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Research Article
Histomorphological and Histochemical Study of the Small Intestine of the Striated Scope Owls (Otus Scors Brucei)

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Abstract

Objective: This project aimed to study the histomorphological and histochemical structures of the wall of the small intestine of Striated Scope Owls (Otus Scors brucei) in Iraq. **Methodology:** To conduct this investigation, 12 healthy owls were collected from local suppliers. Birds were euthanized, dissected and then specimens were processed for histological and histochemical staining techniques. **Results:** 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 one longitudinally set fibers. 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 showed blunt apical part and wide basal part with absence of muscularis mucosa. In the ileum, both simple columnar cells and goblet cells were observed and similar to jejunum, muscularis mucosa was absent. Histochemical findings showed that the columnar cells were negatively 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. **Conclusion:** Because the bird is carnivores species, 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. Infact, 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: Owl, small intestine, histochemistry, avian carnivorous

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There are about 8600 kinds of birds distributed throughout the world, out of them the order Passeriformes found the largest, whereas, the smallest one was the order Struthioniformes[1]. During the previous century, different kind of birds was studied in Iraq by several investigators[2-4].

Several studies were conducted to explore how the dietary habits have shaped the morphological features and subsequently affect physiological activities on the digestive organs in some rodents species[5-7] and in birds[8]. Barton and Houston[9] mentioned that the extent to which the animal can utilize energy from the ingested food depends highly on both morphological and physiological properties of its digestive tract. In fact, Hilton *et al.*[10] considered digestive organs as machine acts to maintain many activities such as metabolism, production as well as reproduction. Accordingly, the differences in their functional capacities may affect their metabolic rates.

Birds have an extremely high metabolism to correspond with their requirements; consequently bird must eat a large amount of food[11]. Accordingly, such characteristic high metabolic rates in birds lead them to consume greater quantity of food in proportion to their size and such trait was differently greater compared to those reported in other animal species[12]. Previously, Sturkie[13] postulated that the lengths of various parts of the digestive tract vary with size of the bird, its type of ingested food beside other factors, so that, birds that eats coarse and fibrous food tend to have especially long digestive tract and those eating grain have longer tracts than those of the carnivores.

The small intestine in birds consists of three segments. The first segment, the duodenum, extends from the gizzard to the pancreas and forms a loop surrounding most of the pancreas. The second segment, the jejunum, extended from the distal portion of the duodenum loop to the mackle's diverticulum. The third segment, the ileum, extends from mackle's diverticulum to the ileocaecal junction[14]. According to our knowledge there were no local studies conducted to study histomorphological and histochemical aspects of the wall of the small intestine of Striated Scope Owls (Otus Scors brucei).

MATERIALS AND METHODS

Bird's collection and study design: Twelve Striated Scope Owls (Otus Scors brucei) were collected to conduct the current study. They were bought from specific markets at Baghdad province from the local suppliers. Birds were housed at animal house of the Veterinary Medicine College/Baghdad 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 (80 mg kg^{-1})[15], 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 2 h (70, 80, 90 and 100%) and then specimens were cleared in xylene for 1 h after that embedded in paraffin wax and then the blocks were sectioned at 6 μm 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[16].

Histochemical processes for the collected specimens: To conduct the histochemical study, specimens were fixed in Bouin's solution. Sections of 6 μm 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, combind 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.

RESULTS AND DISCUSSION

The small intestine of the owl was distinctly divided into 3 segments, namely the duodenum, jejunum and ileum. The 3 grossly divided parts of small intestine were similarly

