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RESEARCH ARTICLE

Histomorphological Study and Scanning Electron Microscopy of the Duodenum in the Iraqi Black Partridge (*Francolinus francolinus*)

Ola Al-Bhadly[1*], Intidhar Mnati[2]

Department of Biology / Collage of Education for Pure Science (Ibn Al- Haitham) / University of Baghdad Iraq.

Abstract

Objective: This project aimed to study the Morphological description and histological structure by using light and scanning electron microscope of duodenum in both male and female Iraqi black partridge (*Francolinus francolinus*) Methods: To conduct this investigation, 20 healthy Iraqi black partridge were collected from local suppliers. Birds were euthanized, dissected and then specimens were processed for histological and histochemical staining techniques. Results: Morphological study showed the duodenum consists of descending and ascending limbs forming (U) shaped tube called duodenal loop extend from the right side of the dorsal surface of the gizzard. The pancreatic and bile ducts opened in the end of the ascending limb. Male had significantly higher than Female in the mean length and weight of duodenum. Histologically, the wall of the duodenum was consisted of four tunicae mucosa, submucosa, muscularis and serosa. The mucosa had a distinctive feature by the presence of villi had finger shape and the crypts of lieberkuhn which covered by simple columnar epithelium with goblet cells. The goblet cells gave positive reaction with PAS stain. Duodenal villi appeared by SEM very long and finger-like shaped with two organized orientations, primary and secondary villi. No significant differences between sexes in the villi height, crypts depth and the number of goblet cells but significantly difference in the mean thickness of tunica. Conclusion: the results showed that the significant difference in the mean length, weight of duodenum and thickness of tunica between two the sexes may be related to differences in food intake amounts. Goblet cells neutral mucopolysaccharide secretions; in fact, the latter stain is an indicator mucin substance which are very important in digestion and absorption and subsequent body growth of the bird.

Keywords: *Duodenum, Histomorphology, IRAQI Black Partridge, Scanning electron microscope.*

Introduction

The Iraqi Black Partridge belongs to a family (Phasianidae) of order (Galliformes). This family all wild birds and called the same name on the male and female and have most of their diet on grains (Granivorous) [1]. The birds' bodies need fuel to go about their daily activities, and this is where the digestive system plays its role [2]. Small intestine is the first site concerned with the breakdown of enzymes, in addition to absorption of carbohydrates, fatty acids, and amino acids [3]. Nutrients absorption is important at all stages of life.

The small intestine, especially the duodenal crypts and villi of the absorptive epithelium, play significant roles in the final stages of nutrients digestion and assimilation. Studies on the duodenum have revealed that the size of the duodenum and its digestive activities are altered during development in birds [4].the small intestine in birds consists of three parts: the first part, duodenum extends from the gizzard forming a loop which surrounds most of the pancreas and the second part, the jejunum extends from distal portion of the duodenum loop to the Meckel's diverticulum and the third part ,the ileum extended from the which is initiated from the Meckel's diverticulum to the ileo-caecal junction [5,6].

The aim of study is designated to determine the Morphological description and histological structure of duodenum in both male and female Iraqi black partridge (*Francolinus francolinus*) and compare between them, in addition to using in this study scanning electron microscope.

Materials and Methods

Birds Collection

Twenty male and female Iraqi black patridge (*Francolinus francolinus*) including (10) from each sex were in this study. These birds were collected from local suppliers in Baghdad province.

Morphological Study

Gross morphology, topographical relationship in situ and other anatomical observations were studied. The length of duodenum was measured after removed and they laid out in a straight line using the Tapeline [7]. Weight of duodenum was taken by sensitive electrical balance after its contents were emptied and cleaned [8]. The duodenum identified and photographed in situ using Digital Sony camera.

Histological and Histochemical Preparation

For histological study, the specimens were fixed in neutral buffered formalin of 10% concentration for 48 hours. After well fixation the specimens were dehydrated by passing them through a series of ascending ethanol concentrations (70, 80, 90 and 100 %) and then the specimens were cleared by xylene, after that they were embedded in paraffin wax, then the blocks were sectioned at 6 µm thickness and stained with the following stains: Hematoxylin and eosin routine stain for general features identification.

The histological examination was done by using light microscope and photographed using (14.1) mega pixels power digital camera. In the histochemical study, sections were stained with Periodic Acid Schiff (PAS) was used for the illustration of the goblet cells and the basement membranes of the epithelial lining of the duodenum, Masson's trichrome stain for the staining of the collagenous and smooth muscle fibers [9].

Morphometric Measurements

The morphometric measurements were including the villus height that was measured from the villus tip to the villus-crypt junction, while the crypt's depth was defined as the depth of invagination between two villi, and for number of the goblet cells, it was calculated per villus, and thickness of tunica was measured [10]. All these measurements were calculated by program Image J. Statistical analysis of data was performed using analysis of variance test (T-test) and significant differences limited on $p \leq 0.05$.

Scanning Electron Microscope Examination:

One-millimeter slice of tissue from the middle portion of the duodenum were fixed in 2.5% glutaraldehyde. Tissue samples for SEM were processed as described previously [11].

Results

Morphological Study

This study revealed that the duodenum was similar in both sexes, the duodenum formed the first loop of small intestine which is situated in the caudal part of the right side in the abdominal cavity, it arises from the right side of the dorsal surface of the gizzard (Fig.1). The duodenum consists of descending and ascending limbs that bind together by mesenteric membrane forming (U) shaped tube called duodenal loop. The pancreas lies between the limbs (Fig.1and Fig.2).

There are two pancreatic ducts and two bile ducts opens into the end of the ascending limb of the duodenum (Fig.2). The results of the visual examination revealed that the internal lining of the duodenum was rough; this is due to containing longitudinal tortuosity (Fig.3). The present study showed significant differences at $p \leq 0.05$ between two the sexes in length and weight, the length and the weight of the duodenum in male were more than in female (Table1).

Table1: The mean and standard error (Mean ± SE) for length and weigh of duodenum of males and females in Iraqi black partridge

Measurement	Male mean ± SE	Female mean ± SE
Duodenum length (cm)	10.08*±.18	9.40*±.16
Duodenum weight (gm)	2.67*±.05	2.31*±.07

(*) denoted that there were significant differences between male and female in Iraqi black partridge at $p \leq 0.05$.

