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Morphological and His tochemical Study of Small Intestine InIndigenous Ducks (*Anasplatyrhynchos*)

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Summary: *Histomorphological study of small intestine in indigenous ducks Anasplatyrhynchoswas carried out for thirty adult indigenous ducks (male and female). Duodenum, jejunum and ileum were studied grossly and histologically. Anatomically, the small intestine appeared as a smooth, uniform through entire length, light pinkish and glistening. The duodenum has a U shape loop extend caudal to the gizzard. The pancreatic and bile ducts opened in the end of its ascending limb.The Jejunum and ileum were arranged in large parallel U shaped loops. The mucous membrane of the small intestines has a velvety appearance. Female had significanthigher than male in the mean length, weight and volume of total small intestine and for each intestinal segment separately. The celiac artery and its branches supply the small intestine.Histologically, the wall of the small intestine was consisted of four tunicae mucosa, submucosa, muscularis and serosa.The mucosa of the small intestine had a distinctive feature by the presence of villi and the crypts of lieberkuhn which covered by simple columnar epithelium with goblet cells. The villi had different shapes in different segments of small intestine.The goblet cells were increased in number towards ileum with PAS and AB positive reactions. No Paneth cells were observed. No sex differences in the shape of the villi, height of columnar cells, villus height, crypts depth, ratio of villus height to crypts depth and the number of goblet cells in the three segments of small intestine.*

Keywords: *Small intestine,In Indigenous Ducks, Duodenum, jejunum, ileum.*

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I. Introduction

Poultry and eggs represent an excellent nutritional source and make up a significant part of peoples diets[1]. Feed represents a major component in the cost of poultry production, considerable efforts have been made in recent years to select more efficient birds[2]. The *Anasplatyrhynchos* Mallard, one of the ducks species from the Anseriformes order, this birds accommodation for water living, and its flesh are very delicious from any water fowl flesh, for this reason become as a hunters target[3].The structure of bird alimentary system is characterized by large individual variability as a result of digestive tract adaptation of these animals to environment condition [4]Avian digestive tract modifications may occur as an adaptation to diet [5]. The small intestine is one organs and it is a primary sit of absorption of the digestive system for most nutrients in birds and mammals [6]. The animal growth depends on its capacity to digest and assimilate ingest macromolecules and any impairment of this is expected to constrain growth[7] . Therefore this study designated to determine the normal anatomical and histological observation of small intestine in both male and female indigenous ducks and compared between them.

II. Materials And Methods

***Birds collection and study design*:** Thirtymale and female indigenous adult ducks (*Anasplatyrhynchos*) including [15]from each sexes were in this study. These birds were collected from local supplier in Baghdad province. The average weight of male ducks was (1706 gm) , and female ducks was (1238 gm).These ducks were divided into three equal groups, first ten used for anatomical study: gross morphology, topographical relationship in situ and other anatomical observations and the second used for study the blood supply of small intestine. All bids were sacrificed by decapitation and laparotomy was performed, the small intestine was lifted out of the abdominal cavity after photographed in situ then grossly described and the total(length,weight and volume)of small intestine and the(length, weight and volume) of each segment of small intestine were measured separately.

The small intestine comprises of three segments: the duodenum, extend from the pylorus to the pancreas and forms a loop surrounding most of the pancreas, the jejunum extend from the distal portion of the duodenal loop to the meckels diverticulum and the ileum extend from the meckels diverticulum to the ileo-cecal junction, with its distal portion connected to a pair ceca[8].

The length of small intestine was measured after removed and they laid out in a straight line using the Tapeline [7]. Weight of small intestine was taken by sensitive electrical balance after emptied its contents and cleaned. Volume of small intestine was done using water displacement method [9].

To study the blood supply of the small intestine: After sacrificing the birds, the heart was catheterized from the left ventricle tile the aorta in order to inject the mixture of latex with carmine. These specimens were fixed in 10% formalin solution for 2days, then dissected very carefully [10].For histological study, the specimen were obtained from the middle point of each segment of the small intestine, fixed in 10%, neutral buffered formalin then dehydrated in ascending grades ethanol (70%, 80%, 90%, 95% and 100%), cleared in two changes of xylem, embedded in paraffin wax and sectioned at 6 μm[11]. Different **s**tains were used Harris Hematoxylin and Eosin Stain,Periodic Acid Schiff (PAS),Van-Gieson stain, Alcian blue [12]. The histological examination was done by using light microscope and photographed by using with(14.1) mega pixel power digital camera. Different histological parameters were studied under x40 by ocular micrometer and the following data were recorded [13]. Different parameters include the villus height was measured from the villus tip to the villus-crypt junction , while the crypts depth was defined as the depth of invagination between two villi [14] and number of goblet cells was calculated per villus [15] all these parameters were done in the three segment of small intestine.

