



HISTOMORPHOLOGICAL AND HISTOCHEMICAL STUDY OF THE SMALL INTESTINE OF THE MALLARD (*ANAS PLATYRHYNCHOS*) IN SOUTH IRAQ

¹Eyhab R.M. Al-Samawy, ²F.J. Al-Saffar, ³Wafa Abdulmutalib Naji and ⁴Ahmed Sami Jarad

¹Department of Anatomy, Histology and Embryology, College of Medicine, Al-Muthana University, Iraq

²Department of Anatomy, Histology and Embryology, College of Veterinary Medicine, Baghdad University, Baghdad, Iraq.

³Department of Biology AL-Muthanna University - College of Science

²Department of Pathology and poultry disease, College of Veterinary Medicine, University of Fallujah , Al-Anbar, Iraq.

ABSTRACT

This project aimed to study the histomorphological and histochemical finding of the structures wall of the small intestine of Mallard (*Anas platyrhynchos*) in south Iraq. To conduct this investigation, 10 healthy mallards were collected from local suppliers. Birds were euthanized, dissected and then specimens were processed for histological and histochemical staining techniques. Gross findings showed that the small intestine was consisted of 3 organs (duodenum, jejunum and ileum). Histologically, the small intestine was lined by simple columnar with goblet cells. The muscularis mucosa in the duodenum arranged in two thick layers of smooth muscle arranged into inner circular and outer longitudinal bundles. The submucosa was thick layer of loose connective tissue. Tunica serosa was thin layer of areolar loose connective tissue. In the jejunum, the mucous membrane was thrown into large numerous villi arranged in a finger like projections with poorly developed and composed of only a few bundles of circular muscle fibers. In the ileum, both simple columnar cells and goblet cells were observed and similar to jejunum. Histochemical findings showed that the columnar cells were positive reacted with the PAS stain, whereas the goblet cells were strongly reacted with this stain. On applying the combined PAS-AB (pH 2.5), the wall showed epithelial cells stained negatively with this stained while the goblet cells gave strong positive reaction (dark blue). The combined PAS-AB (pH 1) showed strong reaction with the goblet cells for their neutral mucopolysaccharides but the columnar epithelium showed poor reaction. Intestinal mucosal lining revealed no response toward mercury bromophenol blue staining, while, the submucosal connective tissue revealed positive reaction for this technique. The columnar cells of small intestine gave the negative reaction with PAS, PAS-AB (pH 2.5) and PAS-AB (pH 1), whereas, the goblet cells were strongly positive reacted. The columnar cells of small intestine gave the positive reaction with PAS, PAS-AB (pH 2.5) and PAS-AB (pH 1), whereas, the goblet cells were strongly positive reacted. Infect, the latter stain is an indicator for sulfated acidic mucin substances which are very important in digestion and absorption and subsequent body growth of the bird.

KEY WORDS: Mallard, small intestine, histochemistry, avian omnivorous, histology.

INTRODUCTION

There are about 8600 kinds of birds are distributed throughout the world, in which the order Passeriformes is the largest. Whereas, the smallest one was the order Struthioniformes (King and Mclelland, 1975). During previous century, different kind of birds was studied in Iraq by several investigators such as Allouse (1961), Shefeq (1983) and Al-Ali (1986). Previously, several studies were conducted to explore how the dietary habits have affected on the morphological features and subsequently on physiological activities of the digestive organs in birds (Caviedes-Vidal and Karasov, 2001) and some rodents (Naya *et al.*, 2008). The small intestine in birds consists of three parts: the duodenum extends from the gizzard forming a loop which surrounds most of the pancreas. Then the jejunum is extends between the duodenum and the ileum when is joined by the Mackle's diverticulum. The third part is the ileum which is initiated from the diverticulum till the ileocaecal junction (Yamauchi *et al.*, 2010). According to our knowledge there were no local studies conducted to study

histomorphological and histochemical aspects of the wall of the small intestine of Mallard (*Anas platyrhynchos*).

MATERIALS & METHODS

Bird's collection and study design: Ten Mallard (*Anas platyrhynchos*) was collected to conduct the current study. They were bought from specific markets at Al-Muthanaa province from the local suppliers. Birds were housed at animal house of the Veterinary Medicine College/ Al-Muthanaa University in suitable cages. They were fed as well and giving them water *ad libitum* before their euthanasia and dissection.

Dissection and morphology study: Birds were euthanized prior to its dissection with an intravenous injection of sodium pentobarbitone (120 mg/kg) (Mitchell and Smith, 1991). Then after, dissected by fixing them on a dissecting board. A mid-line incision was made in the abdominal wall to view the coelomic viscera. The duodenum, jejunum and ileum of the small intestine were identified and photographed in situ using digital camera (pupil cam. ken-a-vision). Locations and relationships of these organs were well illustrated in figures. The organs then after

washed by normal saline solution to remove blood or other adhering debris. The contents of the small intestine eviscerated by gentle pressure on each of them and then washed by normal saline again.

