Histological and Histochemical Study of Small Intestine at Neonatal Stage of Cats (Felis catus)

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Abstract

This study was occurred to detect histological and histochemical details of small intestine in neonatal cats. Pregnant queens in good health condition collected by hunting and cage in the animal house till their delivery to obtain six kittens from them when kittens reach one week age separated from mothers and euthanized killed. The small intestine was lined by simple columnar epithelium with goblet cells. The general architecture was similar to other mammals with few peculiarities: the shape of the villi varied from leaf-like in the duodenum to pointed in the jejunum and leaf-shaped in the ileum due to the type of feed at this age. Brunner's gland was present in the initial part and caudal of duodenum and the initial part of jejunum act to neutralize the acidity of the food coming from the stomach. Secretion materials from these glands contribute to layer of mucus which was the consistency of slimy, viscoelastic lubricating the lining small intestine. The paneth cells few numbers in this age in the duodenum with rather epithelial cells at this part. The crypts of lieberkuhn were simple branched, coiled tubular glands lined with simple cuboidal in the duodenum to the low columnar epithelium in the jejunum. Payer's patch was present at the termination of jejunum and ileum. Goblet cells and Brunner's glands secrete mucous into the lumen of small intestine, the reaction of combined PAS–AB indicated the presence of mixed mucin (neutral and acidic) and in the brush border of small intestinal parts.

Keywords: *Duodenum, Jejunum, Ileum, Neonatal, Histology.*

Introduction

The intestinal epithelium performs many important functions key to the growth and survival of organisms which allow nutrient absorption and acts as a barrier to the risks posed by responses to the external environment, pathogenic injury, secretes mucin and lubricating mucus and survives a constant turnover of cells (Barker, 2014; Peterson and Artis, 2014), secretion hormones (Gunawardene et al., 2011). The small intestinal epithelium is regular into crypt-villus domains where the villus epithelium includes absorptive enterocytes, hormone secretion enteroendocrine cells and goblet cells that secrete mucus. The intestine is considered the caudal part of the alimentary canal. It's subdivided into the small intestine beginning from the pylorus to the cecum and the large intestine starting from the cecum and ending at the anus (Nickel et al., 1979; Bailey et al., 1997). The small intestine is consist of three parts duodenum, jejunum and ileum (AL-Saffar and Al-Zuhairy, 2016; Hamza and

Al-Mansor, 2017). Microscopically, the small intestine is containing numerous "finger-like" projections known as villi that increase the intestinal surface area to assist in nutrient absorption (Parish, 2011). Pách (2000) mentioned that the development of the gastrointestinal tract after birth was divided into three periods that are birth and early suckling, suckling and the weaning period. In postnatal, the gastrointestinal tract was developed depending on some factors such as age, diet, and hormones that are secreted from the intestine or even from other organs associated with it such as the pancreas and liver. The small intestine of feline and guinea pigs grows little through the first days after birth (Weaver and Lucas, 1987; Buddington and Diamond, 1992).

Materials and methods

Samples

Six kittens were used in this study which separated from pregnant queens at one week of age; the pregnant queens in good health condition will be collected by hunting and cage in the animal house in the department of anatomy, and histology at the College of Veterinary Medicine/ University of Baghdad till their delivery. The kittens were euthanized by intra-cardiac injection of an overdose of sodium pentobarbital. The representative specimens of one cm were cut from all parts of the small intestine. The specimens of small intestine parts were rinsed with normal saline and then directly soaked in 10% neutral buffered formalin for 48 hours (Al-Haaik, 2 019).

Histological examination

Specimens were processed by the routine histological process then 6 µm paraffin sections were obtained by using a rotary microtome (Culling et al., 1985; Hamza, 2018; Hussein and Khalid, 2019). The specimens of neonatal age were stained with Harris's hematoxylin and eosin and Masson's trichrome stains for a general histological feature and Periodic acid Schiff stain (PAS) was used to detect the glands and cells of the small intestine (Taha, 2021). The micro morphometric measurements such as epithelial height and thickness of all tunics of each part of the small intestine at neonatal ages were studied.

Statistical analysis

Statistical analysis was carried out using the ANOVA test in the SPSS, and differences were considered significant at P□0.05 (Gharban, 2022).