Figure 1: Showing shape and position of duodenums, (D) Duodenum, (G) Gizzard, (L) Liver, (GB) Gall bladder, (C) Caeca, (J) jejunum, (R) Rectum

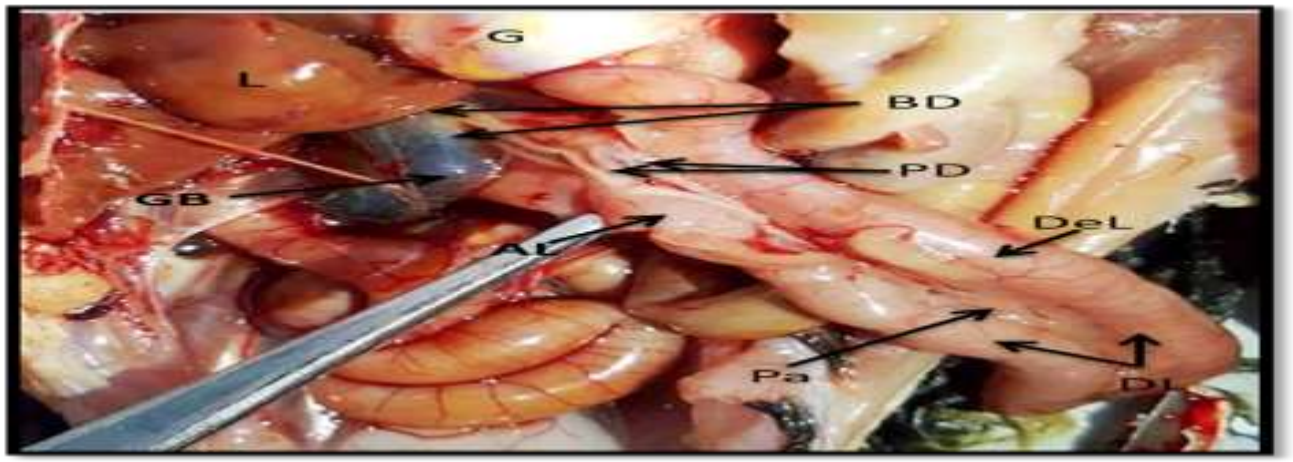

Figure 2: Showing (DL) Duodenal loop, (DeL) Descending limb, (AL) Ascending limb, ,(PD)Pancreatic ducts,(BD) Bile ducts, (G) Gizzard, (L) Liver, (GB) Gall bladder

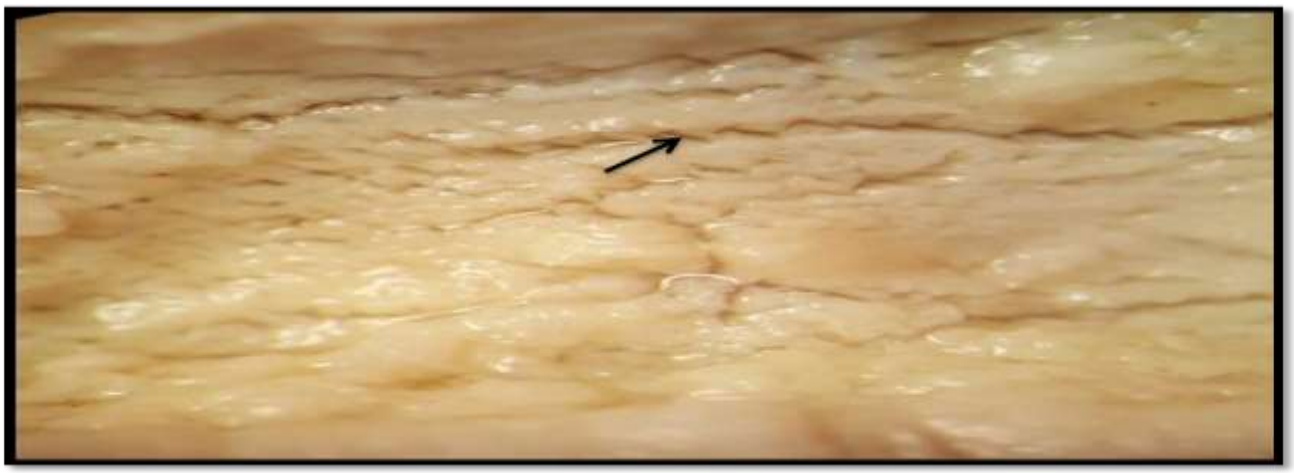

Figure 3: Showing longitudinal tortuosity of internal lining for the duodenum

Light Microscopic Observation

The histological observations in both sexes revealed that the duodenum wall containing four tunica from the lumen to the external layer, the tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa (Fig.4).

Tunica Mucosa

This tunica consists of three sub-layers represented by lining epithelium, lamina propria and muscularis mucosa (Fig.5). The lining epithelium was characterized by a very long finger like villi (Fig.6), that covered by simple columnar epithelium formed of columnar cells or absorptive cells with a striated border and large oval nucleus lie in the lower half of these cells, these cells are based on basement membrane, scattered between these cells mucous goblet cells with narrow basal part and basally located nucleus and swollen upper part, the goblet cells showed positive reaction of PAS stain and appeared magenta color (Fig.7). Lamina propria consists of loose connective tissue

containing several blood vessels, lacteal lymphatic vessels, smooth muscle fibers and nucleus of connective tissue cells (Fig.8). The mucosa shows invaginations at the bases of villi to form the Crypts of Lieberkühn or intestinal glands occupying most of the lamina propria. These crypts appear as simple, branched tubular gland. The glands in the lamina propria appear as a group of columnar and goblet cells that rest on the basement membrane around small or wide lumen (Fig.9).

In the basal portion of the glands some enteroendocrine cells are present and appear as a pyramidal cell with round nucleus, below the nucleus and against the basement membrane granules are found. These cells have broad base narrowing towards the apex of the cell (Fig.10). As well as containing the basal portion of the glands some Paneth cells are also found. That has pyramidal shape, central nucleus, and its cytoplasm contains granules clustered around the nucleus which receptive for acid dyes (Fig.11). Muscularis mucosa is composed of a narrow part of smooth muscle fibers longitudinally arranged, it extends inside the mucosal villi and between the intestinal glands (Fig.9).

Tunica Submucosa

The tunica sub mucosa was reduced into thin layer of loose connective tissue separating the muscularis mucosa from the tunica muscularis (Fig.12).

Tunica Muscularis

Composed of smooth muscle layer oriented in thick inner circular and thin outer longitudinal layers and between them fibrous connective tissue containing elastic fibers, blood and lymphatic vessels and nerves (Fig. 12, 13 and14).

Tunica Serosa

The last outer layer is the tunica serosa that appears as a loose connective tissue containing blood vessels, adipose tissues and covered by simple squamous epithelium (Fig.14).