Histological processes for the collected specimens: For the histological aspect of the study, the specimens were fixed in neutral buffered formalin of 10% concentration. After well fixation the specimens were dehydrated by passing them through a series of ascending ethanol each for 2h (70, 80, 90, 95 and 100%) and then specimens were cleared in xylene for 1h after that embedded in paraffin wax and then the blocks were sectioned at 6 μ m thickness and stained with either one of the following stains: Mayer's hematoxylin and eosin routine stain for general features identification, masson trichrome stain for the staining of the collagenous and smooth muscle fibers (Widhi Dubey and Trivedi, 2012).

Histochemical processes for the collected specimens: To conduct the histochemical study, specimens were fixed in Bouin's solution. Sections of 6 μ m were prepared and stained with one of the bellow stains and subsequently examined and photographed by olympus BH-2 microscope, using dino-eye camera. For the determination of the acidic mucin, combined PAS-alcian blue (AB) (pH 2.5) was used and for the determination of the neutral mucin, combined AB-PAS (pH 1). The PAS alone was used for the illustration of the goblet cells and the basement membranes of the epithelial lining of the small intestine. The last stain, Mercury Bromophenol Blue (MBB) was conducted to determine the protein content (Bancroft and Stevens, 2010).

RESULTS & DISCUSSION

Morphological aspect: The small intestines of mallard were distinctly divided into three segments, namely the

duodenum, jejunum and ileum (Fig. 1). The three grossly divided parts of small intestine in the current studied birds were similarly observed in other avian species such as ostrich (Wang and Peng, 2008), chicken (Yamauchi *et al.*, 2010) but in contrary, Klasing (1999) documented only duodenum and ileum in the small intestine of avian species. The duodenum consisted of descending and ascending limbs forming U-shaped tube called duodenal loop (Fig. 1). The pancreas observed between these limbs (Fig. 1). The U-shape of duodenum in the current birds was commonly observed in the other avian species (King and Mclelland, 1984; Bailey and Brown, 1997). The jejunum of the mallard was organized grossly in the form of cone-shaped of spiral coils. The cone had centripetal coils, a sigmoid flexure and centrifugal coils (Fig. 1). This jejunum shape was similar to other avian species such as domestic fowl (King and Mclelland, 1984), in most birds (Dyce *et al.*, 2002) and in African pied crow (*Corvus albus*) (Igwebuik *et al.*, 2010). It comprised most of the space of the coelomic cavity and such trait being common in all avian species (Caceci, 2003). The third segment of the small intestine of the mallard was the ileum which appeared the shortest part of the small intestine. It joined the jejunum cranially and extended caudally to join the cecum (Fig. 1). Obviously, the point at which the ileum can be demarcated from the jejunum was the last branch of the cranial mesenteric artery that supplies the small intestine in the mallard. The presence of arterial branch to distinguish the jejunum from the ileum was also recently documented in other avian species such as Pacific swallow (*H. tahitica*) and greater flameback (*C. lucidus*) (Andertont and Rassmussen, 2005).

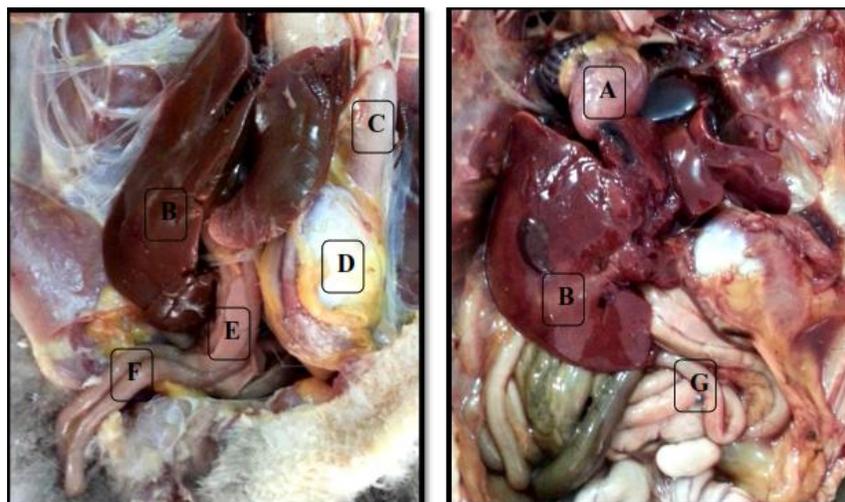


FIGURE 1. Visceral of Mallard showed: Heart (A), Liver (B), proventriculus (C), Gizzard (D), Pancreas (E), Duodenum (F), Jejunum (G) and Ileum (H).

Histological aspect

The small intestine appeared long convoluted tube, extends from the junction with stomach to joining point with the large intestine. Three segments were found in the small intestine of the studied birds, namely duodenum, jejunum and ileum (Fig. 2).