Results

Duodenum

The microscopic examination of the duodenal wall showed that its structure consisted of four major tunics that were tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa in the kitten. The tunica mucosa had various finger-like projections (villi) which appeared long and crowded and were leaf-shaped. Each villus was lined by simple columnar epithelium which had large oval nuclei that were centrally located; the epithelial cells displayed thick line demarcation which represented the brush border associated with circular goblet cells spread through them (Fig. 1A). The epithelium rested on thin lamina propria, the lamina propria of each villus consisted of a thin layer of cellular loose connective tissue contained collagen fibres and lymphocyte and occupied by crypts of Lieberkühn (Fig. 1B). The mean of the thickness of tunica mucosa was 231.24 ± 3.6 µm, the mean height of epithelial was

14.07±0.20 µm, the mean of Villi length was 141.27±4.3 µm, the width of villi was 32.25 ± 0.85 µm, and the crypt depth was 90.25 ± 1.6 µm.

The crypts of Lieberkühn were detected in the lamina propria of neonatal kittens with the underneath muscularis mucosa of many circular bundles of smooth muscle fibers (Fig. 1A). The goblet cells were prominently circular with their nuclei enforced toward the bases of these unicellular glandular cells. These cells were more numerous in the villi compared to their number in the crypts. Numerous folds were noted which were structured from both mucosa and submucosa called plicae circularis. The paneth cells were few numbers which record compared with epithelial cells at this stage of age (Fig. 1D). Submucosa was thinner than mucosa and was constructed of irregular dense bundles of connective tissue fibers richly supplied with blood vessels the mean thickness of tunica sub mucosa was 66.13±1.34 µm. The tunica submucosa consisted of irregular connective tissue and connective tissue cells; fine blood capillaries along with yellow, white and reticular fibre. The Brunner’s glands occupied the tunica submucosa which high number in the cranial part than the caudal part of the duodenum, the submucosa of the cranial part of the duodenum was relatively thicker than those of the middle and caudal parts as due to found Brunner's glands in the cranial part of duodenum it's as aggregated mucous secretory units invested in the connective tissue which was stained moderately with PAS stain (Fig. 1C). The tunica muscularis was found thick in neonatal age of cats compared to the other structural tunicae of the wall of the duodenum. It was constructed of two layers that were thicker inner circular and thinner outer longitudinal layers of smooth muscle bundles (Fig. 2). Connective tissue fibres were interspersed between bundles of the inner circular layer of muscle. The connective tissue also separated the two layers of tunica muscularis which included Auerbach's nerve plexuses associated with rich blood vessels (Fig. 2). The mean thickness of tunica muscularis was 341.77 ± 2.6 µm. Tunica serosa, which is the outermost layer of the intestinal wall, was composed of a thin layer of loose connective tissue blended with a tissue of the mesentery. The histochemical findings of the wall structure of duodenum showed the presence of goblet cells in the duodenal villi which gave with the combined PAS-AB (pH 2.5) dye vigor positive reaction (magenta color) toward PAS and moderate reaction (blue color) with the AB (pH 2.5) (Fig. 1C).

Jejunum

The wall of the jejunum in kitten was made from the following tunics: tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa (Fig. 3B). The first tunica mucosa had villi of various shapes and sizes that were lined by simple columnar epithelium and had few goblet cells. The villi were tip-pointed and elongated in their anterior portion while villi were broad and blunt-shaped in the posterior portion near the ileum surface of the villi containing transverse furrow (Fig. 3C). The mean thickness of tunica mucosa was 175.24 ± 5.6 µm, and the height of epithelial was 13.09 ± 0.30 µm. The number of goblets cells varied from few to moderate from the cranial to caudal end of the jejunum. The villi length was 131.25±7.3 µm, the villi wide were 42.45 ± 2.85 µm and the crypt depth was 70.25 ± 4.6 µm (Table1).

The lamina propria beneath the villi was a thick layer has secretory units of crypts of Lieberkühn which were small in size and lined

by simple columnar cells that contained acidophilic cytoplasmic granules (Fig. 3D). The Intestinal glands or crypts of lieberkuhn were simple branched, coiled tubular glands lined with simple cuboidal to low columnar epithelium. The muscularis mucosa varied in thickness and was made up of smooth muscle fibers. In a few places, it was interrupted due to the presence of a large amount of lymphoid tissue and the extension of crypts of lieberkuhn. The tunica submucosa was formed by loose irregular, connective tissue having connective tissue cells fine blood capillaries along with elastic, collagen and reticular fibers the Brunner glands less number than the duodenum which was found in the cranial part of the jejunum and decrease toward the ileum (Fig. 3A and C). Payer's patches were positioned in the posterior end of the jejunum near the Jejuno-ileal union (Fig. 3A). The mean thickness of tunica submucosa was 56.13±2.34 µm.