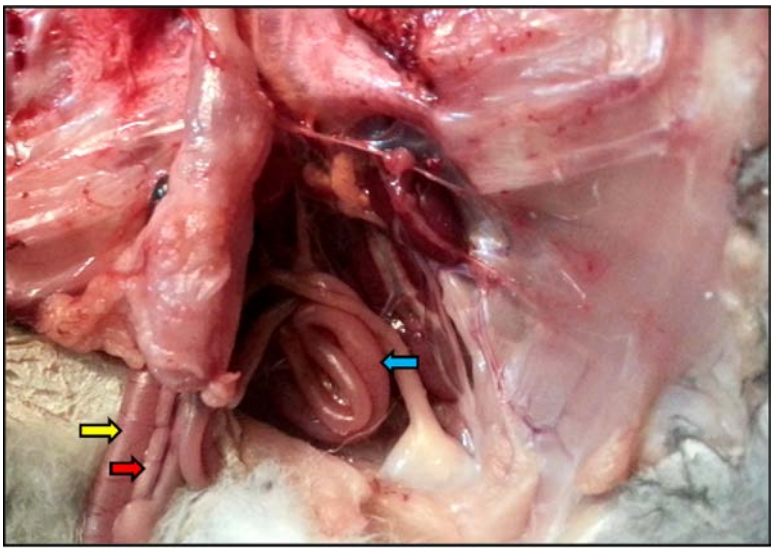

Fig. 1: Gross picture of the small intestine of owl *in situ* showed duodenum (yellow arrow), jejunum (blue arrow) and pancreas (red arrow)

observed in other avian species such as ostrich[17], chicken[14], but in contrary, Klasing[18] documented only duodenum and ileum in the small intestine of avian species. The duodenum consists of descending and ascending limbs forming U-shaped tube called duodenal loop. The pancreas was observed between these 2 limbs (Fig. 1). Currenttlly, the U-shape of duodenum was commonly observed in the other avian species[1,19].

The jejunum of the owl was organized grossly in the form of cone-shaped of spiral coils. The cone had centripetal coils, a sigmoid flexure and centrifugal coils. The shape of this organ was similar to other avian species such as domestic fowl[1], in most birds[20] and in African pied crow (*Corvus albus*)[21]. It filled most of the space of the coelomic cavity and such trait being common in all avian species[22].

The third segment of the small intestine was the ileum which appeared the shortest part of the small intestine. It joined the jejunum cranially and extended caudally to join the cecum. Grossly, there was no marked difference between the jejunum and initial part of the ileum except in term of color in which the jejunum has yellow, while the ileum has dark green. Infact, in other avian species, the point at which the ileum can be demarcated from the jejunum is the last branch of the cranial mesenteric artery that supplies the small intestine[23].

Histological aspect

Duodenum: The organ showed microscopically the 4 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[24], rock dove[25], African pied crow (*Corvus albus*)[21] and pigeon (*Columba livia*)[26]. The duodenal mucous membrane showed 3 different parts, that were lining epithelium (simple columnar cells), lamina propria (loose connective tissue with the presence of mucosal glands) and muscularis mucosa (arranged only in one longitudinally set smooth muscle fibers) (Fig. 2). Differently, two layers of muscularis mucosa in the duodenal mucosa observed in ostrich (*Struthio camelus*)[27]. Whereas totally absent in the duodenal mucosa of African pied crow (*Corvus albus*)[21].

The duodenal villi which were finger-shaped mucosal projections were constructed of lamina propria, smooth muscle fibers as well as the lacteal. The latter was blind ended lymphatic capillary that was lined by simple columnar epithelium. The lining epithelium of these villi was similar to those observed previously in the same organ in ostrich (*Struthio camelus*)[28], blue and yellow macaws[29]. Crypts of Lieberkühn were simple tubular glands 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). Goblet cells which were globed-shaped unicellular mucous glands scattered among the columnar lining of both villi and crypts of lieberkühn (Fig. 3). Their nuclei were close to the bases and they appeared vacuolated on staining with hematoxylin and eosin. These cells were reacted positively with the PAS as they gave rise the magenta color. Current finding was in a good agreement with those previously observed in the *Struthio camelus*[28] but