Duodenum: The organ showed microscopically the four classic known layers of a tube organ: mucosa, submucosa, muscularis and serosa (Fig. 2). These four layers appeared in the duodenum and other parts of the small intestine in all avian species such as quail (Zaher *et al.*, 2012), Rock dove (Albideri *et al.*, 2011), African pied crow (*Corvus*

albus) (Igwebuikwe *et al.*, 2010) and pigeon (*Columba livia*) (AL-Sheshani, 2006).

Tunicae mucosa : The duodenal mucous membrane in the mallard showed three different parts (Fig. 2), that were lining epithelium (simple columnar cells) (Fig. 2), lamina propria (loose connective tissue with the presence of mucosal glands) (Fig. 2,3) and muscularis mucosa (two thick layers of smooth muscle arranged into inner circular and outer longitudinal bundles). The presence of two layers of muscularis mucosa in the duodenal mucosae of mallard was similar to the findings observed in Ostrich (*Struthio camelus*) (Bezuidenhout and Vanswegwn, 1990). But conversely, the muscularis mucosa in the duodenal mucosa in African pied crow (*Corvus albus*) was absent (Igwebuikwe *et al.*, 2010).

Duodenal Villi: They were finger-shaped mucosal projections which constructed from the lamina propria, smooth muscle fibers as well as the lacteal. The latter was blind ended lymphatic capillary that is lined by simple columnar epithelium in studied birds (Fig. 2).

The lining epithelium of the villi was similar to those observed previously in the same organ in Ostrich (*Struthio camelus*) (Cornila *et al.*, 2008), Blue and Yellow macaws (Rodrigues *et al.*, 2012). The irregularity that observed in the mucosal surface could be due to the presence of duodenal villi intervening between the bases crypts of Lieberkühn. Duodenal Crypts of Lieberkühn: These were simple tubular glands called intestinal glands that were extended from the muscularis mucosa till the bases of the villi. They were lined by a simple columnar epithelium similar to the lining epithelium of the duodenal lumen (Fig. 2&3). As mentioned by (Hamdi *et al.*, 2013) in avian, the crypt covered by columnar epithelium. Tunicae Submucosa: It was formed irregular dense connective tissue situated, beneath the muscularis mucosa, and the layer composed of large blood, lymphatic vessels (Fig. 2 & 3). Absence of Brunner glands that found in submucosa in mammals concert in chicken when Aitken (1988) mentioned that the Brunner's glands are apparently lacking in submucosal layer.

