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RESEARCH ARTICLE

Comparative study of large intestine histochemical and histomorphometrical in sheep (*Ovis aries*) and rabbits (*Oryctolagus Cuniculus*)

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ABSTRACT

The current article aims to compare the colon and the large intestine's histochemical and histomorphometric characteristics. The study included 20 animals, ten healthy male sheep and ten male rabbits; their weight were from 35-45 kg and 1.5-2.5 kg. Haematoxylin and eosin, a standard tissue approach, and periodic acid Schiff for demonstrating neutral mucopolysaccharides. Histologically, showed similarity in epithelium tissue of mucosa lining the cecum and colon in both animals, the epithelium lining was composed of simple columnar epithelium. Well-developed intestinal glands were abundant in the mucosa layer. These are glands simple tubular straight unbranched, and these glands compose goblet cells and columnar cells. In both animals, the submucosa layer of cecum and colon does not possess glands and lymphatic nodules. The mean thickness regarding these layers sub mucosa and muscularis in sheep cecum and colon were significantly ($p<0.05$) larger comparison to rabbits' cecum and colon. There have been non-significant ($p>0.05$) differences among thickness mucosa sheep and rabbit in cecum and colon, where thickness mucosa of cecum in sheep and rabbit was (272.96±36.55), (237.96±24.09), while thickness mucosa of colon (604.24±252.58) (564.58±47.69). Intestinal glands showed a strong pas' positive materials. Conclusions: The findings indicated similarities of herbivores species in and histochemical features in cecum and colon of the sheep and rabbit.

Keywords: Large Intestine, Histochemical and Histomorphometrical

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INTRODUCTION

The colon, caecum, and rectum are the three segments of large intestine, which is a hollow organ with a considerably larger diameter than small intestine (Pérez et al., 2008). The large intestine, whose function is breaking down the ingested food into small units which could be absorbed into tissues and utilized for maintaining the body, also absorbs many vitamins, water, electrolytes, and mucus production (Kadam et al., 2007). The digestive system is the only system that meets the body's energy requirements by nutrient absorption, forging a strong bond with nature by digesting various types of foods. Because of their higher energy and protein requirements per unit mass than giant herbivores, small herbivorous animals are constrained by their nutritional equipment systems. Young herbivores therefore require special digestive techniques to get beyond the limits of their modest body mass in comparison to large animals. The pattern of digestion's flow and mixing in large intestine varies across small herbivorous mammals, even though they typically have bigger cecum (Sakaguchi, 2003). The rabbit cecum is larger than the stomach and produces 40% of intestinal contents (Calamar et al., 2014). The colon, which is the longest segment of the large intestine, is made up of descending, ascending, transverse, and sigmoid colons. Most of the water and salt absorption occurs in the proximal portion of the colon. The distal portion of colon is primarily used for processing and storing feces, with a limited amount of absorption activity. The surface of colon is smoother than that of the small intestine because it lacks villi. Four types of layers are found throughout the large intestine, namely, tunica mucosa, submucosa, tunica muscle and serosa tunica (Kadam et al., 2007). However, the anatomical difference between the sheep and rabbits is the presence of large cecum as well as the different fermentation sites between these two animals was the reason behind this study.

MATERIALS AND METHODS

Surgical Procedures

Ten adult male sheep were collected from local slaughterhouses, weighing between 30-40 kg and aged approximately one year. Also, a total of ten rabbits, weighing between 1.5-2.5 kg and aged approximately three months from an adult male, were collected from the city of Maysan, southern Iraq**.** The animals have been raised in accordance with accepted practices and euthanized in accordance with euthanization protocol. Prior to the euthanasia, each animal underwent a physical assessment to ensure they were all in good health. Previous studies reported that the euthanizing techniques with anahal chlorophorm ethically not used according to The Institutional Animal Care and Use Committee (ICUC) involved putting 2.0mL of the chloroform ($CHCl_3$) on cotton and placing it on animal's nose (Blackshaw et al., 1988). Each specimen was subjected to a regional gross dissection using the necessary instruments, including scissors, scalpels, and tweezers. The large intestine was removed from the abdomens of the rabbits and sheep. After that, 1 cm segments of each large intestine were removed from various parts (cecum, colon).

Histological examination

Sheep and rabbit colon and cecum samples have all been quickly fixed in 10% neutral buffered formalin 72hrs. The tissue samples were dehydrated in ascending grades of ethyl alcohol. All the samples have been cleaned by using xylene for an hour to create paraffin blocks, which were after that embedded in paraffin wax. Tissue sections were then treated with two stains and cut to a thickness of 7 micrometers (Luna, 1968). Hematoxylin and Eosin were utilized for general histological structural descriptions, Periodic Acid-Schiff (PAS) to detect the neutral mucopolysaccharides (glycoprotein and glycogen) in the intestinal glands (Luna, 1968).