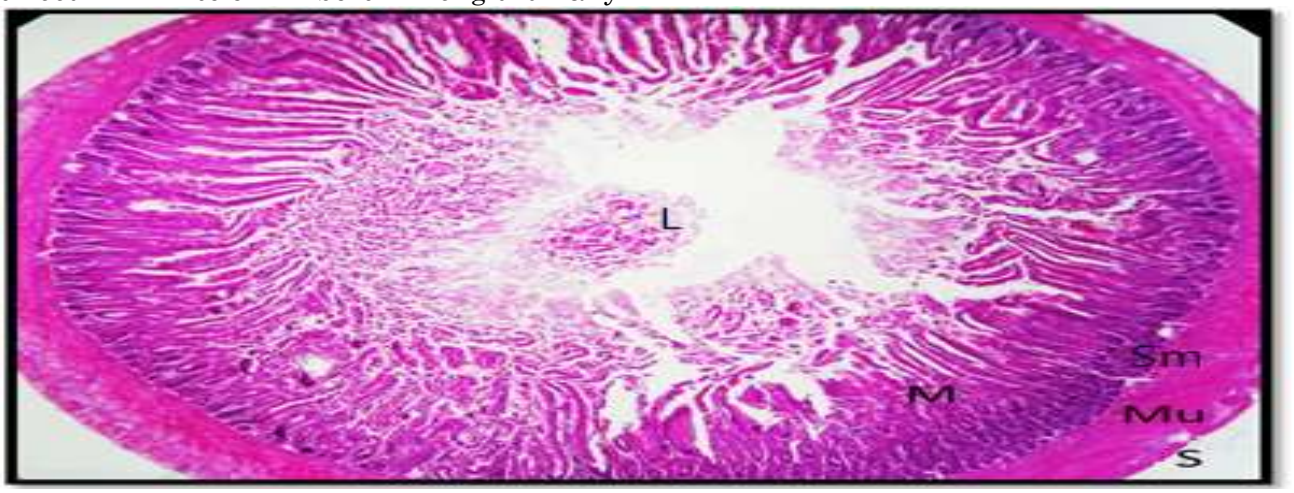

Figure 4: Cross section of duodenum showing the four tunicae, (M) Mucosa, (Sm)Submucosa, (Mu) Muscularis mucosa, (S)Serosa, (L) Lumen (4X H&E)

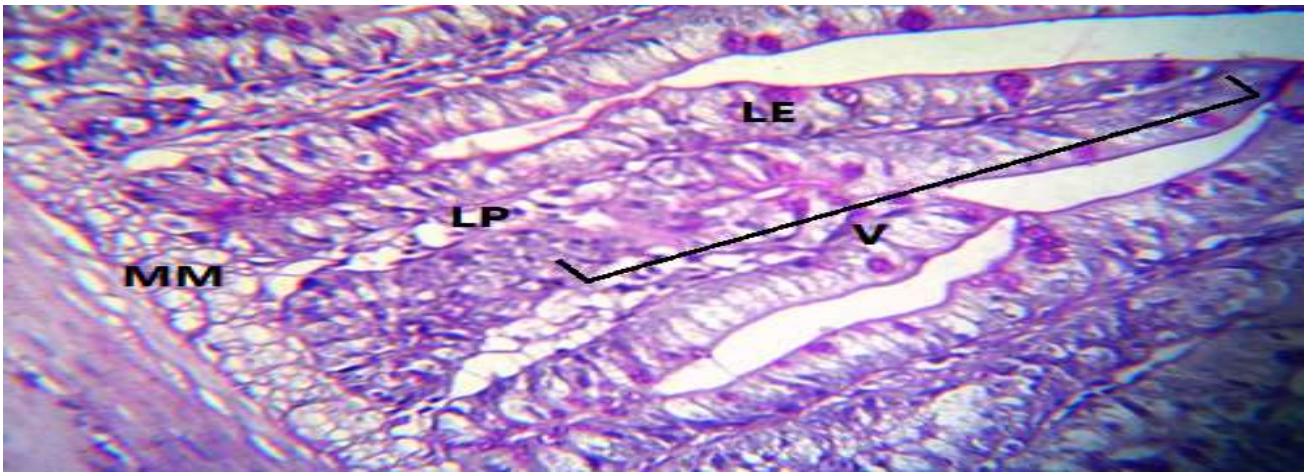

Figure 5: Cross section of duodenum showing three sub-layers of mucosa (LE)Lining epithelium (LP) Lamina propria, (MM) Muscularis mucosa (40X PAS)

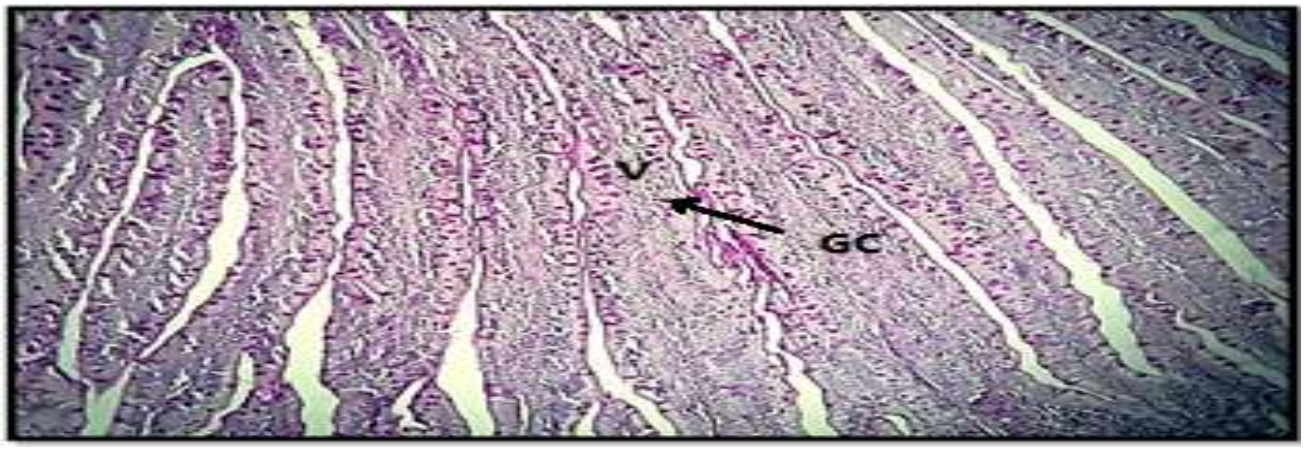

Figure 6: Cross section of duodenum showing finger shaped villi (V) and positive reaction of mucopolysaccharides in (GC) goblet cells with PAS stain (10X PAS)

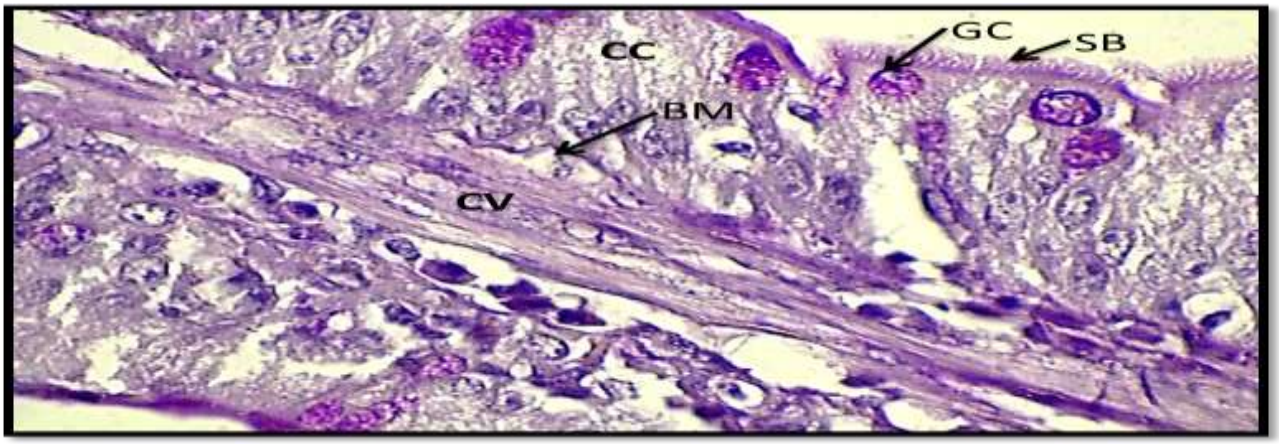

Figure 7: Cross section of duodenum showing (CC) Columnar cell, (GC) Goblet cell, (BM) Basement membrane, (SB) Striated border, (CV) Core villus (100X PAS)

