Research article

Histomorphological and Histochemical Study of Ileum and Jujenum of the Camel (*Camelus dromedarius*)

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Abstract | This study aimed to investigate the histological and histochemical features of the camel's ileum and jejunum in light of the camel's special metabolic needs. A total of 12 mature camels' digestive systems were used in this investigation. General histological staining including used periodic acid-Schiff (PAS), PAS and alcian blue, and Crossman stains were used. Through microscopic examination, we discovered that the length of the mucosal folds (villi) decreases from the jejunum to the ileum. The camel jejunum and ileum anatomy revealed mucin distribution throughout the gastrointestinal tract.

Keywords: Camel, Histochemistry, Histomorphology, Small Intestine, Ileum, Jujenum

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INTRODUCTION

The camel's digestive tract stands out above other animal guts in Saudi Arabia and beyond because it has the highest proliferation rate in the human body (Mohamed *et al.,* 2018). Although it is a ruminant, the camel feeds on dry hay and cores. Although similar in function, the stomachs of domesticated animals have distinct anatomical differences from those of wild ruminants and human.(Tharwat *et al.*, 2012).

The jejunum is a significant portion of the abdominal cavity and is distinguished by the presence of villi, goblet cells, intestinal glands, and Payer's patches, which exhibit gradual structural modification. From a histological perspective, it resembles the duodenum, albeit it exhibits rounder villi and a higher concentration of goblet cells. The ileum is distinguished by a large concentration of goblet cells, along with Payer's patches and villi that are club-shaped and poorly differentiated. The duodenum is characterized by leaf-shaped villi, low goblet cells, and the absence of Payer's patches. The camel's duodenal glands are located in the submucosa around two meters posterior to the pylorus. These glands release mucosubstances that are both neutral and acidic (He *et al.*,2018).The wall of the small intestine in camels consists of four histological layers: mucosa, submucosa, muscularis, and adventitia. The mucosa comprises three distinct sublayers: the epithelial lining, the lamina propria, and the T muscularis mucosae. The lining of the epithelium consists of a single layer of columnar cells, with an increasing number of goblet cells as it approaches the large intestine. The lamina propria is composed of lax connective tissue that is infiltrated by lymphocytes. It contains intestinal glands called crypts of Lieberkuhn, which are uncomplicated tubular glands that open between the villi. The muscularis mucosae comprise an inner layer that runs in a circular direction and an outer layer that runs longitudinally. The mucosa comprises varying degrees of connective tissue, from loose to dense. The muscular layer of the small intestine is composed of two distinct layers: an inner circular layer and an outer longitudinal layer. These layers are separated by connective tissue and surrounded by the peritoneum's visceral layer (Hyder *et al.,* 2023).

Although camel shares certain similarities in anatomy with other ruminants, its stomach and duodenum (El-Bahr, 2017) are distinct. The small intestine plays a crucial role in digestion and absorption. To do this, the endocrine glands release digestive enzymes combined with the meal before digestion. Duodenum, jejunum, and ileum all make up the small intestine in domestic animals, and all three have a similar histological pattern with their unique fea-

tures (Junqueira and Mescher, 2013).

Mucosa, submucosa, muscularis, and serosa are the four tunics that make up the histological characteristics of the small intestine. Epithelium, lamina properia, and muscularis inside the mucosa layer were all visible under a light microscope. Villi, on the other hand, are characteristic of the wide projecting mucosa of the intestinal glands. The small intestine is divided into three sections, each with its unique set of villi processes (the duodenum, jejunum, and ileum). Lamina propria with crypts houses the digestive glands. Lieberkuhn's crypt is a cavity lined with simple columns of epithelium. Situated beneath the mucosa lies a stratum of compact connective tissue called the submucosa. The muscularis layer, often known as the third layer, comprises circular and longitudinal muscle fibers. Both tunics terminate with a serosa layer comprising loose connective tissue and enveloped mesothelium. (Suhaib *et al.,*2023).

The jejunum is positioned as the intermediate segment within the small intestine between the duodenum and the ileum. The jejunal arteries derive the vascular supply of this structure, while the celiac and superior mesenteric plexi provide its innervation in conjunction with the vagus nerve. The jejunum assumes a significant part in digestion, comprising approximately 40% of the total length of the small intestine. This entity's primary roles encompass water absorption and essential nutrients. From a histological perspective, the mucosa of the structure in question comprises a layer of simple columnar epithelium. Additionally, it possesses the distinctive crypts of Lieberkuhn, which are accompanied by villi (Lema *et al.,* 2020).

