.12). The histometric study of duodenum show that the villi length, the submucosa measurements, The muscularis thickness and serosa thickness were (373±25.8, 115.6±19.9, 56.6±3.0, 15±1.0) µm respectively (Tab.2).

Table 2- Histometric parameters of duodenum

Bird type	Villi length(um)	Submucosa thickness(um)	Muscularis thickness(um)	Serosa thickness(um)
Owl bird	306.4±82.5 SE	73.2±12.6 SE	77.4±5.3 SE	24.8±3.9 SE
Moorhen bird	373±25.8 SE	115.6±19.9 SE	56.6±3.0 SE	15±1.0 SE
Budgigar bird	614.6±76.8 SE	423±10.6 SE	140±4.0 SE	33.2±0.7 SE

The histological study of the duodenum in budgie bird show that the duodenum was such the two other birds in the current study, it composed of 4 layers (mucosa, sub mucosa, muscularis and serosa) (fig.13), the mucosa consists of relatively low villi, each one covered by simple columnar epithelium which covered the core of C.T, the fewer numbers of goblet cells situated among the mucosa. between the bases of each two villi there are the crypt of leberkuhen which invaginated extend deeply as intestinal gland Tunica mucosa less than that of moorhen and contain special lymphatic nodules. Tunica serosa also thinner than that of moorhen. The serosa is outer layer of flattened simple squamous epithelium (Fig.14).

The histometric study of duodenum show that the villi length, the submucosa measurements, the muscularis thickness and serosa thickness were (614.6±76.8, 423±10.6, 140±4.0, 33.2±0.7) µm respectively (Tab.4). The histometric statistical analysis of the villi length, the submucosa measurements, the muscularis thickness and serosa thickness in budgie was higher than that of the two other birds (diagram 8,9).

The wall of the duodenum in Owls, Moorhen and Budgerigar consists of four-layer tunica mucosa, submucosa, muscularis and serosa. Tunica mucosa was composed of the lamina mucosa lined by simple columnar epithelia, lamina propria and lamina muscularis (this result observed by Albideri *et al*. (2015) in the Rock Dove, Igwebuike et al. (2010) in the African Pied Crow (*Corvus albus*) and by Albideri *et al.* (2015) in the African Ostrich (*Struthio Camelus*). The tunica mucosa is divided in to fingerlike projections (villi) which is folded and of different shape, size which were lined with simple columnar epithelium having more goblet cells in proximal part in contrast middle and distal part. The goblet cells are distributed between enterocytes and secrete mucus that covers the mucosa (this result also observed by Taki-El-Deen (2017) in spur-Winged Lapwing (*Vanellus spinous*); Kadhium *et al*. (2018). The mucosa was simple columnar with goblet cells and folded into a villi), The apical part of villi of proximal duodenum were slightly sharp, pointed while the basal and middle parts of villi were thicker than middle and apical duodenum. The lamina propria had loose connective tissue with few lymphoid cells in proximal duodenum in Contrast the middle and distal duodenum. The crypt of Lieberkühn is located between the bases of two villi and invaginated extend deeply as intestinal gland (Most birds have digestive glands called crypts of Lieberkühn, which are found between the base of the villi and the muscular (this result also found in African pied crow by Igwebuike & Eze (2010). Lamina muscularis mucosa is various in thickness in all parts of duodenum and was made up of smooth muscle fibers. Tunica submucosa in duodenum of owl is consist of loose connective tissue rich with Lymphoid fibers and duodenal glands. The lamina muscularis is consists of two smooth muscle layer outer longitudinal layer and a thick circular muscle layer that allow mixing and propulsion of the digestion through the intestinal tract. The serosa is outer layer of flattened simple

squamous epithelium. The tunica muscularis is made up of thick, circular inner and thin. Outer longitudinal layers of smooth muscle bundles in the majority of avian species. A thin or restricted connective tissue that houses blood vessels and nerve plexuses lie between these layers (Zghair and Khaleel, 2019). Finally, the layers in the three birds that used in this study are the same but the difference only in the parameters of this layers.