Tunica muscularis was constituted by inner circular and outer longitudinal layers of smooth muscles in between these layers there were blood vessels, and nerves bundle the mean thickness of it was 341.77 ± 4.6 µm. Tunica serosa was involved of loose irregular connective tissue having collagen, elastic and reticular fibre along with a varying amount of fatty tissue (Fig. 3D). The histochemical result of the wall structure of jejunum showed the presence of goblet cells in the villi which gave with the combined PAS-AB (pH2.5) dye vigor positive reaction (magenta color) with PAS and moderate reaction (blue color) with the AB (pH2.5), (Fig. 3C).

Ileum

The wall of the ileum consists of four tunics: tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. The villi in the ileum were wider and shorter than in the duodenum and jejunum (Fig. 4A). The villi were flattened finger-leaf shaped (Fig. 4B). The entire surface of ileum was lined by simple columnar with numerous goblet cells. The crypts depth was significantly less than the crypt of duodenum and crypt jejunum because the mucosal glands found superficial arrangement in both the jejunum and ileum while in the duodenum the mucosal glands were positioned in several layers, the presence of payer's patches in mucosa which extend to the tunica submucosa. The presence of the payer's patches which showed diffuse lymphatic tissue in the lamina propria extend to the submucosa, and the payer's patches about the aggregation of lymphatic nodules to showed well-developed with pale centers and darkly stained marginal regions (Fig. 4A). The mean thickness of tunica mucosa was (163.24 ± 8.6 µm), the mean height of epithelial was (12.24±0.20 µm), the Villi length, villi width and crypt depth were 124.27 ± 2.3 µm, 35.25 ± 2.85 µm, and 80.25 ± 3.6 µm, respectively (Table 1).

The tunica submucosa thickness was 76.13 ± 4.34 µm which was formed by loose irregular, connective tissue having connective tissue cells and fine blood capillaries along with elastic, collagen and reticular fiber (Fig. 4A). The tunica muscularis made from two layers of smooth muscles fibers inner circular layer and outer longitudinal layer, the mean thickness of it was 361.77± 8.6 µm (Table 1). The histochemical result of the dye with a combination of PAS-AB appears that the presence of apparently acidic mucin more than the neutral. The acidic goblet cells of the ileum villi and crypts proceeded with amounts of neutral mucin (Fig. 4B).

Discussion

Duodenum

The wall duodenum consists of four tunics: mucosa, submucosa, muscularis and serosa, the morphometric measurement of tunics varies in different tunics, the thickness of tunica mucosa was higher than tunica submucosa and less than the thickness of tunica muscularis, the tunica mucosa had various finger-like projections. These differences indicated the higher functional mucosa of duodenum of newly kittens of one week of age because the kittens in this age feed on milk and some solid food, these finding was similar to those (Al-Saffar and Al-Zuhairy, 2016) when compared between weaned and neonatal kittens. The weaning stage of the animals appeared critical period through which morphological changes occurred in the digestive organs such as the duodenum. The height villi were excess in length, width villi and crypt depth associated with well-developed intestinal glands. These various results are due to the type of nutrition of kittens which feed on milk and some solid food kind at this stage. These results are similar to those mentioned by Wołczuk et al. (2011) who notice the small intestine in rats through their postnatal developmental periods which were extended from birth until adulthood period. the paneth cells were very few at this period of age compared with absorptive cells at this region because these cells consider defense line antimicrobial in the bowel and increase in number in progressive of age and these cells live roughly 20 days, these results same finding by (Verburg et al., 2000). Selective sparing of goblet cells and Paneth cells in the intestine of methotrexate-treated rats. The Brunner glands were present in the tunica submucosa, also these glands were found at the gastrointestinal junction. These glands act to neutralise the acidity of the food coming from the stomach with foods. Secretion materials from these glands contribute to a layer of mucus which was the consistency of a slimy; viscoelastic gel lubricating the lining of the proximal part of the small intestine in all animals, this finding parallel with Vigueras et al. (1999) noticed the Brunner's glands found in submucosa of duodenum in all studied ages of rats.