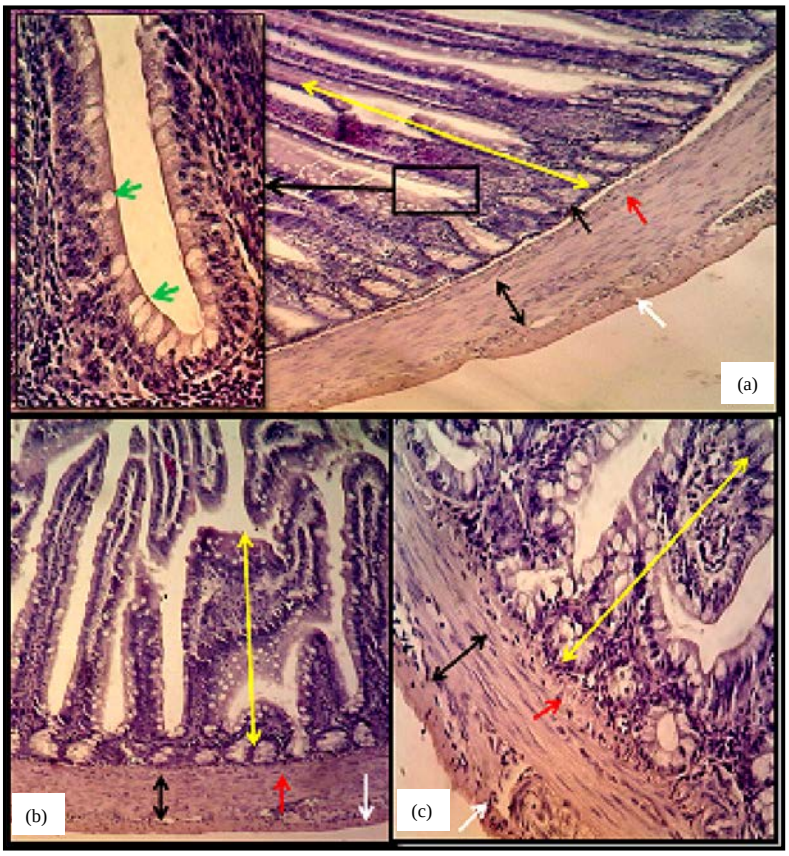

Fig. 2(a-c): Cross section of the small intestine wall of male owl (a) Duodenum, (b) Jejunum and (c) Ileum showed T mucosa (yellow arrows), T Submucosa (red arrows), T Muscularis (black arrows), T serosa (white arrows). This showed epithelial linning with goblet cells (green arrows). The H and E, X100, X400 (magnified rectangle)

being different to those of african pied crow (*Corvus albus*)[21] in which the occurrence of the goblets were not very prominent in the duodenal mucosa.

Tunica submucosa was formed of irregular dense connective tissue situated beneath the muscularis mucosa and the layer composed of large blood, lymphatic vessels. Underneath submucosa the muscular coat consists of the smooth muscles fibers arranged into 2 layers (inner circular and the outer longitudinal layers) (Fig. 4). Evenly the inner layer was thicker than the longitudinal layer over all parts of the duodenum. This finding was agreed with Zaher *et al.*[24] in common quail (*Coturnix coturnix*) and Rodrigues *et al.*[29] in digestive tract of blue and yellow macaws that described 2 layers in this tunic. The muscularis was covered by a thin layer of tunica serosa constructed by loose connective tissue covered by mesothelial cells (Fig. 2, 4). These findings were similarly recorded in other avian species such as African pied crow (*Corvus albus*)[21] and pigeon (*Columba livia*)[26].

Jejunum: The mucous membrane was thrown into large numerous villi that were with blunt apical part and wide basal part (Fig. 2). The epithelial lining represented by single layer of tall columnar cells of both villi and crypts which was in a good agreements with what was recorded by Zaher *et al.*[24] 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, 4). The lamina propria consists of loosely packed connective tissue containing blood vessels and muscle fibers and such findings were comparable with those observed by Rodrigues *et al.*[29] in the blue and yellow macaws. The muscularis mucosa was absent in owl's jejunum. The absence of muscularis mucosa was similarly documented in other previous studies in carnivorous birds[26].