Statistical analysis of data was performed and used analysis of variance test (T- test) and significant differences limited on $p \leq 0.01$[16].

III. Results And Discussion

***Anatomical Study*:** This study revealed that the small intestine was similar in both sexes, and appear relatively long, smooth , uniform through entire length, light pinkish and glistening and consists of duodenum, Jejunum and ileum (Fig.1). The duodenum formed U-Shaped loop, with proximal descending and distal ascending limbs, the pancrease lies between the limbs (Fig.2), there are three pancreatic ducts opened into ascending loop of the duodenum, these ducts are opened near the two biliary ducts . Similar finding by [17] on local ducks, but this study disagrees with I G [18] on pied crow *Coryus albus* showed that there is two pancreatic ducts only.

The jejunum and ileum in both sexes was similar, the jejunum extend from the termination of the duodenal loop to the meckles diverticulum while the ileum extened from mecklesdiverticulum to the ileocecal junction (Fig. 1).The jejunum and ileum arranged in a large parallel U shaped loops supported by mesentery in the right part of the abdominal cavity and terminating at the ileo-caecal junction (Fig. 2). Meckel's diverticulum divide the jejunum from the ileum, this finding agree with that observed by[19] in chicken, but differ from that found by [20] who noted that the jejunum is arranged in a number of short garland-like coils at the edge of the long dorsal mesentery in the broiler chicken.

In this study the male duck had Significantly higher body weight than in female(Table1). This finding was agree[21].The small intestine total weight in female ducks was more higher than in male ducks, there was a Significant difference between them at p 0.0≤1(Table 1).There is appositive correlation between the total length of the small intestine and its weight, as observed by[22]on the broiler chickens. The present study noticed the relative small intestine weight to body weight in female was higher than in male.

The mean length of small intestine in female was longer than that in male ducks, there was a significant differences between them at $p \leq 0.01$(Table 1).[23]that the sex had a significant effect on the digestive tract.

The mean volume of small intestine in female ducks reach a higher value than in male ducks (Table1), there is a significant difference between male and female , this may be due to appositive correlation between small intestine weight and its volume.

There were a significant differences between two sexes in length of all segments ofsmall intestine, in female is longer than that in the male. This results were in parallel with [24] they mentioned that these features are connected with the amount and type of food,sex and species characteristics,housing type and also the current investigate that the jejunum is the longest segment in the small intestine (Table 2). This finding was inagreement with [25].

The weight of each region of small intestine in male were less than in female(Table2), these may be related to increase in whole intestine weight in female than in male due to a differences in food intake amounts[26]. The mean volume of the duodenum, jejunum and ileum in male was less than in female(Table2).There is a correlation between weight and volume of each segment of small intestine, there are a considerable anatomical differences between male and female due to sex of birds. The blood supply of small intestine in both sexes via the celiac artery which originate from the descending aorta, the duodenum and pancreas are supplied by pancreatico-duodenal artery which is the continuation of the right branch of the celiac artery.The pancreatico-duodenal artery extend caudally to the duodenum in the mesentery between the descending and ascending parts of the duodenum and give many branches at varying levels to the duodenum and pancreas (Fig.3), the Proximal part of Jejunum is supplied by duodeno-jejunal artery which is a branch from celiac artery and by the jejunal arteries, the branches of the cranial mesenteric artery that extend in the mesentery toward the jejunum. The Ileal arteries arise from cranial mesenteric artery, supplied the anterior segment of ileum (Fig.4), the medial segment of ileum is supplied by the terminal branch of ileo-cecal artery of pancreatico-duodenal artery. The ileal segment near the ileo-cecal junction is supplied by another branches from ileo-cecal artery which anastomos is with branches of caudal mesenteric artery which is arise from celiac artery.This study showed no sex specificdifferences in the blood supply of the small intestine, as reported by[27] who mentioned that the branches and distribution of the celiac artery were not gender dependent on domestic fowl.