The point at which the extraperitoneal ascending portion of the duodenum transitions to the intraperitoneal jejunum is known as the duodenojejunal flexure, which is located at the level of L2. The distinction between the ileum and its adjacent regions must be distinctly delineated and can only be observed at a microscopic level. The jejunum constitutes approximately 40% of the whole length of the small intestine, which ranges from 1.5 to 3.5 meters. Evident at a macroscopic level are the numerous circular folds in the mucosa, commonly referred to as the valves of Kerckring. Like other intraperitoneal organs, the jejunum and ileum are connected to the posterior abdominal wall through the mesentery. Through this mechanism, the entirety of the small intestine is flexibly positioned in the abdominal cavity, albeit surrounded by the colon (Dubbelboer *et al.,* 2022) The intestinal mucosa is home to many different types of microorganisms, both commensal and pathogenic, and they can perform substantial metabolic activities, such as the fermentation of complex sugars that aid in the host's metabolic process. Goblet cells produce and secrete mucin glycoproteins, which coat the gastrointestinal epithelium in a protective mucous gel. Goblet cells in both the small and large intestines play a crucial role in producing and releasing mucins, which are glycoproteins with a high molecular weight (Kim and Khan, 2013). The mucosal epithelia need this mucus coating to protect them from the acidic and corrosive conditions of digested food, digestive fluids, and bacteria. The small intestinal mucosa provides one more selective barrier for absorption (Korkmaz and Kum, 2016).

Glycoconjugates, vital constituents of the intestinal mucosal barrier, fulfill various functions within the gastrointestinal tract. These functions include facilitating digestion, promoting nutrient absorption, safeguarding the mucosa against ingested substances, and mediating interactions between cells and pathogens in the intestinal lumen. Neutral glycoproteins lack any acid groups, while acidic mucosal substances contain only trace amounts of carboxyl and sulfate radicals. Proteoglycans and glycoproteins, which include carboxyl and sulfate groups, react significantly with alcian blue because of their carbohydrate moiety (Grondin *et al.,*2020).

Tissue sections that react with the periodic acid-Schiff (PAS) reagent can be examined for neutral glycoproteins. Compared to neutral mucins, acidic mucins are more stable and have a higher pH, making them less susceptible to destruction by bacterial glycosidases and host proteases (Gurina and Simms,2020). This research examines the camels' jejunum and ileum for unique histological and histochemical characteristics.

A prior study conducted multiple investigations to examine the influence of dietary choices on the physical characteristics and subsequent physiological activities of the digestive organs in camel species. The small intestine consists of three different segments. The initial section, the duodenum, arises from the gizzard and creates a loop surrounding a substantial part of the pancreas. The jejunum is located anatomically between the duodenum and the ileum. The third section, the ileum, stretches from the diverticulum to the ileocaecal junction. Nevertheless, material regarding the Ileum and Jujenum in camels is scarce. Therefore, this study focused on examining the histomorphology and histochemistry of the Ileum and Jujenum.

This project aimed to study the histomorphological and histochemical structures of the ileum and jejunum of the Camel in Iraq.

MATERIALS AND METHODS

Animals

12 camels were healthy and devoid of apparent pathological alterations. The ages of the animals ranged from two to twelve.

Methods

Tissue samples from the jejunum and ileum of 12 healthy adult camels of both sexes were dissected and then fixed by immersing them in 10% neutral formal saline for 24 hours. Subsequently, we subjected the tissue samples to drying, cleaning, and paraffin embedding. Histochemistry: Conventional histochemical methods were used to detect glycoconjugates in the goblet cells of the small intestine of camels. The PAS method was used to stain glycogen and neutral mucosubstances, while alcian blue pH 2.5 was used to stain the carboxyl group of acidic mucosubstances. In addition, a mixture of periodic acid-Schiff (PAS) and alcian blue pH 2.5 was employed to colorize neutral and acidic mucosubstances, a process known as PAS/AB staining. Histometry: Crossmon's variation of Mallory's trichrome procedure was used to stain 6-µm-thick slices of jejunum and ileum (Denk *et al.,* 1989) . The Leica DMLB light microscope, along with a Leica DC200 CCD camera and Q-win image processing software, was used to capture images of the villi at a 20X objective. The height, diameter, and crypt depth of the villi were subsequently measured. For each segment, we conducted measurements of the height (HV), depth (DC), and diameter (D) of three distinct villi. An analysis of variance (ANOVA) was conducted on histometric data, namely the measurements of villus length, crypt depth, and villus diameter. The statistical software used for this analysis was the Statistical Package for the Social Sciences (SPSS) version 14.01. Additionally, Duncan's multiple-range test was included in the analysis. The method of orthogonal comparison was employed to examine the least squares means, and differences with a significance level of P0.001 were considered to be significant.