The submucosa was a thick layer of loose connective tissue possessed many blood vessels. Tunica muscularis was constructed of a thin outer longitudinal and a thick inner circular layers. Between these muscle bundles, fine dispersed narrow connective tissue layer containing many large blood vessels. The presence of two muscular layers in the current bird was similarly recorded in other avian species[22]. Tunica

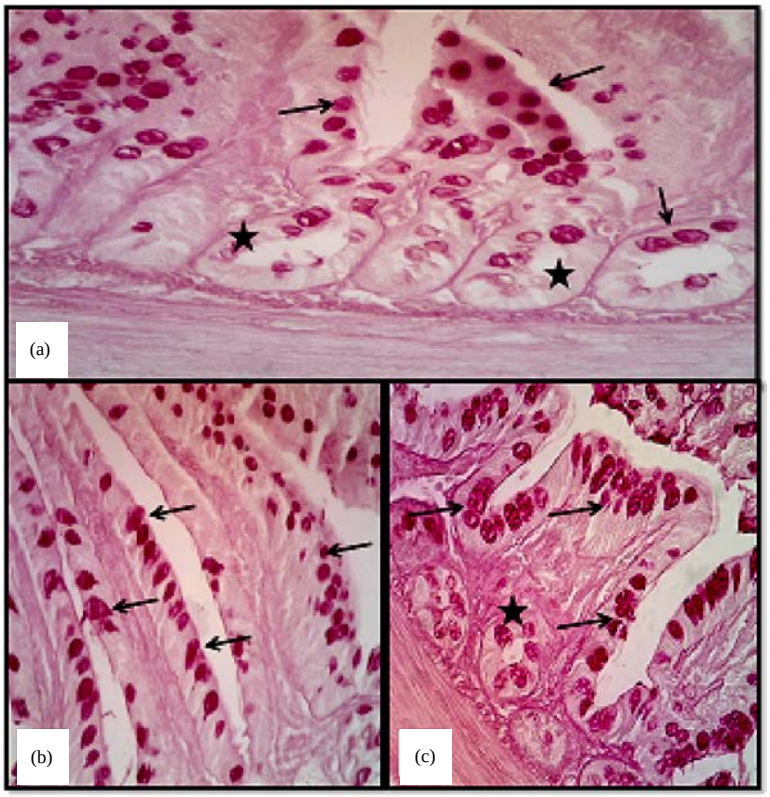

Fig. 3(a-c): Cross section of the small intestine wall of male owl (a) Duodenum, (b) Jejunum and (c) Ileum showed goblet cells with mucin granules (black arrows), crypts of lieberkühn (black stars). PAS, X400

serosa was formed by layer of simple squamous epithelium under which was a thin layer of loose connective (Fig. 2, 4).

Ileum: The mucous membrane was similar to that of the jejunum, thrown into large numerous villi that were with blunt apical parts and wide basal parts (Fig. 2). Each villus was lined by an epithelium while its center contained connective tissue core and such construction was agreed with results of Igwebuike *et al.*[21] 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. The lining epithelium was simple columnar which 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 which was similar to records of Zaher *et al.*[24] in the common quail (*Coturnix coturnix*). The muscularis mucosa was absent in owl in which the connective tissue of the lamina propria was continuous with that of the submucosa. Differently, in the ostrich, Bezuidenhout and Aswegen[27] mentioned that the muscularis mucosa is made up of three layers in the ileum.

Tunica submucosa was formed of loose connective tissue with blood vessels and these findings agreed with those recorded in in pigeon[25] and duck[30]. Tunica muscularis was made up of an inner circularly and an outer longitudinally arranged layers of smooth muscles bundles. This muscular arrangement was similar to that in fowl[31]. This layer appeared thickest in the ileum and thinnest in the jejunum. Tunica serosa was a thin layer of loose connective tissue covered externally by simple squamous epithelium (Fig. 2, 4).

Histochemical aspect: The organs such as duodenum, jejunum and ileum were well studied histochemically by applying the following 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.

Duodenum: Microscopic examination of its wall showed that the mucosal layer as well as the villi possessed 2 types of cells that were the columnar cells and goblet cells. The