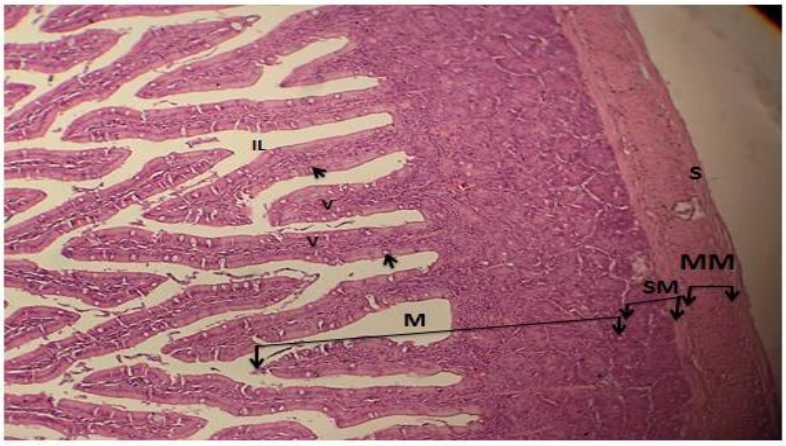

Fig.11. Histological section of Moorhen duodenum shows mucosa(M), submucosa (SM), muscularis (MM), serosa(S), goblet cells (black arrows), intestinal lumen (IL).H&E 10X

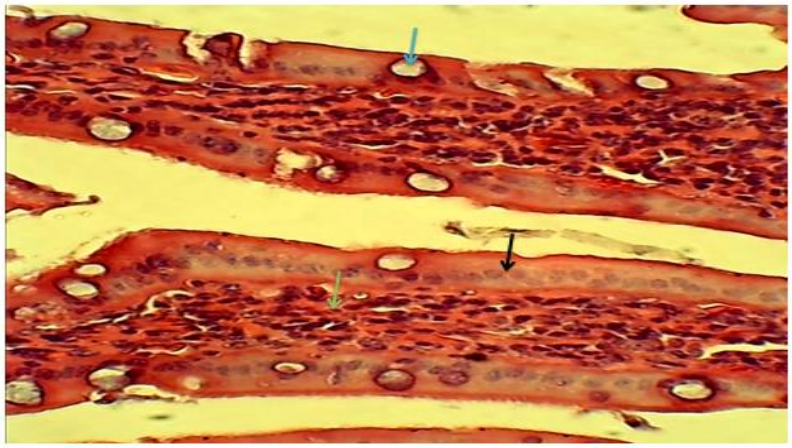

Fig.12. Histological section of Moorhen duodenum show mucosal cells (black arrow), core of villus (green arrow), goblet cells (blue arrow).H&E 40X

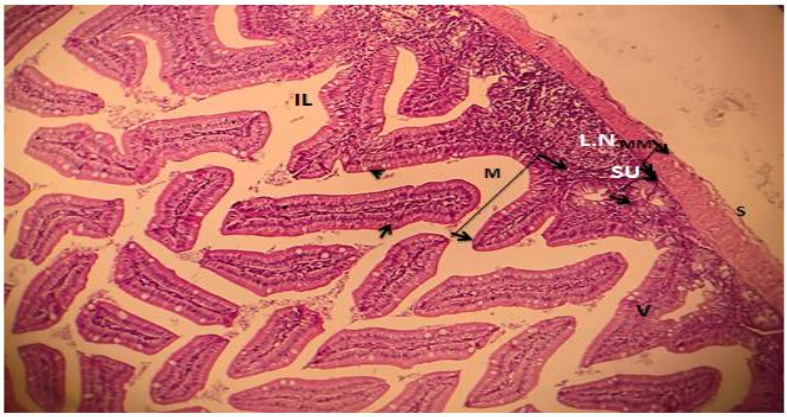

Fig.13. Histological section of Budgie duodenum shows mucosa(M), submucosa (Su), muscularis (MM), serosa(S), goblet cells (black arrows), lymphatic nodule (LN), villi (V). intestinal lumen(IL).H&E 10X

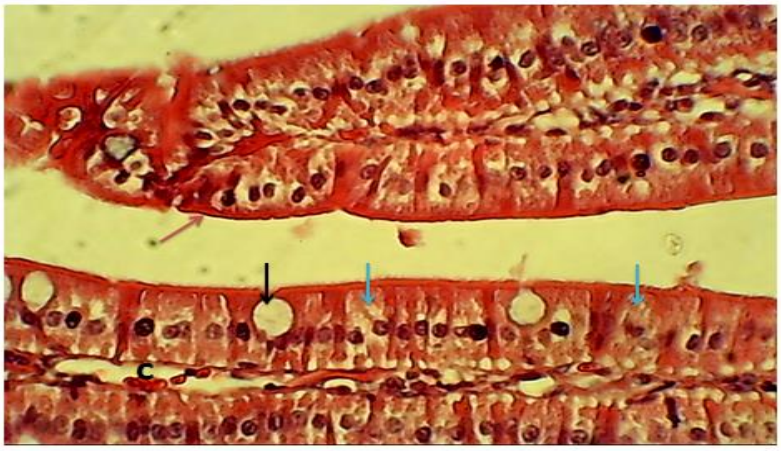

Fig.14. Histological section of Budgie duodenum shows mucosal cells (blue arrows), goblet cells (black arrow) .H&E 40X