Jejunum

The jejunal wall consists of four tunics: tunica mucosa, submucosa, muscularis and serosa same finding by (Hasanzadeh and Monazzah, 2011) in buffalo. The tunica Mucosal growth included distension of the villi, Vacuolated enterocytes with aggregation of colostral proteins are consistent with macromolecular absorption. Same finding (Clarke and Hardy, 1970) when mentioning the Structural changes in the small intestine associated with the uptake of polyvinyl pyrrolidone by young domestic animals. The small intestine of neonatal mammals has lined with enterocytes with an apical tubular network this result resemblance to Wilson et al. (1991) in the rat, which is related to endocytosis and intracellular ingestion or transferal of whole macromolecules to the general circulation. the presence of oblique grooves just after birth suggests that we should consider not only dynamic growth usually related to increasing the number of epithelial cells but also mechanisms causing an increase of villus length within a short time of the postnatal period. This opinion was similar to Skrzypek et al. (2005) in mucosal development in pigs mentioned the extension is related to the presence of numerous oblique grooves on the villus body shortly after birth, We theorized that the small intestine mucosal surface was compressed and prepared to unfold directly

after birth and colostrum intake. The presence of a great number of transverse furrows on the villi at birth prolonged the sustenance of the great number of vacuolated fetal cells.

The Brunner's glands were found in the anterior part of the jejunum in the tunica submucosa of the jejunum, this result was parallel to the notice in pigs and large herbivores by Verdiglione et al. (2002), in rabbits by Ergun et al. (2010) whom mention that the Brunner glands in duodenal submucosal glands were spread from the pyloric-duodenal junction and to reach to the anterior of jejunum these glands act to neutralise the acidity of the food coming from the stomach with foods. Payer's patches were found towards the posterior end of the jejunum near the Jejuno-ileal union in the tunica submucosa it's about the aggregation of lymphocytes to form lymphatic nodules this finding parallel to the observation in goats (Bello and Danmaigoro, 2019).

Ileum

The wall of the ileum had a similar histological structure to duodenum and jejunum, the villi in the ileum were wider and shorter than in the duodenum and jejunum and the finger-leaf-shaped surface of the villi contain transverse furrows same finding (Bello and Danmaigoro, 2019) in goats, the shape or morphological arrangement of the intestinal villi may return the state of the functional face of the mucosa. Moore et al., (1988) mentioned that villous morphology does not return the grade of health. The payer's patch about small aggregations of lymphocytes that appeared in the lamina propria of tunica mucosa extends to the tunica submucosa of this organ, we opinion slow development of payer's patch after birth affected by some such factors like type feeding surrounding environment breeding of animal. Really the capability of neonates toward immune responses may need a considerable time period and appropriate environmental factors to be developed (Bailey and Haverson, 2006). Present theory supposed the effect of nutrition nature considered an important factor to cause such developmental changes in the lymphoid structures in the wall of ileum. The depth of the crypts was significantly less than the crypt of duodenum and crypt jejunum because the mucosal glands found superficial arrangement in both the jejunum and ileum this results in the same finding by (Hamza and Al-Mansor, 2019) in indigenous gazelle. The tunica submucosa was increased in thickness compared with tunica submucosa of duodenum and jejunum due to the presence of aggregation of lymphocytes which form payer's which consider the immune line in the digestive system this finding corresponds (Al-Saffar and Al-Haaik, 2016).

Histochemical

Goblet cells and Brunner's glands were able to secrete a high amount of mucous into the lumen of the small intestine. The distribution of mucins secreted by goblet cells distinguishes by histochemical dyes which showed the presence of neutral mucin in the mucosal lining of the small intestine in neonatal. The sulfated acidic mucin was abundant in the neonatal kittens the non-sulphated (carboxylated) mucin was the dominant type. The reaction of combined PAS–AB indicated the presence of mixed mucin (neutral and acidic) in neonatal kittens and in the brush border of all small intestinal segments(duodenum, jejunum & ileum). Recent results were in agreement with Zanuzzi et al (2010) in New Zealand and Bello and Danmaigoro (2019) in goats.

In conclusion, the histomorphological observations of a newborn cat's small intestine look just like that of the carnivores to which it belongs.