Table(1). The comparison of body weight, small intestine total weight , Small intestine total weight to body

Measurement	Male mean ± SE	Female mean ± SE
Body weight/ gm	a 1706	b 1238
Small intestine Total weight/ gm	a 25.12 ± 1.27	b 31.42 ± 1.97
Percentag $\frac{\text{Total weight}}{\text{Body weight}}$ %	a 1.472	b 2.537
Small intestine Total length/ cm	a 123.41 ± 2.73	b 131.11 ± 2.18
Small intestine Total volume /cm^3	a 25.09 ± 1.43	b 30.09 ± 1.90

The different letters in one raw denoted that there were significant differences between male and female indigenous ducks, in all traits at p≤0.01.

weight, Small intestine total length and Small intestine total volume

Table (2) . The comparison of lengths, weight and volume of various parts of the small intestine (duodenum, jejunum and ileum) in males and females indigenous ducks (Mean ± SE).

Intestine regions	Length (cm)		weight (gm)		Volume (cm^3)	
	Male mean ± SE	**Female mean ± SE**	**Male mean ± SE**	**Female mean ± SE**	**Male mean ± SE**	**Female mean ± SE**
Duodenum	**a 24.03 ± 0.24**	**b 25.75 ± 0.62**	**a 4.37 ± 0.20**	**b 6.55 ± 0.40**	**a 4.66 ± 0.26**	**b 6.48 ± 0.40**
Jejunum	**a 50.35 ± 0.63**	**b 53.31 ± 0.34**	**a 11.59 ± 0.33**	**b 13.67 ± 0.56**	**a 11.12 ± 0.36**	**b 13.13 ± 0.52**
Ileum	**a 49.03 ± 0.56**	**b 52.05 ± 0.99**	**a 9.16± 0.45**	**b 11.20± 0.43**	**a 9.31 ± 0.51**	**b 10.84 ± 0.38**

The different letters in one raw denoted that there were significant differences between male and female indigenous ducks, in all traits at p≤0.01.

***Histological results and Discussion*:** The general structure of small intestine of both sexes appeared as uniform through its entire Length; although it can be divided into duodenum, jejunum and ileum, all are consist of tunica mucosa, submucosa , muscularis and serosa. The mucosal surface of small intestine has a distinctive feature by the villi which vary in shape, Length and number of goblet cells according to the region of small intestine and by presence of the crypts of Lieberkuhn(intestinal glands) which appear as a short, simple, branched tubular glands that open inbetween the villi and occupy most of the lamina propria between the bases of villi and muscularis mucosa.

***Duodenum region*:** Height of epithelium, villus height, crypts depth. The duodenum appear similar in both male and female. The tunica mucosa was characterized by a very long finger like villi and crypts of lieberkuhn that covered by simple tall columnar cells with a striated border and large oval nucleus lie in the lower half of these cells, between these cells scattered mucous goblet cells with narrow basal part and basally located nucleus and swollen upper part (Fig.5).This findings agree with [28] in pigeon.The mean height of columnar cells was higher in male than in female (Table3).This indicate that the columnar absorptive cells have same function in both sexes which include an absorption of nutrients substance [29].

There is no significant difference between male and female in the villus height, the longest villi are in the duodenum (Table3), As observed by[30]. The mean crypts depth in female was higher than in male (Table3). The intestinal epithelial cells are change constantly and compensate villi cells losses through proliferation and maturation inside crypts and upward migration. The crypts depth was correlated with the intestinal cells turnover rate and the increase in crypts depth indicates the need for enterocyte replacement and higher tissue turnover [31]. The crypt depth may be an important factor that determines the ability of the crypts to sustain the increase in the villus height as well as to maintain the villus structure [32].The ratio of villus height to crypts depth was slightly hieghr in female than in male (Table3).The increase of villus height to crypts depth associated with better nutrient absorption and faster growth [33].