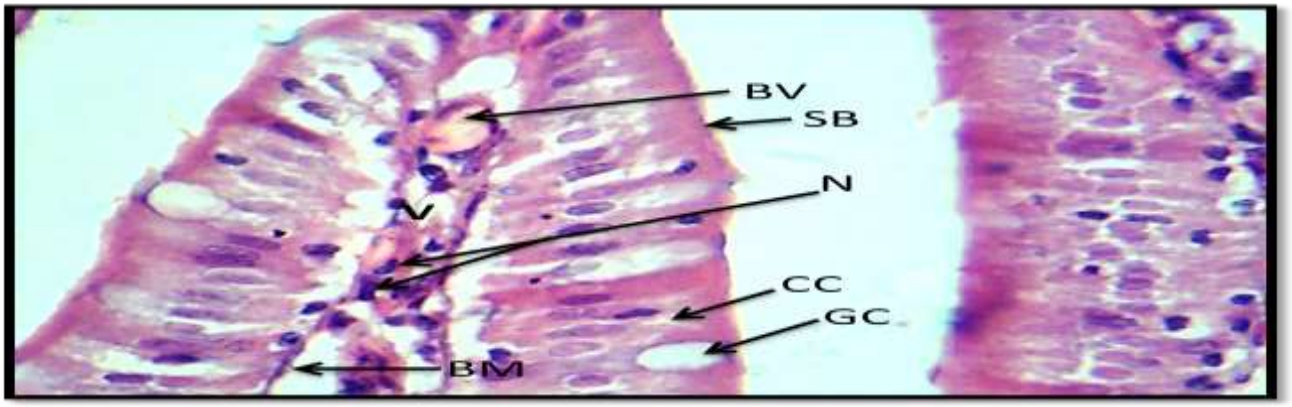

Figure 8: Cross section of duodenum showing (CC) Columnar cell, (GC) Goblet cell, (BM) Basement membrane, (SB) Striated border, (BV) Blood vessel, (N) Nucleus of connective tissue cells (100X H&E)

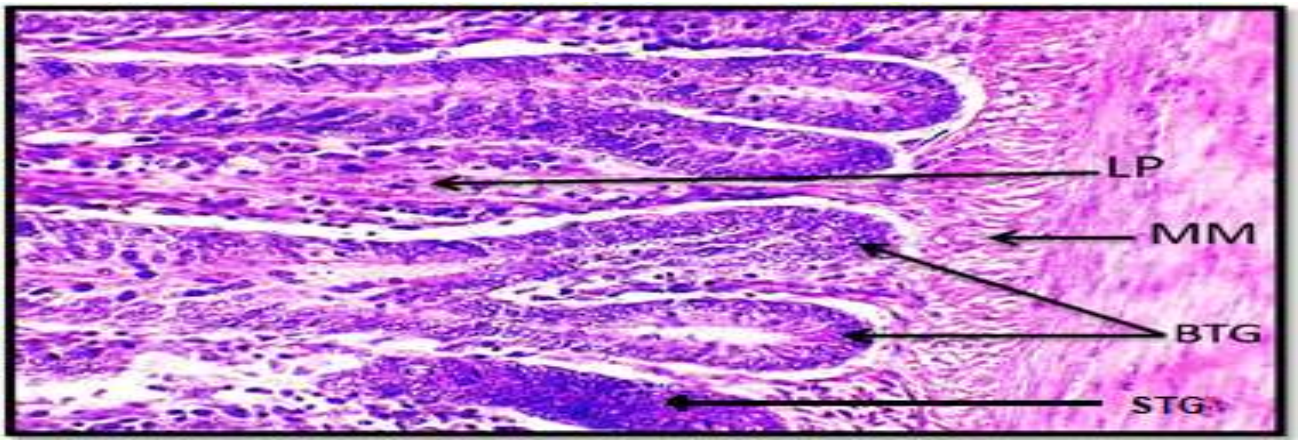

Figure 9: Cross section of duodenum showing (BTG) Branched tubular gland, (STG) Simple tubular gland, (LP) Lamina propria, (MM) Muscularis mucosa (40X H&E)

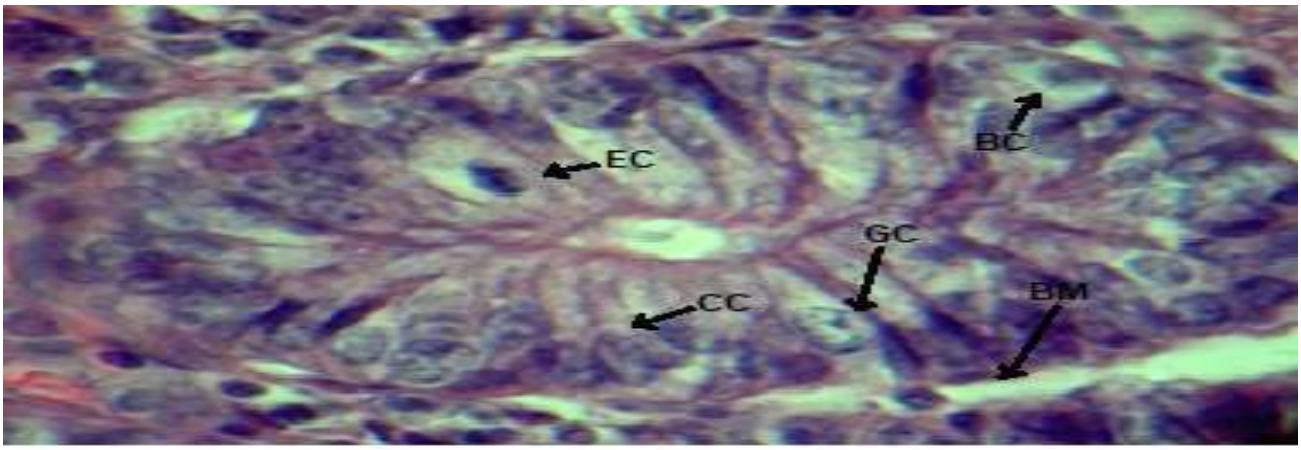

Figure 10: Cross section of duodenum in intestinal gland showing (CC) Columnar cell, (GC) Goblet cell, (BC) Basal cell, (EC) Enteroendocrine cell, (BM) Basement membrane (100X H&E)