Table 1: Shows the measurement in the three parts of small intestine of neonatal cats (µm)

Parameter	Duodenum	Jejunum	Ileum
Tunica mucosa	231.24±3.6	175.24±5.6	163.24±8.6
Height of epithelial	14.07±0.20	13.09±0.30	12.24±0.20
Villi length	141.27±4.3	131.25±7.3	124.27±2.3
Villi width	32.25±0.85	42.45±2.85	35.25±2.85
Crypt depth	90.25±1.6	70.25±4.6	80.25±3.6
Tunica submucosa	66.13±1.34	56.13±2.34	76.13±4.34
Tunica muscularis	341.77±2.6	341.77±4.6	361.77±8.6

Figure 1: (A) Show the four tunics of a wall in the duodenum (red double head arrow), Simple columnar epithelium with goblet cells (black arrow), Brunner glands (yellow arrow), crypts of Lieberkuhn (cl),Tunica muscularis (red star) H&E stain, 40X. (B) Goblet cell (red arrow), Villi (black arrow), crypts of Lieberkuhn (cl), Tunica submucosa (yellow star). H&E stain, 100X. (C) Intestinal crypt (black arrow), Goblet cell (red arrow), Brunner glands (yellow arrow). Combined AB & PAS stains 40X. (D) Paneth cell (yellow arrow), Brunner gland (red arrow) H&E stains, 100x.

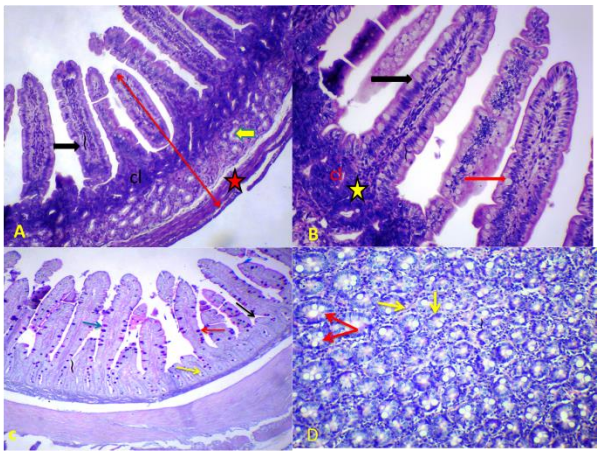


Figure 2: Show duodenum: tunica submucosa (Sb), Auerbach's nerve plexuses (two black arrow),inner and outer muscular layer (yellow arrow), mesentery red arrow. H&E stain X40.

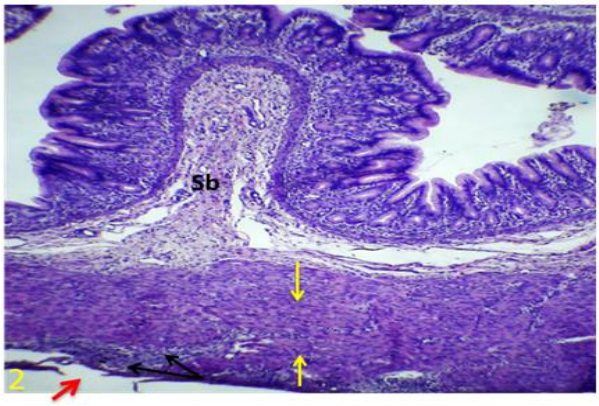


Figure 3: Show the histological structure of the wall in the jejunum (A) The villi (red arrow), Pyres patch (yellow arrow), and tunica musculris (yellow star). H&E stain X40. (B) Show the four tunics of the jejunum (double head black arrow), and goblet cells (red arrow). Combined AB & PAS stains X40. (C) Show the columnar epithelial tissue (black arrow), goblet cell (red arrow), and Brunner gland (yellow arrow). Combined AB & PAS stains X100. (D) Show the lamina propria (double head black arrow), tunica submucosa (yellow star), tunica muscularis (black star), myenteric plexus (blue arrow) Masson's trichrome stain X40.