The epithelium covering the villi is continue with the crypts of lieberkuhn that open at the bases of the villi, these crypts appear as simple, branched tubular invagination. The glands in the lamina propria appear as a group of columnar cells, goblet cells cells rest on the basement membrane around small or wide lumen (Fig.6).In the basal portion of the glands some enteroendocrine cells are present appear as a pyramidal cells, clear cytoplasm, round nucleus and it is granules below nucleus and against the basement membrane with broad base rest on the basement membrane and narrowing towards the apex of the cell. No paneth cells are present (Fig.7).The lamina propria appearfills the spaces between the crypts of lieberkuhn and form the core of the villi. It appear as highly cellular of loose connective tissue and a abundant of lymphocyte that aggregates as lymphatic nodule. Fine elastic networks surrounding the crypts and smooth muscle fibers extending into the villi. The muscularis mucosa denoted as consist of a layers of smooth muscle fibers occur in a longitudinal smooth muscle layer some smooth muscle fibers extend inside the villus,

The tunica submucosa is poorly developed as to be unexistent and obvious only where meissner plexus and large blood vessel were present and its appear as avery thin layer of connective tissue separating the musularis mucosa from the musularisexterna, and the brunner glands are absent. As finding of[20]. The musclarisexterna was composed of smooth muscle layer oriented in a thick inner circular and thin outer longitudinal layers and between them,Auerbachs plexus was present.The last outer layer is the tunica serosa that appear as a loose connective tissue contain blood vessels, adipose tissue and covered by mesothelium.

Jejunum region: Height of epithelium, villus height, crypts depth. The jejunum in both sexes were similar, decrease in height of the villi that become shorter than in duodenum and arranged in a characteristic zigzag pattern, some are long and othersare short and laef like with increase in the number of goblet cells (Fig.8).The epithelium covering the villi was simple columnar cells with numerous goblet cells that extend to lining the crypts of lieberKuhn (Fig.9, 10). The columnar cells or absorptive cells have a columnar shaped with brush border and oval nucleus relatively basally located, while the goblet cells become round as their cytoplasm become filled with mucigen and become hydrated when the mucigen released (Fig.10) similar observation by [34]. There is no respect differences between male and female in mean height of columnar cells of indigenous ducks at $p \leq 0.01$ (Table 3).This result may suggest that there is no respect differences in the nutrient absorption capacity of these cells in both sexes.

The villi in the jejunum are shorter than in duodenum. The morphology of the villi was similar to that reported by [35]and a according to[36], the nutrient absorption is more efficient when villi are organized like this pattern than if they are in parallel or randomly poisoned.There was no significant difference between male and female in mean villus height, crypts depth and ratio of villus height to crypts depth(Table 3).[7]mentioned that the villi and crypts play significant role in final stages of nutrient digestion and assimilation .The lamina propriamuscularis mucosa were similar to that found in duodenum (Fig.11).At the bases of the crypts very few enteroendocrine cells are present, they have same feature as in duodenum, as the observationby[20] .

Tunica submucosa is very reduced and appear as a very thin scattered connective tissue with elastic fibers observed only when blood vessels or miessner plexus present and the Brunner glands are absent,similar observation by [34] .The tunica muscularis appear as consist of a thick inner circular and a thin outer longitudinal layer of smooth muscle fibers. Blood vessels and nerves and Auerbach plexus present within connective tissue between the two layers.The outer most is the tunica serosa showed as a thin layer of loose connective tissue contains blood vessels and covered by a single layer of squamous epithelium.

Ileum region**:**Height of epithelium, villus height, crypts depth.Generaly, the histological structure of the ileum in male and female was similar and same as in the duodenum and jejunum. The characteristic feature of surface absorptive cells and goblet cells are the same as in the duodenum and jejunum. Lieberkuhn crypts were simple branched invaginations of the epithelium around the villi and occupy most of the lamina propria. The crypts lining were single layer of columnar cells, goblet cells and very few of enteroendocrine cells in the base of the crypts which have the same features, of the same cells in the duodenum and jejunum, Similar finding by [30]

There is no significant difference between male and female mean height of columnar epithelium(Table 3). This result may be due to that in both sexes the absorptivefunction of the enterocyte was needed. The small intestine of birds are known to have high absorptive rates for water and electrolytes, the movement of ions responsible for the electrical current across the epithelium are mainly result of the absorption of Na^+ and the secretion Cl^-[37].The villi of ileum appear as zigzag pattern, some are long, broad, with Flattened ends and others are short with pointed ends, leaf like and some are branched (Fig.12).The height of villi and mean crypts depth were higher in female than in male(Table3). No significant differences between them. There was a decrease in the crypts depth from duodenum to ileum, this is compatible with [38].No differences between male and female in ratio of villus height to crypts depth(Table3), that mean both sexes need for replacement of villus cells and maintain of villi. The lamina propria and muscularis mucosa have similar histological structure to that found in duodenum and iliumwith both diffuse and nodular accumulation of lymphoid tissue similar to peyres patches (Fig. 13,14). The lamina propria also forms the core of villi, similar finding by [20].