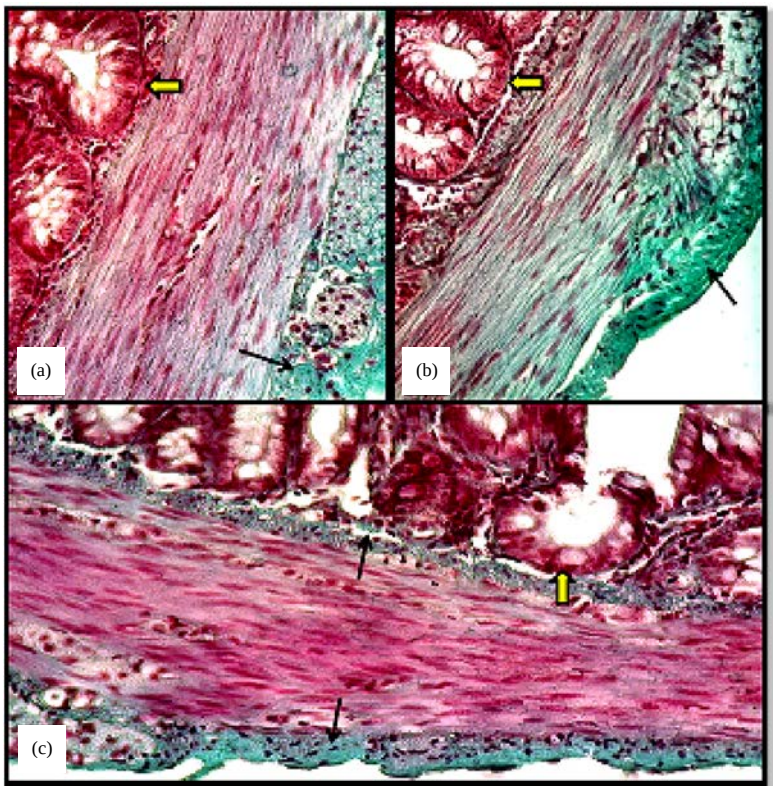

Fig. 4(a-c): Cross section of the small intestine wall of female owl (a) Duodenum, (b) Jejunum and (c) Ileum showed: Crypts of lieberkuhn (yellow arrows), serosa (black arrows) the connective tissue (green), masson's trichrome and X400

columnar cells gave the negative reaction with the PAS stain, whereas the goblet cells were strongly reacted (Fig. 3). These findings were in a good agreement with the recent records of Hamdi *et al.*[32] in the duodenal surface lining and the crypts of lieberkühn of the black-winged kite (*Elanus caeruleus*), which is one of the carnivorous avian species. The connective tissue in the lamina propria, submucosa and serosa negatively reacted with this stain. Additionally, the current findings revealed that the smooth muscle fibers that constitute the muscularis mucosa as well as tunica muscularis gave rise negative reaction with PAS.

On applying the combined PAS-AB (pH 2.5) to stain the tissues sectioned from the wall of duodenum, the epithelial cells stained negatively while the goblet cells gave strong positive reaction (dark blue) (Fig. 5). Such findings were similar to those of Hamdi *et al.*[32] observed in 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.

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 (Fig. 6). In addition to that, the connective tissue of the submucosa gave positive reaction for PAS, but negative toward AB part of the stain. The smooth muscle fibers present in the tunica muscularis showed moderate reaction with this staining technique. Histochemically, the mucosal lining revealed no response toward the mercuric bromophenol blue staining in owl, whereas, the lamina propria and submucosal connective tissue revealed positive reaction for this technique. The tunica muscularis was constituted by layers of smooth muscle fibers which were positively reacted with this stain (Fig. 7). Current findings were inconsistence 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 weak reaction was observed in the goblet cells[32].

Jejunum: The simple columnar cells which lined these villi were stained negatively with the PAS, whereas, the goblet