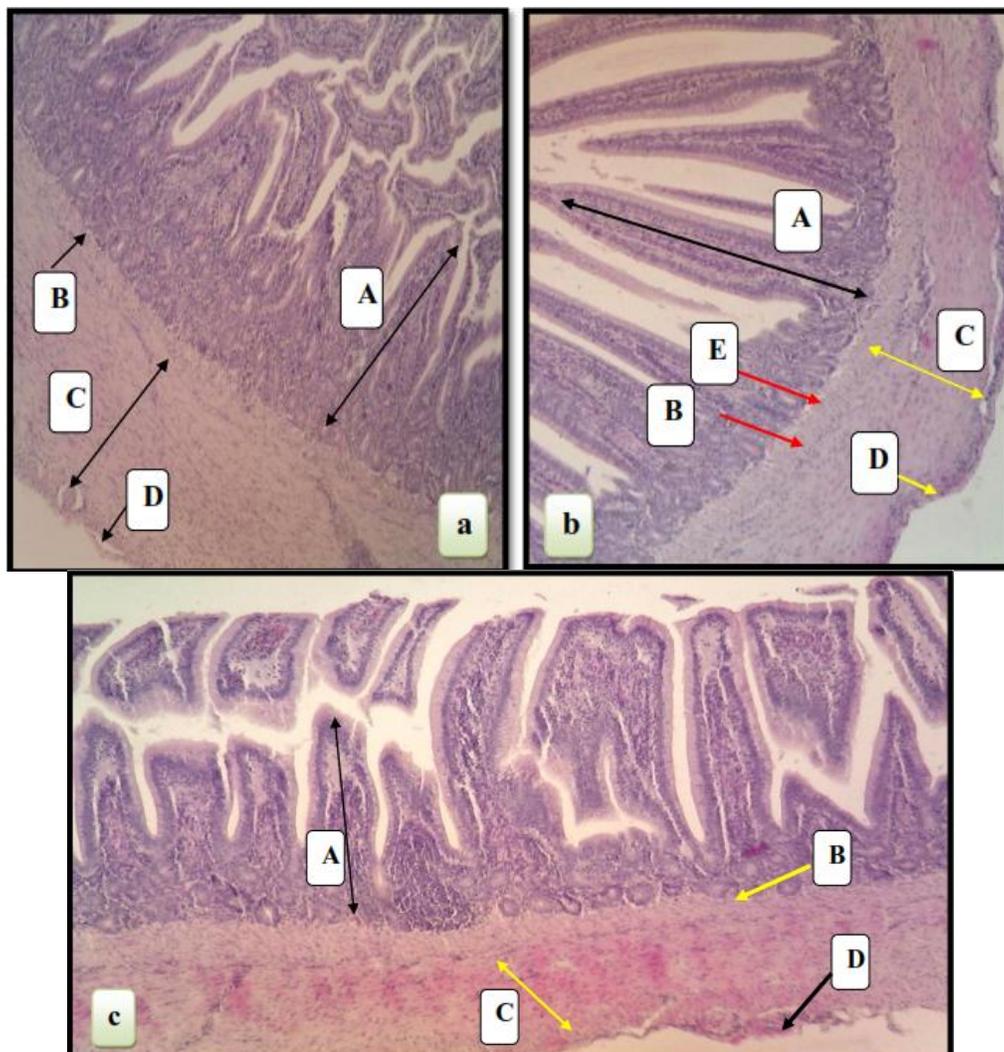


FIGURE 2. Cross section of the small intestine wall of Mallard showed mucosa (A), Submucosa (B), Muscularis (C), and serosa (D), muscularis mucosa (E), (a) duodenum, (b) jejunum, (c) ileum H & E, X100 (a) and (b), X 40 (c)

Tunicae Muscularis Externa: Underneath submucosa the muscular coat consists of the smooth muscles fibers arranged into two layers, the inner circular and the outer

longitudinal layers in the studied bird (Fig. 2, 3). Evenly the inner layer was thicker than the longitudinal layer over all parts of the duodenum. This finding was agreed with

(Zaher *et al.*, 2012) in common quail (*Coturnix coturnix*) and (Rodrigues *et al.*, 2012) in digestive tract of Blue and Yellow macaws that stated this tunica formed two layers.

Tunicae Serosa: The layer appeared thin in thickness constructed by loose connective tissue covered by a layer of mesothelial cells (Fig. 2, 3). The serosa lined externally the muscularis. These findings were similarly recorded in other avian species such as African pied crow (*Corvus albus*) (Igwebuikwe *et al.*, 2010) and pigeon (*Columba livia*) (AL-Sheshani, 2006).

Jejunum: The microscopic examination of jejunum's wall showed similar histological layers of a tube organ (Fig. 2, 3).

Tunicae mucosa: The mucous membrane was thrown into large numerous long leaf-shaped villi that were arranged in a finger like projections in mallard. The epithelial lining represented by single layer of tall columnar cells of both villi and crypts in the studied birds (Fig. 2, 3 & 4) which was in a good agreements with what was recorded by Zaher *et al.* (2012) in the common quail (*Coturnix coturnix*). The crypts of Lieberkühn were short and simple tubular ducts opened at the bases of villi occupying most of the thickness of the lamina propria till the muscularis mucosa (Fig. 2, 3). The lamina propria consists of loosely packed connective tissue containing blood vessels and muscle fibers (Fig. 2, 3&4) and such finding was comparable with that observed by Rodrigues *et al.* (2012) in the blue and yellow macaws.

The muscularis mucosa in the mallard was poorly developed and composed of only a few bundles of circular muscle fibers (Fig. 2, 3).

Tunicae Submucosa: The submucosa was a thicker layer of loose connective tissue possessed many blood vessels (Fig. 2, 3, 4).

Tunicae Muscularis Externa: This layer was constructed of a thin outer longitudinal and a thick inner circular layers in the studied bird. Between these muscle bundles, fine dispersed narrow connective tissue layer containing many large blood vessels (Fig. 2,3). The presence of two muscular layers in the present birds was similarly recorded in other avian species (Caceci, 2003).

Tunicae Serosa: It was formed by layer of simple squamous epithelium under which was a thin layer of loose connective tissue (Fig. 2).

Ileum: Similar to the previous tube like organs the microscopic examination of ileum's wall showed the four layers: mucosa, submucosa, muscularis and serosa (Fig.2).