The tunica submucosa appear as a very reduced layer and appear as scattered loose connective tissue obvious only where the blood vessels are present therefore the brunner glands are absent.The tunica muscularis extern a was composed of a thick inner circular layer and a thin outer longitudinal layer, between them narrow connective tissue layer contain nerve plexus and blood vessels. lymphoid aggregates are extend from lamina propria to the muscularisexterna.As observed previously in other birds by [39] The contractile activity of smooth muscle fibers of the tunica muscularis is responsible for peristalsis that aids propelling the digesta and other materials contained in the lumen of small intestine[40]. Externally the ileum is covered by a thin layer of loose connective tissue contain elastic and collagen fibers covered by mesothelium.

***Number of goblet cells*:**There were no significant difference between two sexes in the numbers of goblet cells per villus in three segments of the small intestine (Table 4), but it was greatest in ileum and lowest in duodenum, similar to that noted by [41]on poultry.

***Histochemical Study*:** PAS stain and Alcian blue (AB). This study revealed that the goblet cells of the villi and intestinal glands secret both types of mucinous substance, an acidic and neutral mucopolysaccharides as indicated by positive reaction to both PAS (Fig. 5,10) and alcian blue (Fig.15). This finding in agreement with[42]on *Varanus niloticus*. The mucous layer plays an important role in protecting the epithelial cells of the small intestinal, lubrication and nutrient transport between the lumen and the brush border [15].

IV. Conclusion

This study revealed that although there were few histological differences between male and female indigenous ducks but they were not significant due to the same feed intake in both sexes and were both in the same environment conditions.

Table (3). The comparison of the heights of columnar cells , villus height , crypts depth and the ratio of villi length to crypts depth of duodenum, Jejunum and Ileum in males and females indigenous ducks (Mean ± SE).

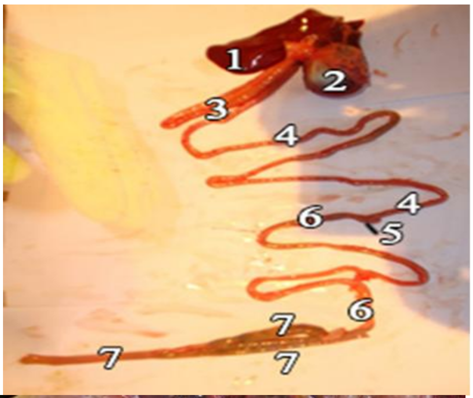
measurement μm	**Duodenum**		**Jejunum**		**Ileum**	
	Male mean ± SE	**Female mean ± SE**	**Male mean ± SE**	**Female mean ± SE**	**Male mean ± SE**	**Female mean ± SE**
Height of columnar cells	**a 28.704 ± 0.702**	**a 27.976 ± 0.832**	**a 33.176 ± 0.858**	**a 32.89 ± 0.754**	**a 32.76 ± 0.832**	**a 32.058 ± 0.884**
Villus height	**a 845.836 ± 37.492**	**a 887.531 ± 25.808**	**a 692.881 ± 28.222**	**a 700.482 ± 25.853**	**a 532.25 ± 21.115**	**a 556.439 ± 19.879**
Crypts depth	**a 81.679 ± 3.914**	**a 84.769 ± 2.369**	**a 74.778± 2.884**	**a 75.256 ± 2.163**	**a 57.989 ± 3.090**	**a 59.800 ± 2.678**
Ratio Villus height / Crypts Depth	**10.355**	**10.471**	9.265	**9.307**	**9. 178**	**9.305**

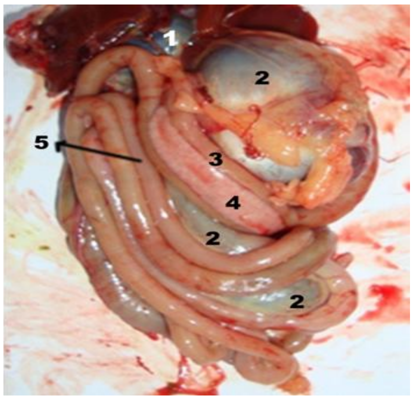
The different letters in one raw denoted that there were significant differences between male and female indigenous ducks, in all traits at p≤0.01.