Micro morphometric measurements

For the colon and cecum of large intestine, ten slides were created. We used an optical microscope and an exact ophthalmic scale (i.e. ocular micro-meter) to measure the thickness of mucosa, submucosa, muscles, and serosa. The exact ophthalmic scale has then been compared to theatrical scale with the use of magnification force (Galigher, Kozloff 1964).

Statistical analyses

Values have been represented in the form of mean ± SD. With the use of SPSS Windows Version 16 to undertake the statistical analysis of the data, the t-test at $P<0.05$ of probability was used to determine whether there were any significant differences (Al-Rawi, Khalaf Allah, 2000).

RESULTS AND DISCUSSION

Microscopic examination of cecum and the colon showing that their wall in the sheep and the rabbits form of four superposed layers: mucosa, sub-mucosa, muscularis and serosa layers (Figures 1-4). The mucosa of the colon was distinguished by presence of folds appeared long shaped, these folds arranged in zig-zag pattern in sheep, while in the rabbit of the colon appeared short and blunt perhaps these folds increase the surface area for absorption, this study similarity with Al-Samawy et al. (2019). While these folds were not found in the cecum of both animals. However, the mucosa lacks villi or plicae in both cecum and colon of both animals, the mucosa in the cecum and the colon are made up of the simple columnar epithelium layer contain mucus-secreting goblet cells and columnar cells are long with long oval intensely basophilic nuclei, in both sheep and rabbits.

Lamina propria is formed of the loose connective contain blood vessel, lymphoid and intestinal glands (crypts of Lieberkuhn), these glands simple tubular straight unbranched in cecum (Figures 5,6) and colon (Figures 7,8) made of many of goblet cells and few of columnar cells, these glands are in cecum rabbit have lumen large. The presence of many mucus secretion cells provides a mucous layer around

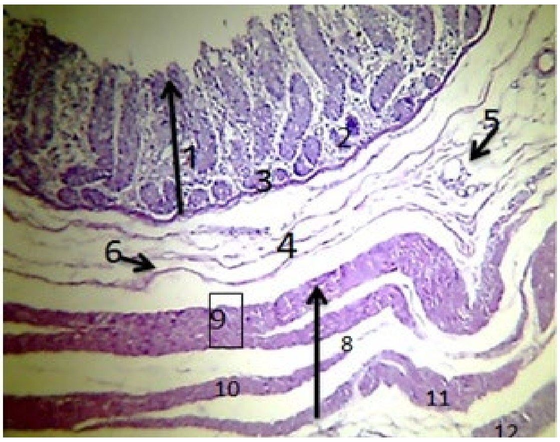

Figure 1. Section of cecum in sheep showing (arrow black) mucosa (1) consist of lamina properia (2), muscle mucosa (3), sub mucosa (4) contains macrophage (5), fiber (6). Muscularis (8) circular muscle (9), longitudinal muscle (10), taeniae coli (11). serosa (12).H &E 100X

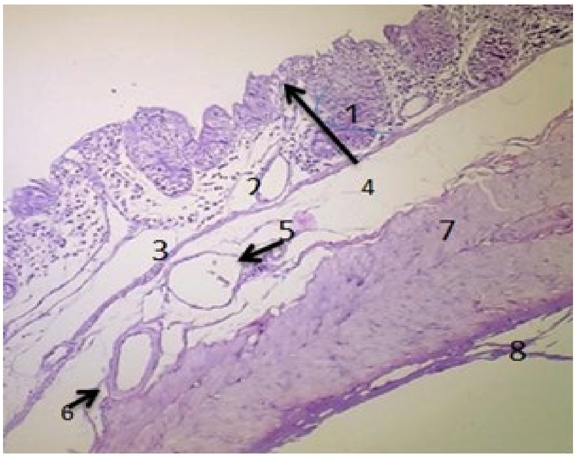

Figure 2. Section of cecum in rabbit showing (arrow black) mucosa (1) consists of lamina properia (2), muscle mucosa (3), submucosa (4) contains macrophage (5). Muscularis (7), serosa (8).H &E 100 X