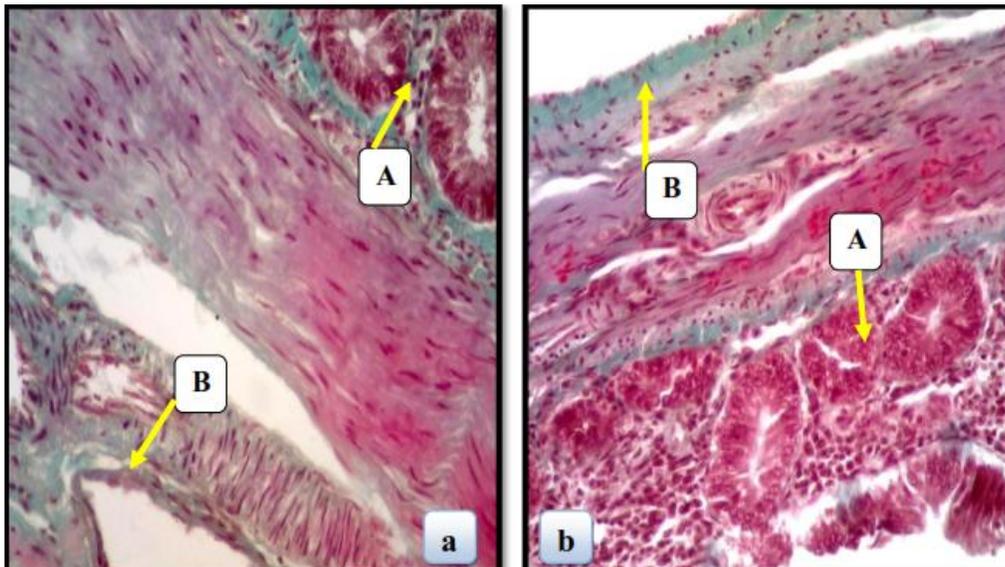
Tunicae mucosa: The villi appeared small leaf-shaped arranged in a zig-zag pattern (Fig. 2, 3). Each villus was lined by an epithelium while its center contained connective tissue core and such construction was agreed with results of Igwebuikwe *et al.* (2010) in the ileum of African pied crow (*Corvus albus*). The villi were short and less numerous compared to those found previously in the jejunum and duodenum of the same investigated bird. The lining epithelium was simple columnar (Fig. 2, 3, 4). The epithelium showed obviously higher number of goblet cells compared to those observed in both duodenum and jejunum. Loose connective tissue observed in the propria just beneath the epithelial lining (Fig. 2, 3) which was similar to Zaher *et al.* (2012) in the common quail (*Coturnix coturnix*) when a simple columnar epithelium supported by underlying connective tissue propria.

The muscularis mucosa was made of a thin outer longitudinal and a thick inner circular layers of smooth muscle fibers. Differently, in the ostrich, Bezuidenhout and Vanswegwn (1990) mentioned that the muscularis mucosa is made up of three layers in the ileum of this bird.

Tunicae submucosa: This layer was formed of loose connective tissue with blood vessels and these findings agreed with that recorded in in pigeon (Albideri *et al.*, 2011) and duck (Applegate *et al.*, 2005).

Tunicae Muscularis Externa: The layer muscularis was made up of an inner circularly and an outer longitudinally arranged layers of smooth muscles bundles (Fig. 2,3,4). This muscular arrangement was similar to that in fowl (Partha *et al.*, 2002).

Tunicae Serosa: Layers serosa was a thin layer of loose connective tissue. Its external surface was lined by simple squamous epithelium (Fig. 2).



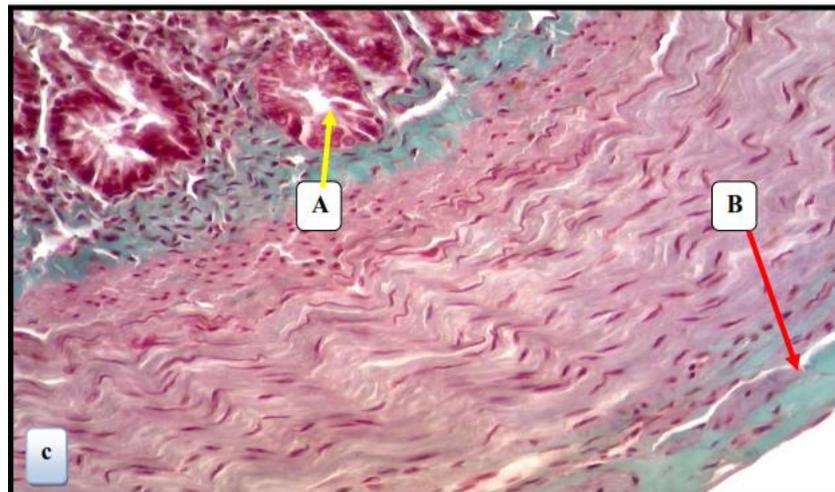


FIGURE 3. Cross section of the small intestine wall of Mallard showed: Crypts of Lieberkuhn (A), serosa (B) connective tissue (green), (a) duodenum, (b) jejunum, (c) ileum, Masson's Trichrome, X400

Histochemical Findings

The organs such as duodenum, jejunum and ileum were well studied histochemically by applying four stains: PAS, PAS-AB (pH 2.5), PAS-AB (pH 1.0) and MBB. These staining techniques were conducted to view the presence or absence of neutral mucins, acidic mucins, sulfated mucin and total protein contents, respectively.

The duodenum: Microscopic examination of the wall of the intestine showed that the mucosal layer as well as the villi possessed two types of cells that were the columnar

cells and goblet cells. The columnar cells gave the negative reaction with the PAS stain in the duodenum of the mallard. Whereas the goblet cells were strongly reacted with this stain in mallard (Fig. 4). These findings were in a good agreement with the recent records of Hamdi *et al.* (2013) in the duodenal surface lining and the crypts of Lieberkuhn of the of the black-winged kite (*Elanus caeruleus*), which is one of the carnivorous avian species.