Table(4). Number of goblet cells per villi in the (duodenum , jejunum and ileum) in male and female indigenous ducks.

Number of goblet cells per villus	Male Mean ± SE	Female Mean ± SE
Duodenum	a 15 ± 3.27	a 17 ± 2.13
Jejunum	a 25 ± 2.801	a 23 ± 3.65
Ileum	a 38 ± 4.121	a 36 ± 7.253

The same letters in one raw denoted that there were no significant differences between male and female indigenous ducks, in all traits at p≤0.01.





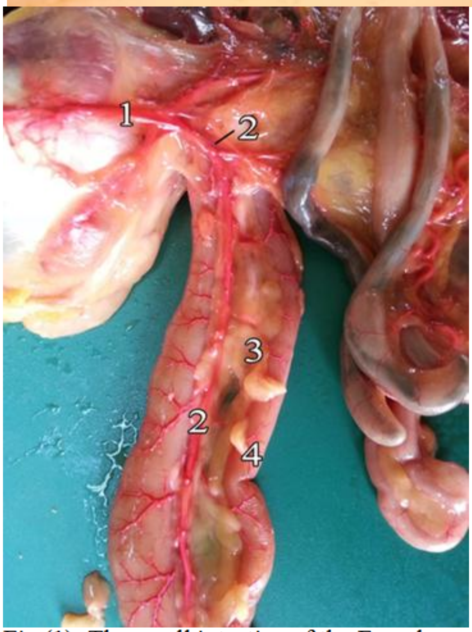


Fig (1): The small intestine of the Female indigenous duck ; 1-Liver 2- Gizzard, 3- duodenal loop, 4- jejunum, 5- Meckel's diverticulum,6-Ileum 7-Large intestine

Fig (2): The arrangement of small intestine of Female indigenous duck.1- Liver 2- Gizzard 3- dudeenum 4- pancreas 5-jejunum 6-Ileum 7- caeca

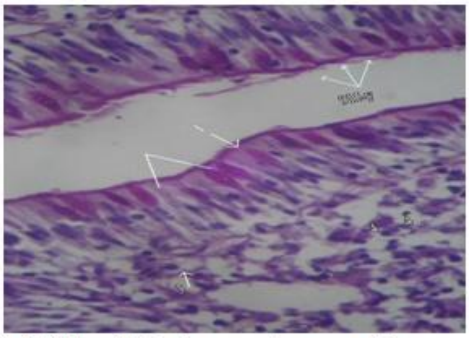


Fig.(5):The villi in the anterior part of the duodenum in female indigenous duke.1- columnar cells 2-goblet cells 3- basement membrane 4- core of the villi PAS stain x400

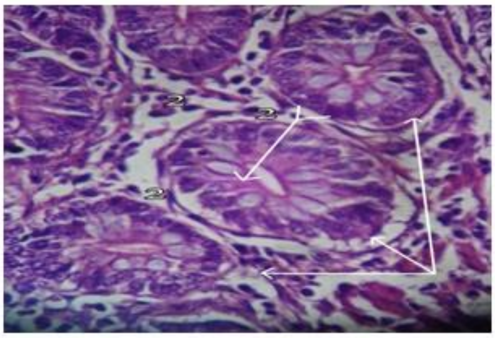


Fig.(6):The intestinal glands in the posterior part of the duodenum in the male indigenous duck1- intestinal glands 2- lamina properia 3- columnar cells 4- goblet cells. H&E (x400)

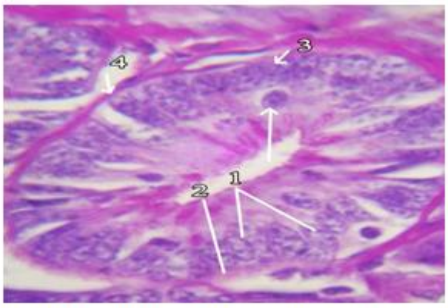


Fig.(7):The intestinal gland in the middle part of the duodenum in the male indigenous duck1- columnar cells 2- goblet cells 3- enteroendocrine cells 4- basement membrane.PAS(1000)