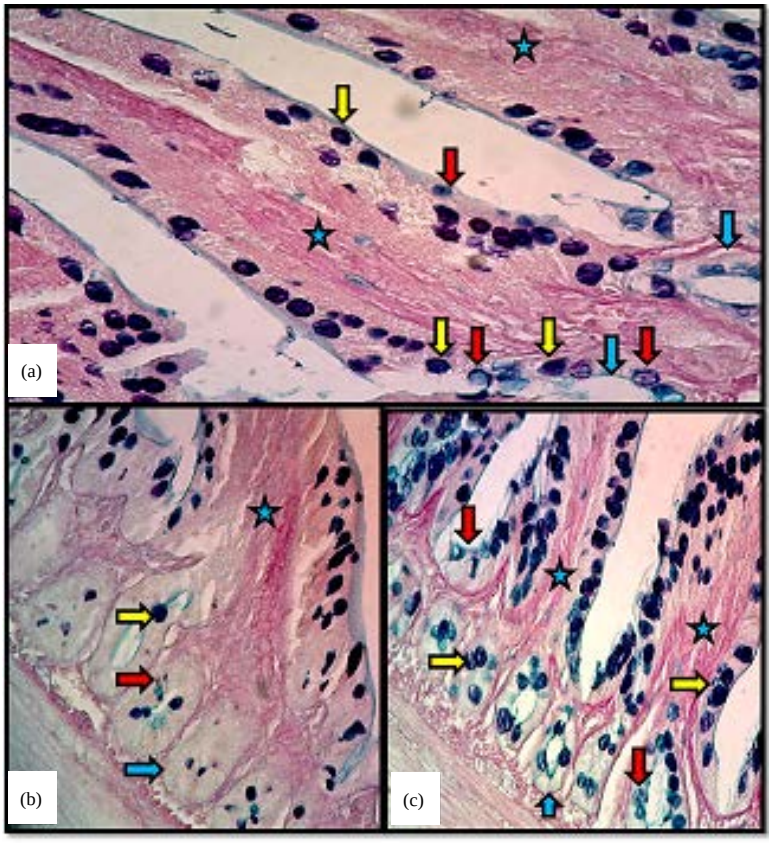

Fig. 5(a-c): Cross section of the wall of small intestine of owl showed neutral (yellow arrows) and acidic mucin (red arrows) in villi's epithelia (blue stars) and intestinal glands (blue arrows) of the (a) Duodenum, (b) Jejunum and (c) Ileum. X400, PAS-AB (pH 2.5)

cells were strongly reacted with this procedure (Fig. 2). The findings were comparable with those recently published by Andleeb *et al.*[33] 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. Regarding PAS-AB (pH 2.5) technique, the mucosal goblet cells were positively reacted, whereas, the rest of the epithelial cells were reacted negatively (Fig. 5). This observation in consistence with that of Aitken[34] who recorded moderate to weak reaction with alcian blue stain throughout the intestine in the chicken. The connective tissue gave with this stain moderate reaction. The smooth muscle fibers in the muscularis tunic afford mild reaction with PAS stain.

The sections which were stained by combined AB-PAS (pH 1) 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. 6). The connective tissue faintly stained with this combined stain. In addition to that the smooth muscle bundles were moderately reacted. 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[35] in some birds.

Ileum: The mucosa revealed 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. The connective tissue and smooth muscle fibers gave mild reaction for the PAS. The positive reaction of the PAS with the goblet cells well recorded previously in the broiler's ileum indicating very important role in lubricating the tract and facilitating the movement of the ingesta[36].