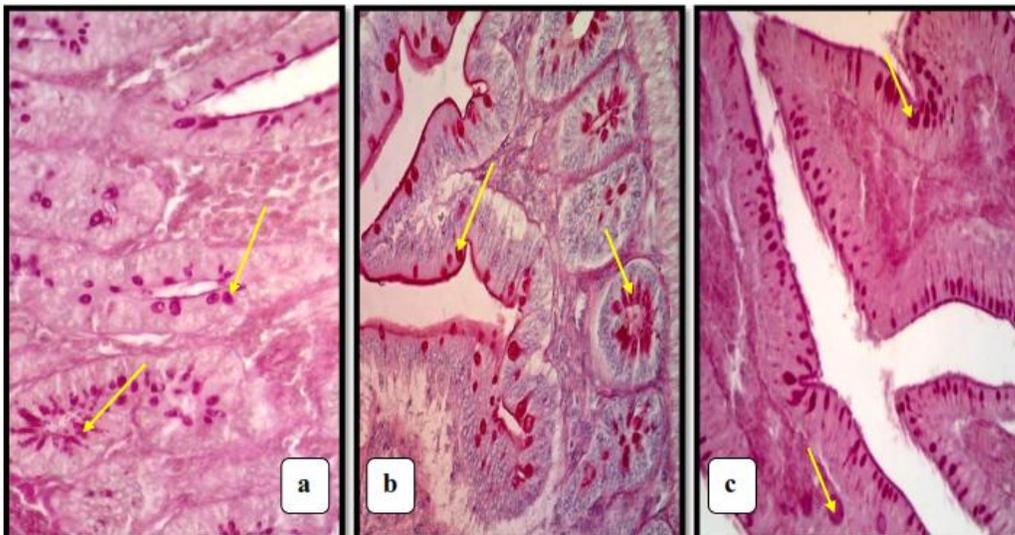


FIGURE 4. Cross section of the small intestine wall of mallard showed the neutral mucin. a : duodenal, b: jujenum and c: ileum. PAS, X 400 (a), X 100 (b) and (c)

The connective tissue in the lamina propria, submucosa and serosa afford mild reaction with PAS in mallard. Additionally, the duodenal wall of the black-winged kite, in which the stain displayed an intense reactivity for acid mucopolysaccharides in the goblet cells. Moreover, red stained neutral mucous in the basal regions of the goblet cells have been encountered. The connective tissue and smooth muscle fibers that were structured the wall of the duodenum were faintly stained with this staining procedure in mallard current findings revealed that the smooth muscle fibers which were constitutes the

muscularis mucosa as well as tunica muscularis gave rise moderate reaction with PAS in mallard (Fig. 4). On applying the combined PAS-AB (pH 2.5) to stain the tissues sectioned from the wall of duodenum, the epithelial cells stained negatively with this stained while the goblet cells gave strong positive reaction (dark blue) in studied bird (Fig. 6). Such findings were similar to those of Hamdi *et al.* (2013) observed in the.

Whereas, on using the combined PAS-AB (pH 1), the goblet cells present in the epithelium showed a strong reaction for neutral mucopolysaccharides but the columnar

epithelium showed poor reaction with this stain in mallard (Fig. 5). In addition to that, the connective tissue of the submucosa gave positive reaction for PAS, but negative toward AB part of the stain in case of mallard (Fig. 5). The

smooth muscle fibers present in the tunica muscularis showed moderate reaction with this staining technique in mallard.