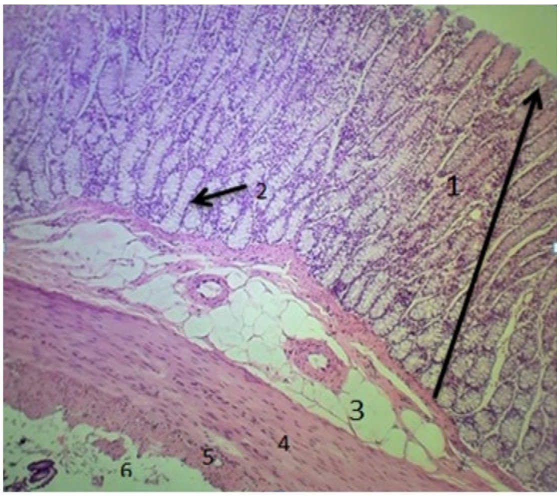

Figure 3. Section of colon in sheep showing (arrow black) mucosa (1), crypts (2), sub mucosa (3). (arrow red) (arrow green). Muscularis consist of circular muscle (4) longitudinal muscle (5) serosa (6) .H &E 100X

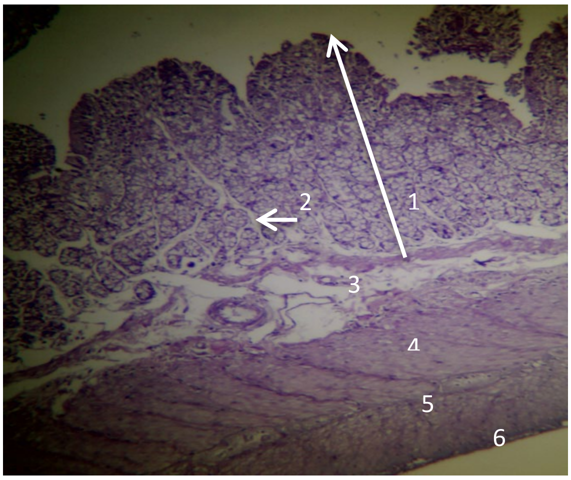

Figure 4. Section of colon in rabbit showing (white arrow) mucosa (1), sub mucosa (3). (arrow red) (arrow green). Muscularis consist of circular muscle (4) longitudinal muscle (5) serosa (6) .H &E 100X

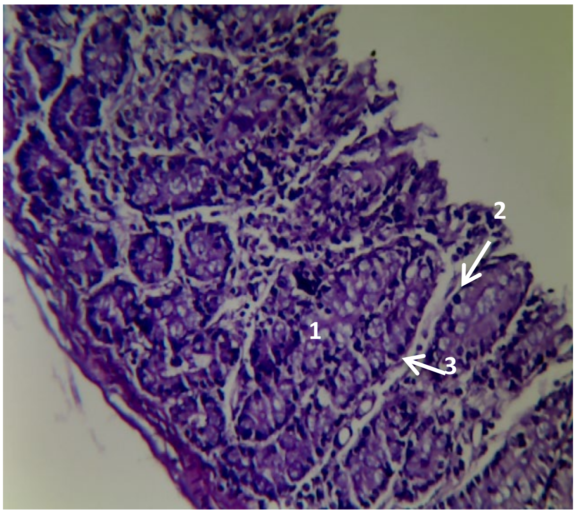

Figure 5. Section of cecum mucosa in sheep showing gland (1), goblet cells (2), columnar cells (3). H &E 400 X.

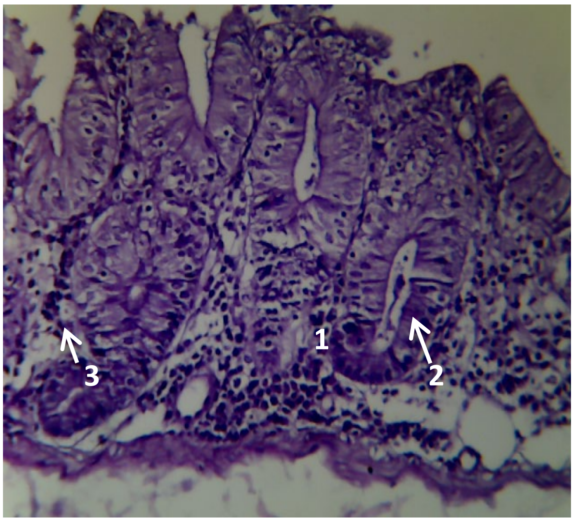

Figure 6. Section of cecum mucosa in rabbit showing gland (1), goblet cells (2), columnar cells (3). H &E 400 X.