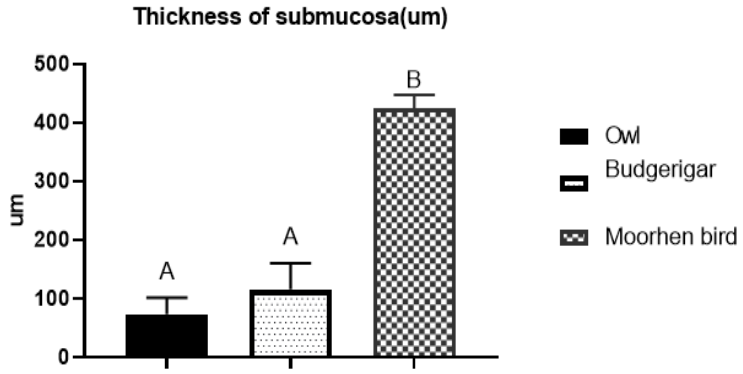

Diagram.9. show there are no significant differences between submucosa layer of duodenum between the owl and budgie. There is significant differ between submucosa layer of duodenum of moorhen bird and the other two birds.

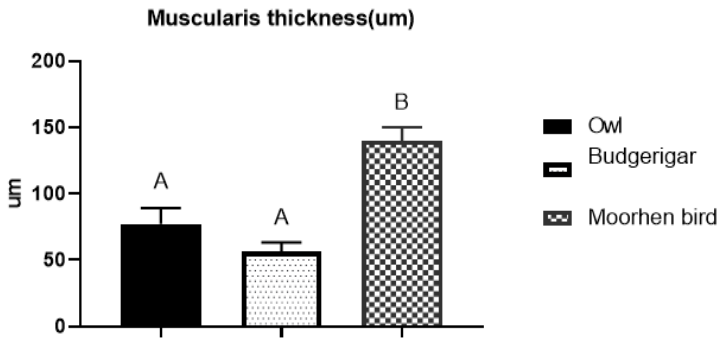

Diagram.10. Show there are no significant differences between Muscularis layer of duodenum between the owl and budgie. There is significant differ between muscularis layer of duodenum of moorhen bird and the other two birds

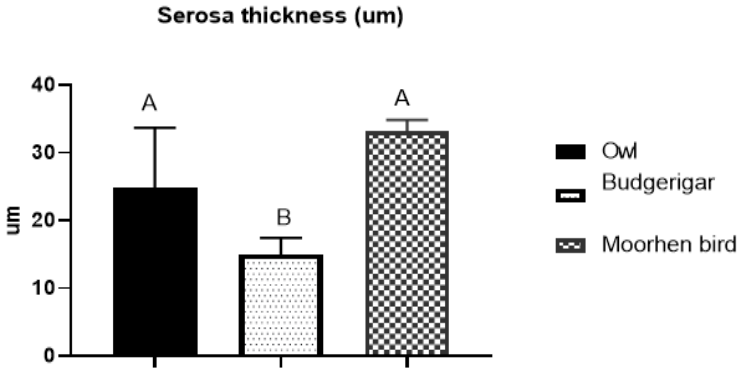

Diagram.11. show there are no significant differences between serosa layer of duodenum between the owl and moorhen. there is significant differ between serosa layer of duodenum of budgie bird and the other two birds.

CONCLUSION

The study revealed distinct histological features in the pancreas and duodenum among Common Moorhen, Barn Owl, and Budgie. The Barn Owl exhibited significant collagen fibers in its pancreatic capsule and septa, while the Moorhen and Budgie showed collagen only in the capsule. Mucin presence was notably higher in the Moorhen's pancreas. In the duodenum, Budgies displayed collagen fibers in various layers, whereas the Moorhen also had collagen in the muscularis. All species tested positive for mucin in goblet cells. These differences highlight the physiological adaptations of these birds related to their dietary needs and environments.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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