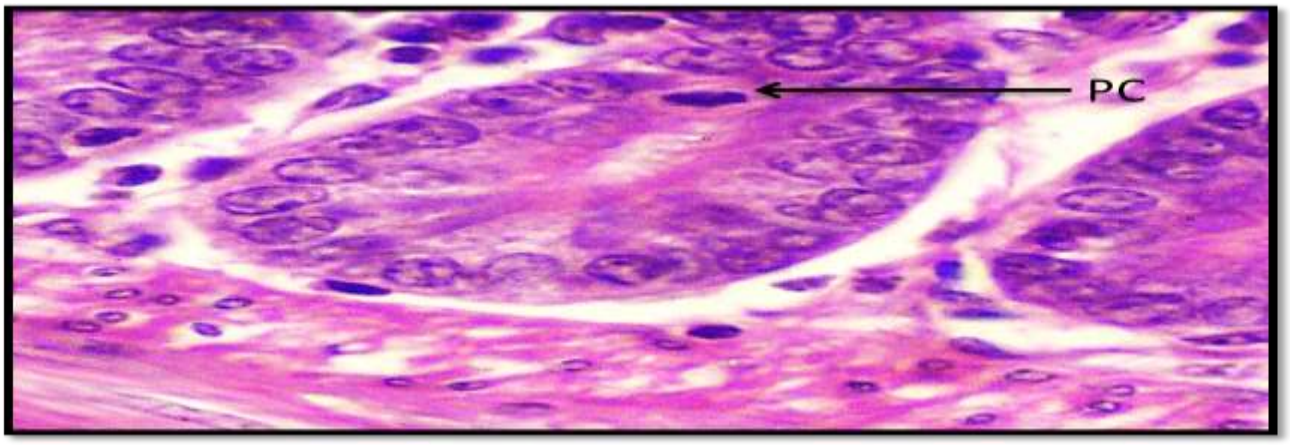

Figure 11: Cross section of duodenum in intestinal gland showing (PC) Paneth cell (100X H&E)

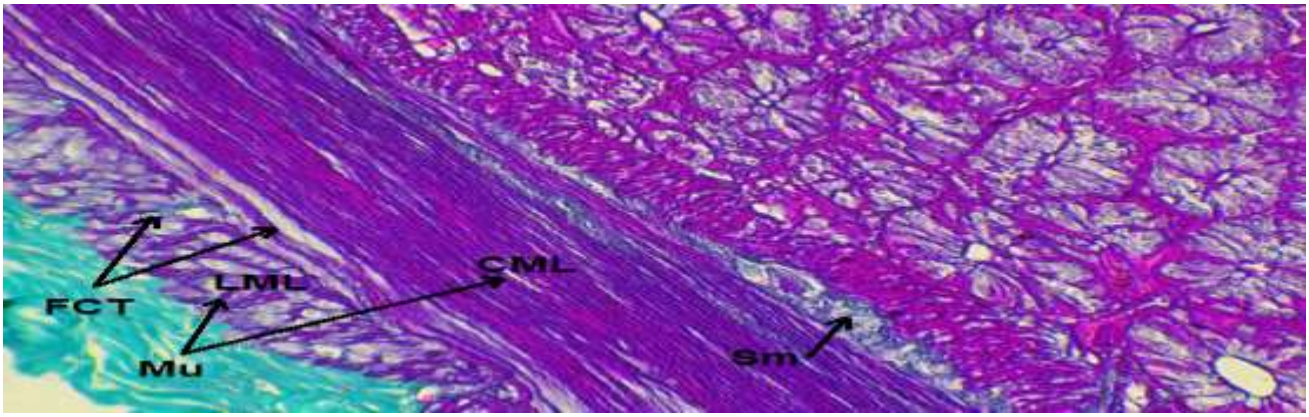

Figure 12: Cross section of duodenum showing (Sm) Tunica submucosa, (Mu) Tunica muscularis, (CML) Circular muscle layer, (LML) Longitudinal muscle layer, (FCT) Fibrous connective tissue (40X Masson's trichrome)

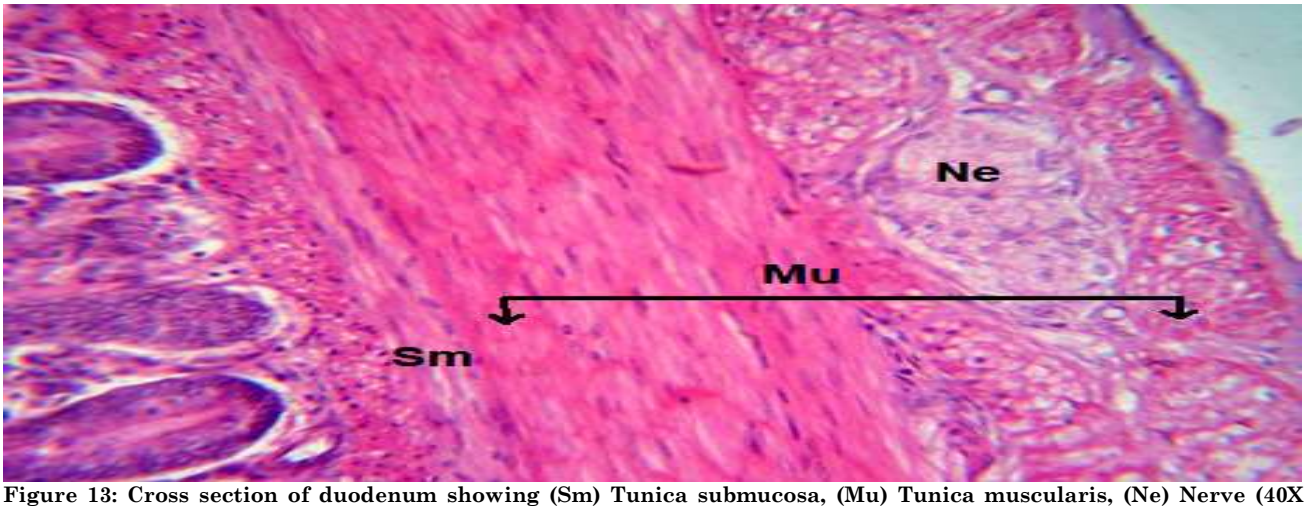

Figure 13: Cross section of duodenum showing (Sm) Tunica submucosa, (Mu) Tunica muscularis, (Ne) Nerve (40X H&E)