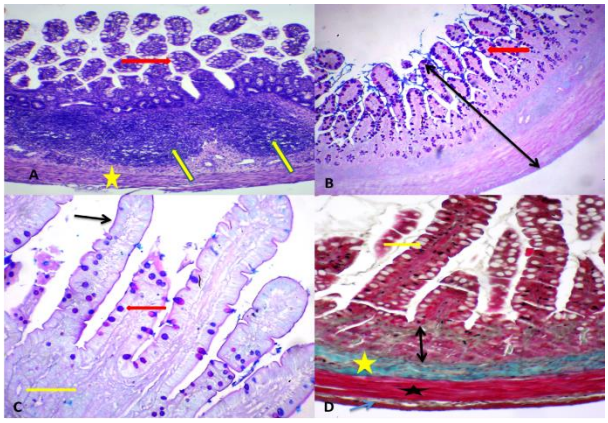
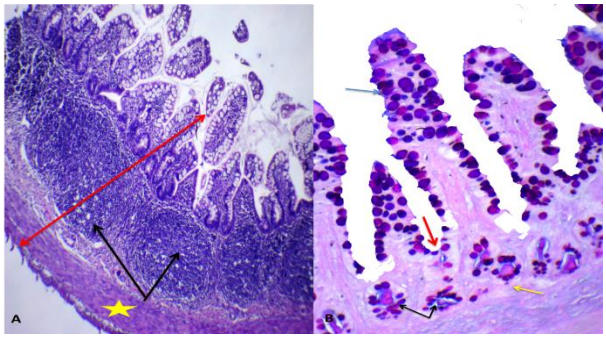


Figure 4: Show the ileum in neonatal cats: (A) Four tunics of the wall in the ileum (red double head arrow), payers patch (two black arrows). Tunica muscularis (yellow star). H&E stain X40. (B) Intestinal crypts (red arrow), villi lined by simple columnar epithelial (blue arrow), tunica submucosa (yellow arrow), intestinal glands (two black arrows). Combined AB & PAS stain X100.



Reference

Al-Haaik(2019). A gross anatomical and histological study of pancreas in adult Kestrel (Falco tinnunculus). Iraqi Journal of Veterinary Sciences, Vol. 33, No. 2, 2019 (175-180).

Alkattan, L. M. Alhasan, A. M. and Albadrany, M. S.(2014). Laparoscopic nephrectomy in Iraqi cat. Iraqi Journal of Veterinary Medicine, Vol.28(1), pp.17-20.

Al-Saffar and Al-Zuhairy(2016). Postnatal developmental Histomorphological and histochemical study of the duodenum in the domestic cat (Felis Catus)..International Journal of Current Research Vol 8, Issue, 12, pp.43681-43690, December, 2016.

Al-Saffar and Al-Haaik(2016).Histomorphological and Immunohistochemical Postnatal Developmental Changes in the Small Intestine and Colon of the Indigenous Rabbits (Oryctolagus cuniculus).PhD

thesis.College of Veterinary Medicine of Baghdad University (Anatomy & Histology).

Al-Saffar, and Eyhab.(2016)."Histomorphological and histochemical study of stomach of domestic pigeon (Columba liviadomestica). The Iraqi Journal of Veterinary Medicine,Vol. 40(1),pp.89-96.

Bailey, M. and Haverson, K.(2006).The postnatal development of the mucosal immune system and mucosal tolerance in domestic animals. Vet. Res; 37: 443–453 443.

Bailey, T.A.; Mensah-Brown, E.P.; Samour, J.H.; Naldo, J.; Lawrence, P. and Garner,A.(1997).Comparative Morphology of the Alimentary tract and its glandular derivatives of captive bustards. J. Anat.: 191, Pp: 387-398.

Barker,N.(2014).Adult intestinal stem cells critical drivers of epithelial homeostasis and regeneration. Nat Rev Mol Cell Biol 15(1):19–33.

Bello,A.and Danmaigoro,A.(2019).Histomorphological observation of the small intestine of Red Sokoto Goat: a review.MOJ Anatomy & Physiology journal Volume 6 Issue 3.

Buddington, RK.and Diamond, JM.(1992). Ontogenetic development of nutrient transporters in cat intestine. Am J Physiol 263:G605–G616.

Clarke,RM. and Hardy,RN.(1970).Structural changes in the small intestine associated with the uptake of polyvinyl pyrrolidone by the young ferret, rabbit, guinea pig, cat and chicken. J Physiol 1970;209:669–687.

Culling, F. A; Allison, F. and Barr, T.(1985). Cellular pathology technique, 4th ed. London. Better Worth Company, pp: 212-214.

Ergun, E. Ergun, L. Ozen A. and Kurum, A. and Bayraktaroglu,A.G.(2010). Histomorphology of the Brunner gland in the Angora rabbit. Journal of Animal and Veterinary Advances 9(5) 887-891.

Gharban, H. A. (2022). Clinical and Serological Diagnosis of Bovine Hypodermosis in Wasit Province. Revista Electronica de Veterinaria, 457-466.