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

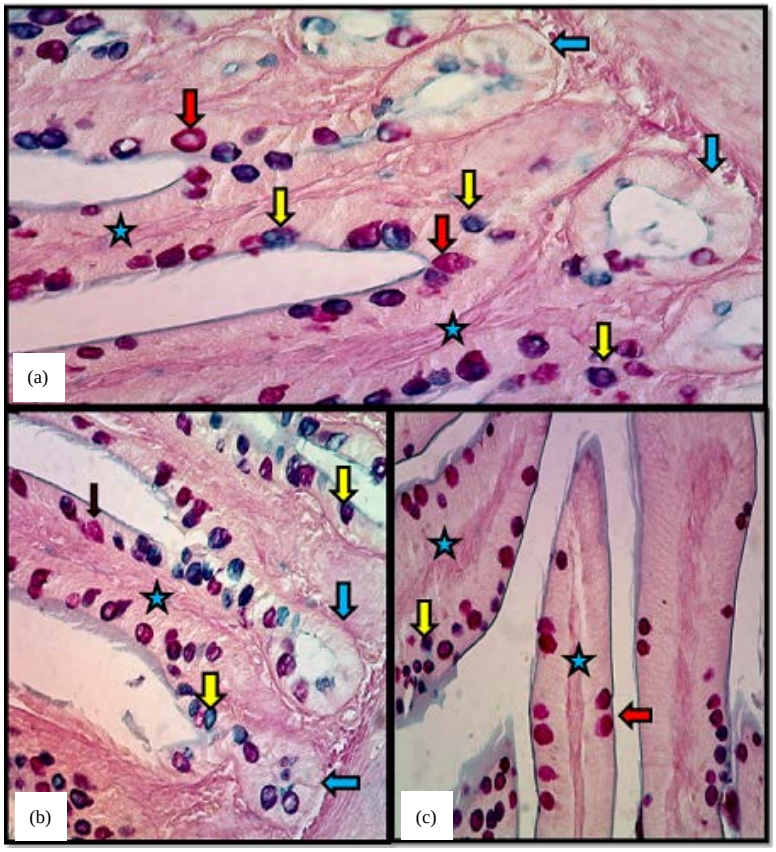

Fig. 6(a-c): Cross section of the wall of small intestine of owl showed sulfated (yellow arrows) and neutral mucopolysaccharides (red arrows) in villi's epithelium (blue stars) and intestinal glands (blue arrows) of the (a) Duodenum (b) Jejunum and (c) Ileum. X400, PAS-AB (pH 1)

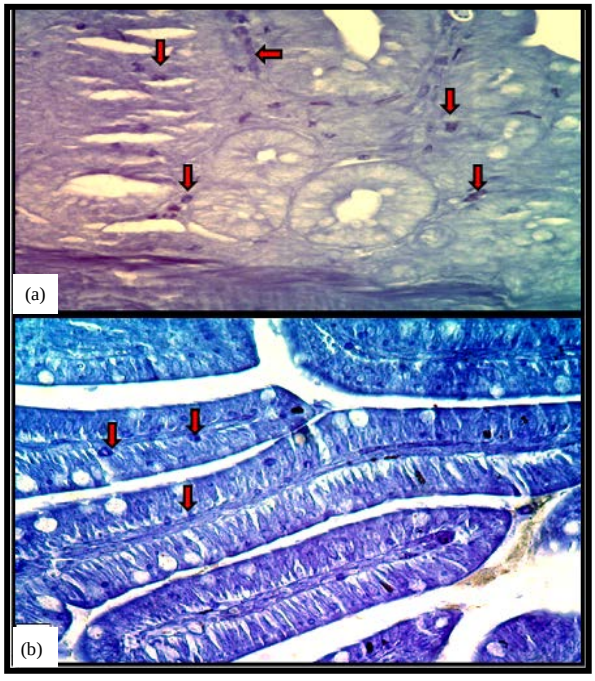

Fig. 7(a-b): Cross section of the wall of small intestine of owl showed positive reaction toward MBB for total protei detection (red arrows) in the connective tissue of lamina propria of the (a) Duodenum and (b) Ileum. X400

cells indicated by strong bluish coloration. Moreover, the red-stained neutral mucous in the basal regions of these cells had been observed (Fig. 5). Similar findings were previously recorded in the ileum wall of the black-winged kite (*Elanus caeruleus*) by Hamdi *et al.*[32]. The connective tissue present in the propria and submucosa showed moderate reaction for PAS part of the PAS-AB (pH 2.5) technique. In addition to that, the tunica muscularis constructed of smooth muscle fibers which were negatively reacted with this staining procedure.

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. 6). 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.

In the ileum, the cellular cytoplasm of the surface lining epithelium of the mucosal folds and goblet cells and the ductular 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 (Fig. 7). Current findings were not comparable with those of Hamdi *et al.*[32] 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.

CONCLUSION

In conclusion, the small intestine of owl was covered by simple columnar cells with goblet cells, with the presence of villi but the different size and shape in the 3 different intestinal segments. The submucosa formed from the irregular dense connective tissue in duodenum, whereas, loose connective tissue in jejunum and ileum. The muscularis mucosa made up of 2 layers of smooth muscles. Because of type of feeding of this bird as a cranivores species, 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. Infact, the latter stain is an indicator for sulfated acidic mucin substances which are very important in digestion and subsequent absorption. The submucosal connective tissue revealed positive reaction for mercuric bromophenol blue technique in all studied birds.

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