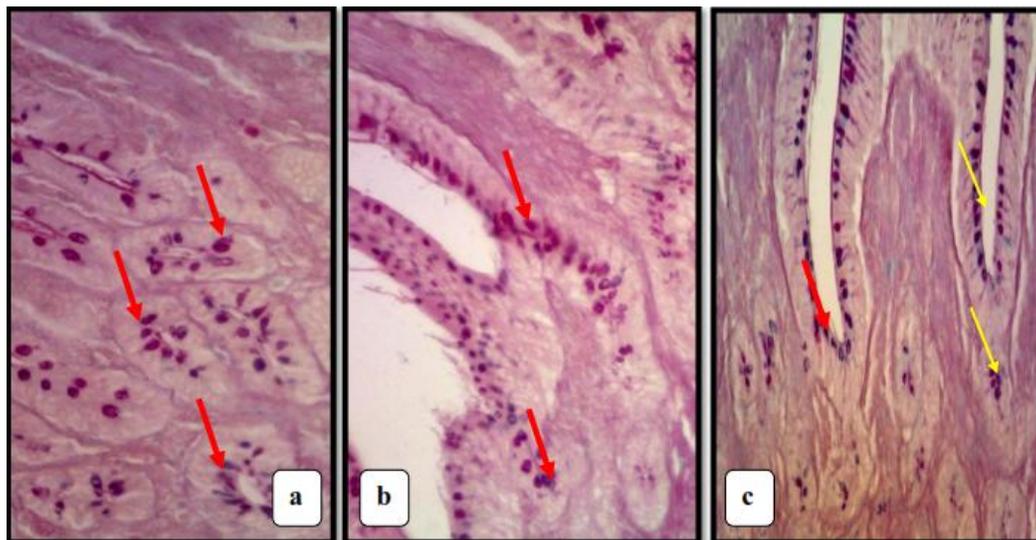


FIGURE 5. Cross section of the small intestine wall of mallard showed sulfated and neutral mucopolysaccharides in goblet cells, a: duodenal, b: jejunum and c: ileum AB-PAS= (pH 1), X400.

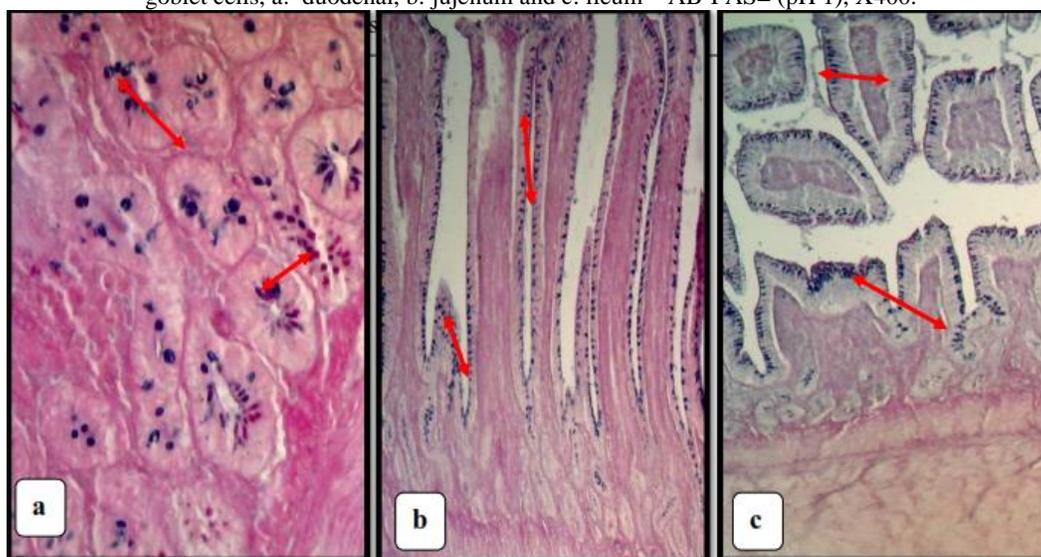


FIGURE 6. Cross section of the small intestine wall of mallard showed the neutral and acid mucin a : duodenal, b: jejunum and c: ileum, PAS-AB=(pH 2.5), (a)X 400, (b,c) X40

Histochemically, the mucosal lining revealed no response toward the mercuric bromophenol blue staining (Fig. 7). While, the submucosal connective tissue revealed positive reaction for this technique in studied bird. The tunica muscularis was constituted by layers of smooth muscle fibers which were positively reacted with this stain. Current findings were inconsistent with those found in the black-winged kite, because Bromophenol blue stain reacts positively with the absorptive columnar cells of the mucosal folds and the lamina propria structures of the duodenum. But a weak reaction was observed in the goblet cells (Hamdi *et al.*, 2013).

The Jejunum: The villi in the mallard jejunum which were characterized by blunt apical part and wide basal part, the goblet cells were strongly reacted with the PAS procedure (Fig. 4). The findings were comparable with those recently

published by Andleeb *et al.* (2009) histochemical studies on the jejunum in which the goblet cells of epithelium were moderately stained with PAS for substances of neutral mucopolysaccharides. The connective tissue which structured the propria, submucosa, serosa and the smooth muscle fibers in the tunica muscularis were negatively reacted with PAS stain (Fig. 4). Regarding PAS-AB (pH 2.5) technique, the mucosal goblet cells were positively reacted, whereas, the rest of the epithelial cells were negatively reacted in the studied bird (Fig. 6). This observation is consistent with that of Aitken (1992) who recorded moderate to weak reaction with Alcian blue stain throughout the intestine. Moderate to weak reaction with Alcian blue stain was in the villi and the basement membrane of the epithelium throughout the intestine as a whole in the chicken. The connective tissue gave with this

stain moderate reaction in case (Fig. 6). The smooth muscle fibers in the muscularis tunic in mallard negatively reacted with PAS stain.