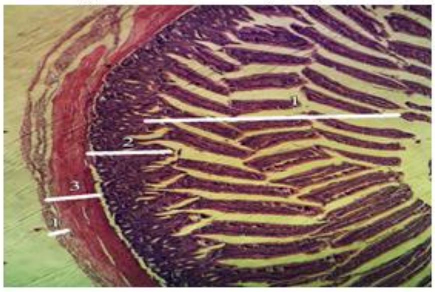


Fig.(8):The wall structure of middle part of the jejunum in the male in indigenous duck.1- Zigzag pattern of villi 2-tunica mucosa 3-tunicamuscularis 4-tunicaserosa. H&E(x40)

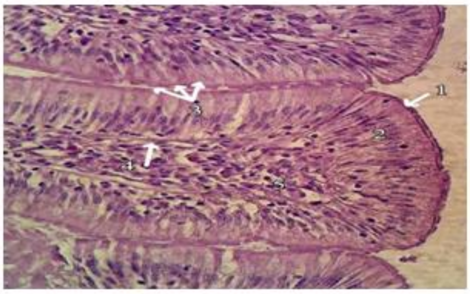


Fig.(9):The villi in the anterior part of the jejunum in the male in indigenous duck1-brush border 2- columnar cells3- Goblet cells 4-basement membrane5- core of villiH&E(x400)

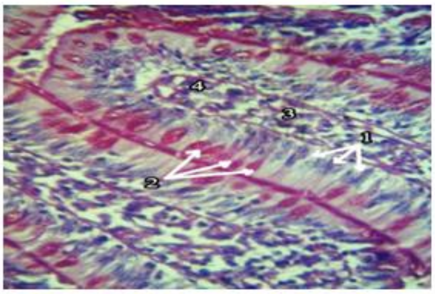


Fig.(10):The villi in middle part of the jejunum in the male in indigenous duck1-columnar cells 2- goblet cells3-basement membrane 4- core of villi. PAS(x400)

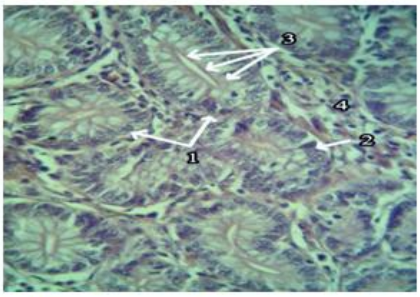


Fig.(11): The crypts of lieberkuhn in posterior part of the jejunum in the female indigenous duck1-crypts of lieberkuhn 2- columnar cells 3- goblet cells 4- lamina properia. H&E(x100)

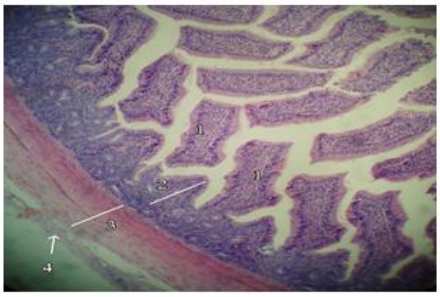


Fig.(12):The wall of the posterior part of the ileum in the female indigenous duck1-villi 2- Tunica mucosa3- Tunica muscularis 4- Tunica serosa. H&E (x100)

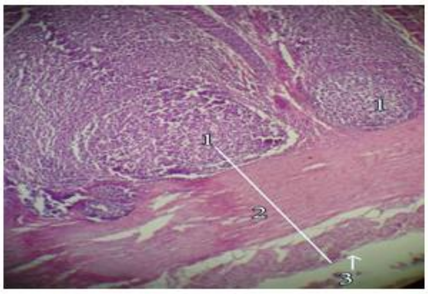


Fig.(13):Lamina properia in the anterior part of the ileum in the female indigenous duck, 1- crypts of lieberkuhun2- lamina properia3- lymphocytes. PAS(x400)

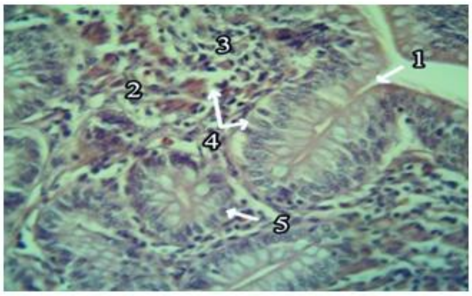


Fig.(14):The aggregated lymphoid tissue in the lamina propria of the posterior part of the ileum in male indigenous duck. 1- nodular lymphoid tissue 2- Tunica muscularis 3- Tunica serosa. PAS(x400)

Fig.(15): The villi in the middle part of the duodenumn in female indigenous duck,the goblet cells give apoitive reaction reaction with AB stain X400.

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