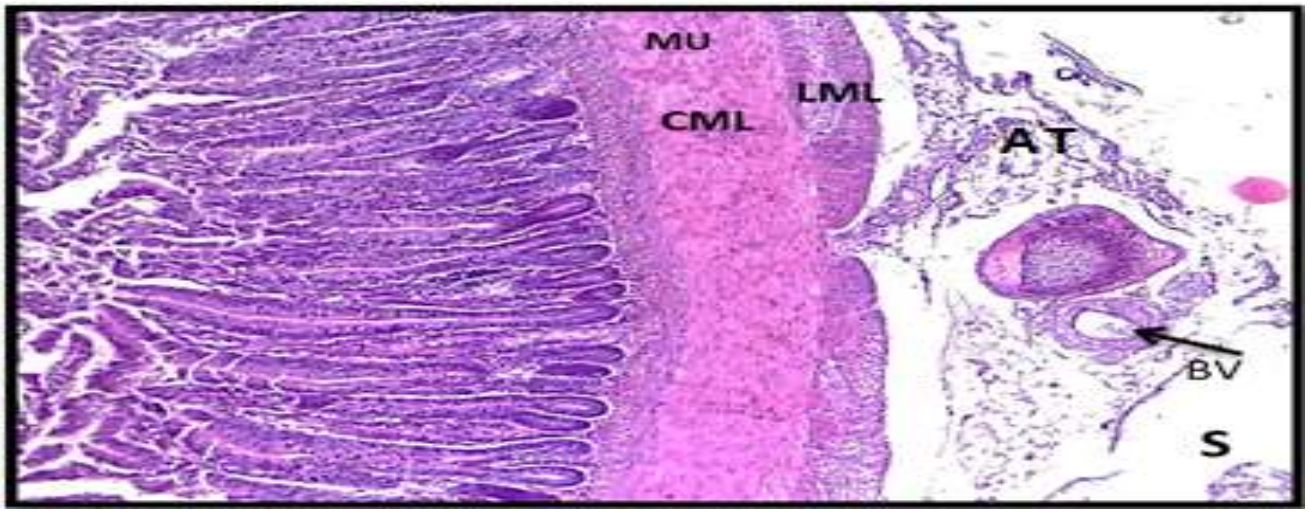

Figure 14: Cross section of duodenum showing (Mu) Tunica muscularis, (CML) Circular muscle layer, (LML) Longitudinal muscle layer, (S) Serosa, (BV) Blood vessel, (AT) Adipose tissue (10X H&E)

Scanning Electron Microscopic Observations

Scanning electron Microscopic micrograph in the present study showed that the inner surface of the wall of the duodenum appears as projections called villi, duodenal villi appeared very long and finger-like shaped with two organized orientations, primary and secondary villi (Fig.15, 16).The high magnification micrograph revealed that the apical surface of the duodenal villi contains goblet cells represented by the pitted view (Fig.17).

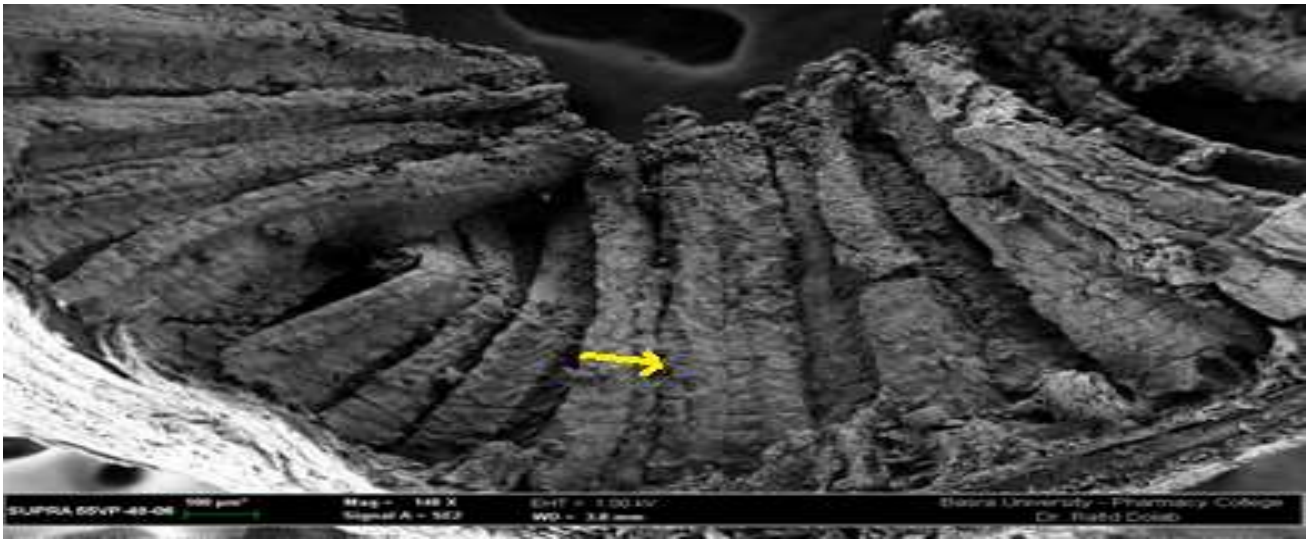

Figure 15: Scanning electron micrograph of the duodenall villi ( )showing finger-like villi (148X)

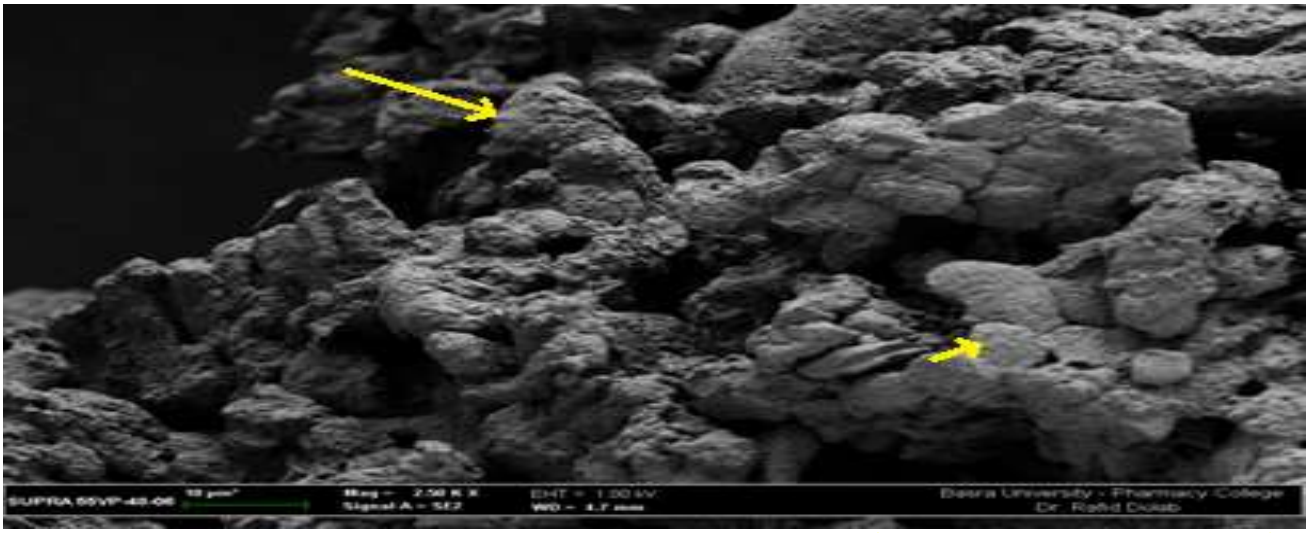

Figure 16: Scanning electron micrograph showing primary duodenal villi ( ), secondary duodenal villi ( ) (2.50 KX)