Gunawardene, AR. Corfe, BM. and Staton CA. (2011). Classification and functions of enteroendocrine cells of the lower gastrointestinal tract. Int J Exp Pathol 92(4):219–231.

Hamza, L. O. (2018). Histological and Histochemical Characters of the Pancreas in the Adult Indigenous Gazelle (Gazella subgutturosa). Indian Journal of Natural Sciences. Vol.8 / Issue 48 / June. 13773-13781.

Hamza, L. O., and Al-Mansor, N. A.(2017). Morphological features of the small intestine in the adult indigenous Gazelle (Gazella Subgutturosa). International journal of science and nature, 8(2), 223-229.

Hamza, L. O., and Al-Mansor, N. A.(2019).Histological and histochemical observations of the small Intestine in the indigenous Gazelle (Gazella subgutturosa). Journal of Entomology and Zoology Studies, 5(6), 948-956.

Hasanzadeh, S. and Monazzah, S.(2011). Gross morphology, histomorphology and histomorphometry of the jejunum in the adult river buffalo. Iranian J. Vet. Res. 12: 99-106.

Hussein Bashar Mahmood and Khalid.K.Kadhim (2019).Histomorphology and Histochemical Study of Esophagus and Stomach in Grey Mongoose (Herpestesed

wardsii) In Iraq. Indian Journal of Natural Sciences. Vol.9 Issue 52. ISSN: 0976 – 0997.

Moore, R.J; Kornegay, E.T; Grayson, R.L. and Lindemann, M.D.(1988).Growth, nutrient utilization and intestinal morphology of pigs fed high fiber diets. Journal of Animal Science, 66: 1570-9.

Nickel, R.; Schummer, A. and Sriferle, E. (1979).The Viscera of the Domestic Mammals, 2nd ed. Springer-Verlag, Germany-Berlin. Pp: 109-112.

Pacha, J.(2000). Development of intestinal transport function in mammals. Physiol. Rev. 80, 1633-1667.

Parish, J. (2011). Ruminant Digestive Anatomy and Function. Beef Production Strategies.

Peterson, LW.and Artis, D.(2014). Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol 14(3): 141–153.

Salih, A. N. and Hamza, L. O.(2022).Histological and histochemical study of stomach in neonatal Cats. Ann. For. Res, Vol.65(1).pp.6441-6452.

skrzypek T, Valverde piedra jl and skrzypek, h.(2005). Light and scanning electron microscopy evaluation of the postnatal small intestinal mucosal development in pigs. J Physiol Pharmacol 2005; 56 (suppl.3):71-87.

Taha, A.M. (2021). Comparative histological and histochemical study of the ileum in two different birds Iraqi Journal of Veterinary Sciences, 35 (3), 479-487.

Verburg M., Renes I.B., Meijer H.P., Taminiau J.A.J.M., Büller H.A., Einerh and A.W.C., Dekker J.(2000).Selective sparing of goblet cells and Paneth cells in the intestine of methotrexate-treated rats. Amer. J. Physiol. 279, G1037-G1047.

Verdiglione,R.Mammola,C.L.and Filotto,U.(2002).Glycoconjugate histochemistry of bovine Brunner glands. Annals of Anatomy; 184 61-69.

Vigueras, R. M; Rojas-Castaneda, J; Hernandez, R; Reyes, G. and Alvarez, C.(1999). Histological characteristics of the intestinal mucosa of the rat during the first year of life. Laboratory Animals, 33: 393-400.

Weaver,LT,Lucas,A.(1987).Upper intestinal mucosal proliferation in the newborn guinea pig: effect of composition of milk feeds. Pediatr Res 22:675–678.

Wilson JM, Whitney JA,and Neutra,MR.(1991). Biogenesis of the apical endosome lysosome complex during differentiation of absorptive epithelial cells in rat ileum. J Cell Sci 1991;100:133–143.

Wołczuk, K; Wilczyńska, B. Jaroszewska, M. and Kobak, J.(2011). Morphometric characteristics of the small and large intestines of Mus musculus during postnatal development. Folia Morphol; 70(4): 252–259.

Zanuzzi, C.N; Barbeito, C.G; Ortı´z, M.L; Lozza, F.A; Fontana, P.A; Portiansky, E.L. and Gimeno, E.J. (2010). Glycoconjugate histochemistry in the small and large intestine of normal and Solanum glaucophyllum- intoxicated rabbits. Res. Vet. Sci. 89:214–222.