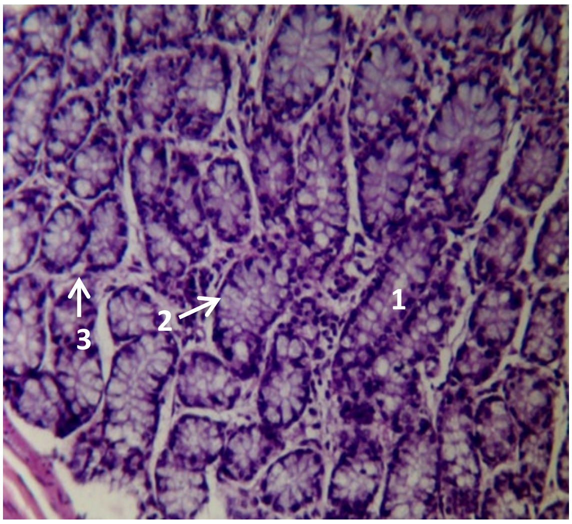

Figure 7. Section of colon mucosa in sheep showing (1) gland, (2) goblet cells, (3) columnar cells H &E 400 X.

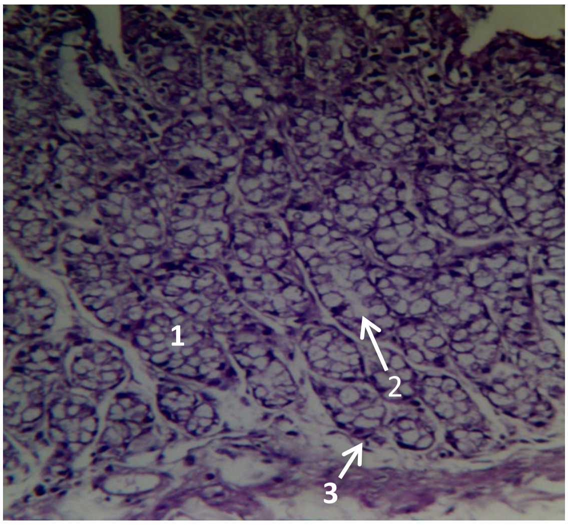

Figure 8. Section of colon mucosa in rabbit showing (1) gland, (2) goblet cells, (3) columnar cells H &E 400 X.

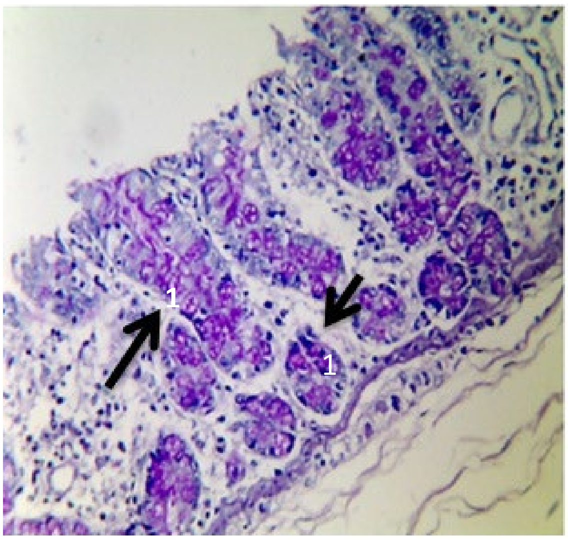

Figure 9. Section of cecum mucosa in sheep showing (1) glands gave reaction strong with PAS.400X

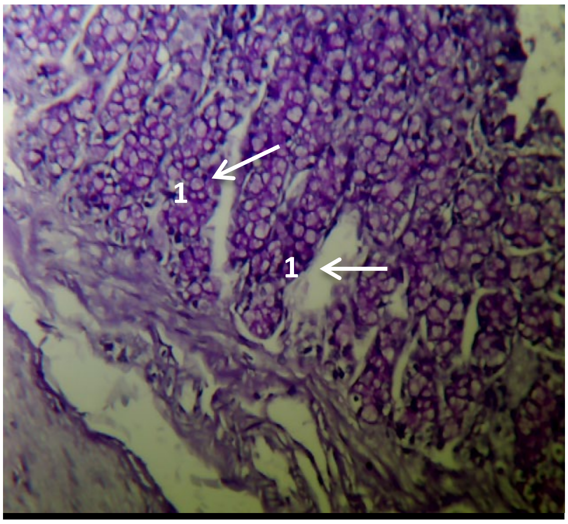

Figure 10. Section of cecum mucosa in rabbit showing (arrow black) glands gave reaction strong with PAS.400X

stool globules, which facilitates their release and protects epithelium as it has been described by Ahmed et al. (2009) and Junqueira, Mescher (2013).