The sections which were stained by combined AB-PAS (pH1) showed positive reaction (dark magenta) in the goblet cells and pink color in epithelial layer of the mucosa, indicating the presence of neutral and sulfated mucopolysaccharides, respectively (Fig.5). The connective tissue faintly stained with this combined stain. In addition to that the smooth muscle bundles were not stained with this stain in mallard, (Fig. 5). Effect of Bromophenol blue stain was positive on the connective tissue and smooth muscle fibers, whereas, the goblet cells and columnar cells of the mucosa gave weak reaction. Such staining character was similar to the previously recorded by El-Sayyad (1995) in some birds such as duck (Fig. 5).

The Ileum: The ileum mucosa revealed a strong red coloration with PAS stain in the goblet cells of both the villi and the crypts of Lieberkühn, whereas, the cytoplasm of the simple columnar cells were slightly stained in mallard. The connective tissue and smooth muscle fibers gave the mild reaction for the PAS (Fig. 4). The positive reaction of the PAS with the goblet cells well recorded in the broiler's ileum indicating very important role in lubricating the tract and facilitating the movement of the ingesta (Van Der Klis *et al.*, 1990).

The histological sections of the ileum wall when subjected to PAS-AB (pH 2.5) technique, they displayed an intense reactivity for acidic mucin substances present in the goblet cells indicated by strong bluish coloration. Moreover, the red-stained neutral mucous in the basal mallard of these cells (Fig. 6). Similar findings were previously recorded in

the ileum wall of the black-winged kite (*Elanus caeruleus*) by Hamdi *et al.* (2013).

The connective tissue present in the propria and submucosa showed negative reaction for PAS part of the PAS-AB (pH 2.5) stain in case of the mallard. In addition to that, the muscularis tunic constructed of smooth muscle fibers which were negatively reacted with this staining procedure in the studied bird (Fig. 6). The sections which were stained with the combined PAS-AB (pH1) showed positive reaction (dark magenta) in goblet cells in epithelial layer of the mucosal lining presence the secretion of neutral and sulfated acidic mucopolysaccharides in this layer (fig. 5). The connective tissue of propria and submucosa showed poor staining with PAS. These observations indicated that the connective tissue in this layer contained trace amount of both the neutral and acidic mucosubstances. The smooth muscles gave mild reaction for PAS part of the stain (Fig. 5). In the ileum, the cellular cytoplasm of the surface lining epithelium of the mucosal folds and goblet cells and the ductile cells that lined the ducts of the mucosal glands showed negative reaction with the mercuric bromophenol blue method. While the connective tissue and smooth muscle fibers in all tunics of the wall of this organ present the positive reaction in mallard (Fig. 5). Current findings were not comparable with those of Hamdi *et al.* (2013) in the ileum wall of the black-winged kite, because Bromophenol blue stain reacts positively with the absorptive columnar cells of the mucosal folds and the lamina propria structures of the duodenum. However a weak reaction was observed in the goblet cells.

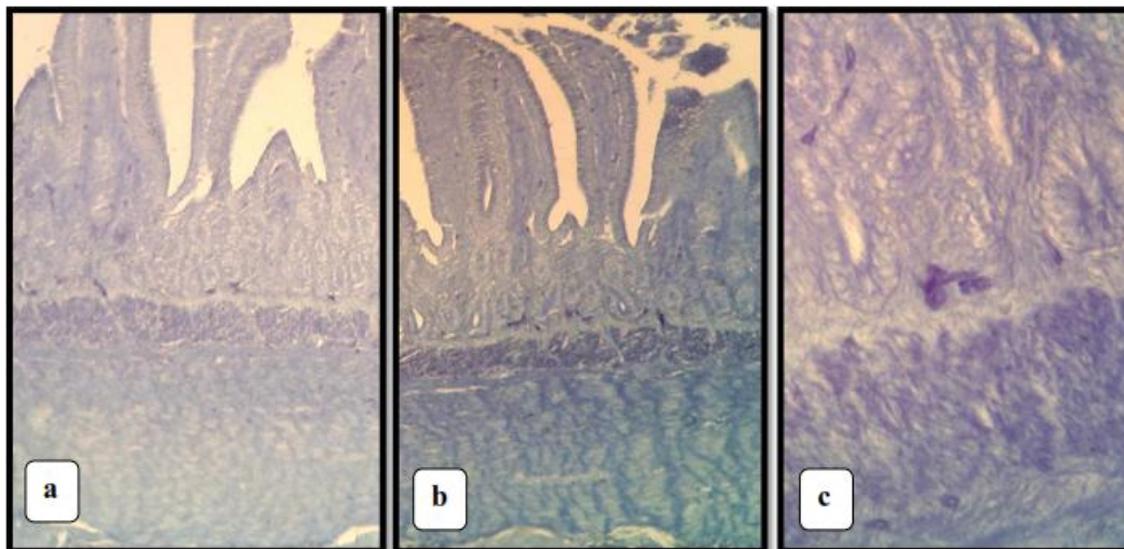


FIGURE 7. Cross section of the small intestine wall of female mallard showed the total protein
a : duodenum, b: jejunum and c: ileum MBB X 100

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