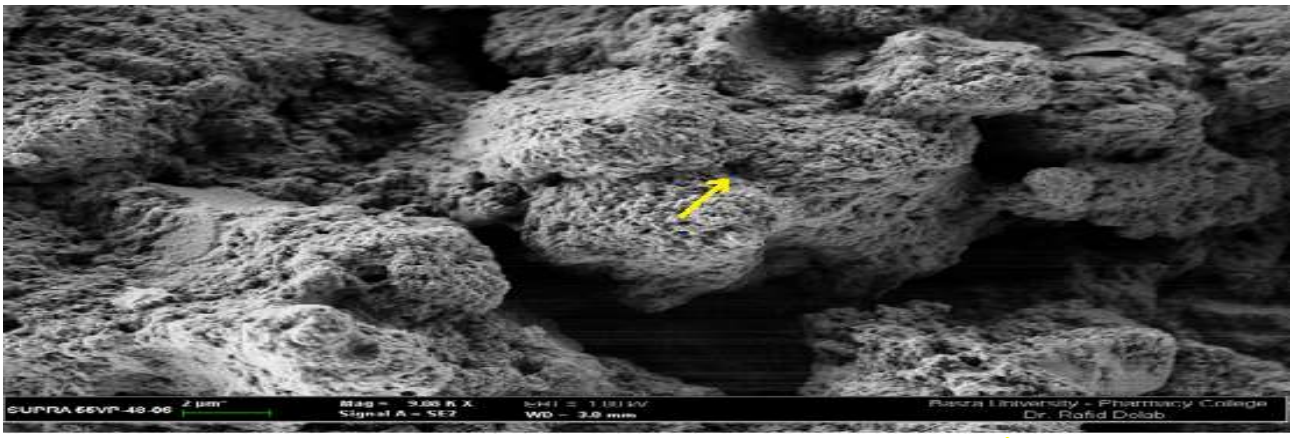

Figure 17: Scanning electron micrograph showing shape and position the goblet cells ( ), (9.08 KX)

Morphometric Measurements

The mean villi height was 822.27±37.47µm, in male it was 835.74±49.15 µm while in female it was 809.69±57.73 µm. The mean crypts depth was 219.59 ±9.40 µm, in male it was 294.88±15.33 µm while in female it was 189±2.369 µm (Fig.18). The present study noticed that there is no significantly difference at p≤0.05 between male and female in the villi height and crypts depth. The mean numbers of goblet cells were 28.20±1.60 µm, they were 28.60±2.76 µm, 27.80±1.75 µm in both male and female respectively (Fig.19), there is no significantly difference between them at p≤0.05. The mean thickness of tunica mucosa was 1097.81±51.83 µm, it was 1194.93±55.03 µm, 997.61 ±43.12µm in both male and female respectively. Mean thickness of tunica submucosa was 9.34±0.11µm; it was 9.66±0.14 µm, 9.19 ±0.16µm in both male and female respectively. Mean thickness of tunica muscularis was 209.18±13.18µm; it was 241.28±20.24µm, 177.08 ±13.51µm in both male and female respectively. Mean thickness of tunica serosa was 53.78±5.48µm, it was 55.55±8.19µm, 52.00 ±7.47µm in both male and female respectively (Fig.20). The present study noticed that there is significantly difference at p≤0.05 only in the mean thickness of tunica mucosa and tunica muscularis between male and female.

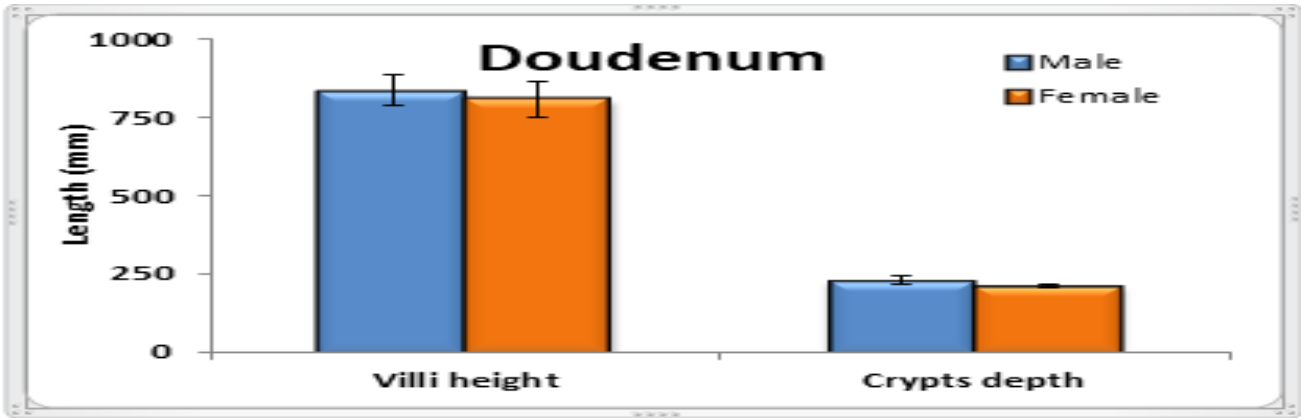

Figure 18: Comparison of the mean and standard error between male and female in length villi and the crypts depth

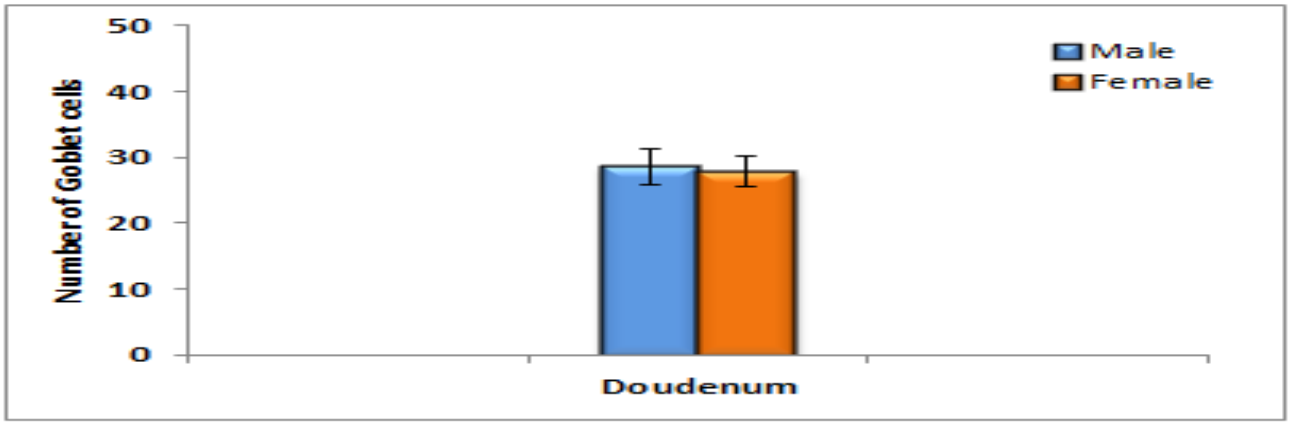

Figure 19: Comparison of the mean and standard error between male and female in number of goblet cells