The muscularis mucosa consists of smooth muscle fiber arrangement longitudinal and it more thickness in sheep than in rabbit. It forms the outer boundary of the mucosa (Eroschenko, 2008). Sub mucosa below mucosa consist of loose connective tissue but this layer lack to lymphaticnodules and glands in both cecum and colon (Figures 1,2), and similarly with study of Mohamed et al. (2018), but this disagrees with Kadam et al. (2011) they seen aggregation lymphoid in cecum sheep. While Yasuda et al. (2006) observed in ruminants, dogs, and pigs, gut related lymphoid tissues have been presented in ileocecal patches only.

The muscularis layer is made from 2 smooth muscle fibre layers: internal-circular more thickness and external longitudinal among them loose connective tissue, in colon rabbit, the outer layer was order into three separate bands, known as taeniae coli in cecum sheep and colon rabbit. Eroschenko, (2008) reported that contractions or tonus in taenia coli forms sacculations in large intestine, which is referred to as haustra. In addition to, serosa layer of thin layer of loose connective tissue, which was containing few small elastics, blood vessel and nerves (Eroschenko, 2008).

Table1. Average thickness of mucosa, sub-mucosa, serosa and muscularis in the Cecum and colon regions of the large intestine of rabbit and sheep.

organ	Thick mucosa		Thick submucosa		Thick muscularis		Thick serosa	
Large intestine	sheep Mean ±SD	rabbit Mean ±SD	sheep Mean ±SD	rabbit Mean ±SD	sheep Mean ±SD	rabbit Mean ±SD	sheep Mean ±SD	rabbi Mean ±SD
cecum	272.96± 36.55 a	237.96± 24.09 a	263.62± 77.80 a	130.64± 27.38 b	368.61± 55.86 a	207.63± 30.01 b	88.65± 24.09 a	60.65± 19.67 a
colon	604.24± 252.58a	564.58± 47.69 a	125.98± 42.87 a	67.65± 23.20 b	520.25± 86.63 a	352.28± 65.48 b	88.65± 21.43 a	48.99± 30.01b

Value represent (mean± SD). Similar letters indicate non-significant (p>0.05) differences between the values. Different letters denote (p<0.05) significant difference between values.

Histomorphometric study: There were non-significant (p<0.05) differences among thickness of mucosa sheep and rabbit in the cecum and colon part as shown in (Table 1). This may be due to the similarity in nutrition. On the other hand, dietary fiber level and sources affect the morphology of the gastrointestinal tract (GIT) mucosa such as villous height, number of the goblet cells and crypt depth. These changes indirectly influence the growth of animals which affect the proliferation of intestinal cells (Yu, Chiou (1997); Desantis et al. (2011)). The thickness of sheep submucosa in the cecum region was (263.62±77.80) and colon (130.64±27.38).

Sections were significantly (p<0.05) larger when compared with the same sections of rabbits' cecum and colon (125.98±42.87), (67.65±23.20) (Table 1) respectively, and this result agree with Mohamed et al. (2018) they observed thickness submucosa in camelus dromedary's cecum (243.2± 44.3)· The thickness of the muscular sheep cecum (368.61±55.86) and colon (207.63±30.01), sections were significantly (p<0.05) larger when compared with same sections of rabbits' (520.25±86.63), (352.28±65.48), (Table 1) respectively, this result disagrees with Putri et al. (2019) who mentioned that thickness muscularis in Aceh cattles colon was (2380±16μm), and this may be related to the function and location of the large intestine. Muscularis thickening was associated with buffering and expulsion of fecal material from this region (Grau et al., 1994). Where there have been non-significant (p>0.05) differences among the thickness serosa in cecum sheep and rabbits' cecum, while thickness of the serosa sheep colon (88.65±21.43) section was significantly (p<0.05) larger compared with the same sections of rabbits'colon (48.99±30.01) as shown in (Table 1).

Histochemical study: The goblet cells of epithelium surface and intestinal glands show a strong reaction with PAS of cecum and colon in two animals (Figures 9,10). This indicator found mucus neutral that facilitates the passage feces, while the columnar cell's poor reaction with PAS. As well as the colon epithelium has been stained by PAS, and the goblet cells and intestinal glands of colon African Giant Rat have been observed PAS positive as well Nzalak et al. (2010). This study, however, disagrees with Gahlot et al. (2018) they concluded that crypts of the Lieberkuhn had acidic muco-polysaccharides predominance in sheep cecum, and this difference may be due the quality of the food.

CONCLUSIONS

These findings indicated similarities between herbivores species and identified the histological features in cecum and colon of sheep and rabbit.

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