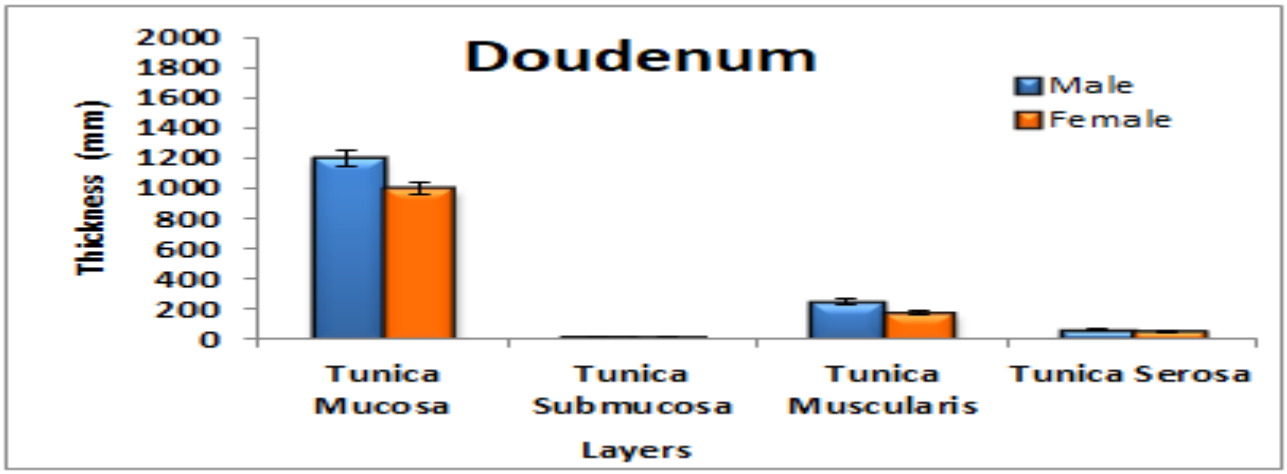

Figure 20: Comparison of the mean and standard error between male and female in thickness of tunica

Discussion

The present results revealed that the duodenum of Iraqi black partridge formed first loop of small intestine which is situated in the caudal part of the right side in abdominal cavity, It arises from right side of the dorsal surface of the gizzard, similar findings have been reported by [12] on *Streptopelia senegalensis* and [13] on Barn Owl (*Tyto alba*) but incompatible with what reported by [14] on African Pied Crow (*Corvus albus*) who reported that the duodenum situated in the caudal part of the left side in abdominal cavity.

The loop consists of a descending and an ascending limb and the pancreas lies between them which was commonly observed in the other avian species such as Domestic fowl [15], Kestrel (*Falco tinnunculus*), White eared bulbul (*Pycnonotus leucotus*) [10] and Buzzard's (*Buteo buteo*) [16]. There are two pancreatic ducts opened into ascending loop of the duodenum, these ducts were opening near the two bile ducts, similar findings were found by [15] on Goose, but present result disagrees with [6] on indigenous ducks (*Anas platyrhynchos*) whom showed that there is three pancreatic and two bile ducts.

The result of the visual examination revealed that the internal lining of the duodenum is rough, this is due to containing longitudinal tortuosity, the present results agreed with the finding of [17] on Common buzzard (*Buteo buteo*) but incompatible with the same researcher on Rock dove (*Columba livia*) which was smooth. The longitudinal tortuosity of the inner lining of duodenum will increase the breadth when food passes through it therefor increment of the surface area of absorption and digestion.

This study showed significant differences at $p \leq 0.05$ between two sexes in length and weight, the length and weight of duodenum in male were more than in female, like finding by [18] on *Ross* broiler chicken, these may be related to the increment in the whole intestine weight in male than in female due to differences in food intake amounts [19].

The histological examination showed that the wall of the duodenum comprised tunica mucosa, submucosa, muscularis and serosa, these four layers appeared in most avian such as blue and yellow macaws (*Ara ararauna*) [20], Common quail (*Coturnix coturnix*) [21], and Buzzard's (*Buteo buteo*) [17].

This result disagreed with [22] on Japanese quail (*Coturnix coturnix Japonica*) who mentioned the absence of the tunica submucosa. The duodenal mucous showed three different parts, the lining epithelium (simple columnar cells) which is the first layer arranged in finger projection like, lamina propria (loose connective tissue with the presence of intestinal glands) and muscularis mucosa (smooth muscle fibers arranged longitudinally), similar finding by [23,24].

While two layers of muscularis mucosa in the duodenal mucosa observed in ostrich (Struthio *camelus*) by [25], whereas totally absent in the duodenal mucosa of African pied crow (*Corvus albus*) in findings by [14].This result indicates that the columnar absorptive cells have the same function in both sexes which include the absorption of sugars, amino acids, fatty acids, monoglycerides, electrolytes, water and many beneficial substances [26].

Goblet cells scattered among the columnar lining of both villi and crypts of lieberkuhn.

These cells reacted positively with the PAS as they gave rise to the magenta color. Current finding was in a good agreement with [23, 24]. The goblet cells excrete a mixture of glycoproteins called mucinogen which act to lubricate the intestinal tract and protect it against infections, acidity of stomach, pathogenic microorganisms and virus [27,28].

The importance of mucous secretion on nutrients helps sliding and moisturizing the inner wall of the digestive tract as well as its role in the process of digestion, absorption and protection of the lining of the spread of parasites and protection of the mucous membrane of mechanical damage resulting from the ingestion of solid materials.

The crypts of lieberkuhn were simple and branched tubular gland opened at the bases of villi occupying most of the lamina propria as mentioned by [6] indigenous ducks (*Anas platyrhynchos*). These glands composed of columnar, goblet, basal, enteroendocrine and Paneth cells, this result disagree with [6] in indigenous ducks (*Anas platyrhynchos*) where the Paneth cells were absent.

These glands secrete a large amount of digestive enzymes that facilitates digestion and absorption of food such as Peptidase and Disaccharidase enzymes which breaks the food bonds.The crypt depth may be an important factor that determines the ability of the crypts to sustain the increment in the villus height as well as to maintain the villus structure [29] The increase of villus height to crypts depth associated with better nutrient absorption and faster growth [30]. The tunica submucosa was reduced into thin layer of loose connective tissue similar to observation [31] on Spur Winged Lapwing (*Vanellus spinosus*) and [32] on common wood pigeon (*Colimba palumbus*) and barn owl (*Tyto alba*) but this result isn't similar with [22] on Japanese Quail (*Coturnix coturnix japonica*) as he mentioned that this tunica was absent.

Tunica muscularis composed of smooth muscle layer oriented in a thick inner circular and thin outer longitudinal layers, the present results agreed with [24] on guinea fowl but disagrees with what has been reported by [22] on Japanese Quail (*Coturnix coturnix japonica*) who mentioned that this tunica consists of middle circular layer between the inner and outer longitudinal layers.

The last outer layer is the tunica serosa that appears as a loose connective tissue containing blood vessels, adipose tissues and covered by mesothelium, this result is like observation of [33,24]. Using Scanning electron, it showed that the duodenal villi appeared as finger-like shape with the apical surface containing goblet cells agreement observation [32] on barn owl (*Tyto Alba*).

Conclusion

The results showed that the significant difference in the mean length, weight of duodenum and thickness of tunica between two the sexes may be related to differences in food intake amounts. Goblet cells neutral mucopolysaccharide secretions; in fact, the latter stain is an indicator mucin substance which are very important in digestion and absorption and subsequent body growth of the bird.

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*Corresponding Author Email: *saedalani@yahoo.com*