RESULTS

Histomorphology

Camels had a three-part small intestine that included the jejunum and ileum. Despite their histological similarities, these areas were distinguished by a few key details. The small intestine's surface area was increased by altering its luminal surface. Studies at a microscopic level showed that the small intestinal mucosa was covered with folds (villi intestinalis), both large and small. Macroscopic fold length decreased from in the ileum (Figure 1). The villi of a camel's small intestine are projections of the mucosa that enter the intestinal lumen. The ileum was found to have the most significant villus diameters (P≤0.001), while the jejunum was found to have the longest villi. Simple tubular glands (crypts) formed at the base of the villi (P≤0.001).

As food, digestive fluids, and bacteria produce acidic and corrosive conditions, the mucosal epithelia require this mucus layer for protection. The mucosa lining the small intestine adds a barrier, this one selective, to whatever is being absorbed.

Table 1: Various parameters of different parts of the small intestine

Group	VL	VD	CD
Jejunum	410,14±5.1[a]	110,35±1.9[c]	240,53±2.1[b]
Ileum	385,22±3.2[a]	136.41±2.5[c]	280.2±3.9[b]

VL represents villi length, VD represents villi diameter, and CD represents crypt depth. These measurements were reported as the mean value ± standard deviation (X±SX). The means within the same column, denoted by distinct superscripts, exhibit substantial differences.

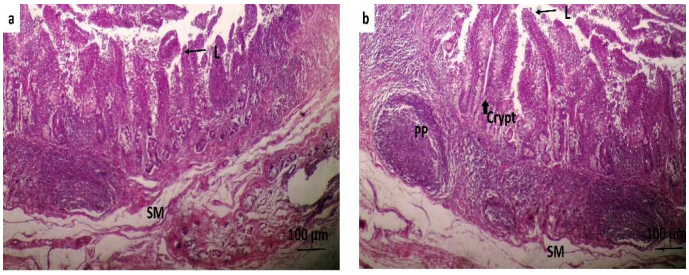

Figure 1: Histological stain: (a) Jejunum, (b) İleum: L: lumen, SM: submucosa, arrow: villus intestinalis, PP: Peyer patches, arrowhead: crypt. Mallory's trichrome method X 20.

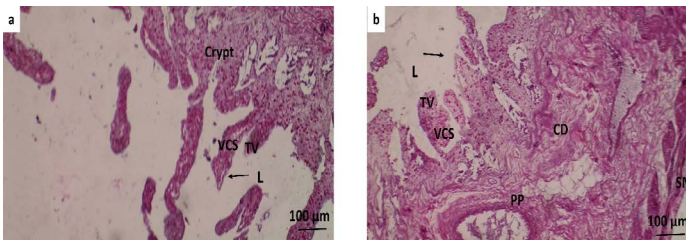

Figure 2: Histological stain: (a) Jejunum, (b) Ileum: TV: Tip of villi, CD: Crypt depth, VCS: Villus-crypt space. PAS stain X 20.

Histochemistry

The gut epithelium comprises three distinct regions: the crypt base, the crypt space between the villi, and the intervillus area. The results of the histochemical staining reactions are presented in Table 2. Goblet cells situated at the apex of the villi exhibited a notable presence of strong PAS-positive, as depicted in Figure 2. Examining sections stained with PAS/AB (pH 2.5) demonstrated distinct color variations in goblet cells at different locations inside the villi. Specifically, goblet cells were pink at the tip of the villi, purple at the villus-crypt gap, and blue at the crypt base, as illustrated in Figure 3. The epithelial cells of the small intestine did not contain glycogen. Goblet cells had significant neutral and carboxylic acidic mucopolysaccharides, although sulfated mucopolysaccharides were detected in deficient concentrations. According to the findings presented in Table 2, it is evident that the concentration of acidic mucopolysaccharides exhibited their maximum lev-

Table 2: Characteristics for histochemical describing in camel small intestine jejunum and ileum goblet cells.

Group	Tip of villi		Villus-crypt space		Crypt base	
	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum
PAS	2	1	2	2	3	2
PAS/ AB (pH 2.5)	2 (R)	2 (R)	2 (P)	3 (P)	3 (R/B)	3 (P/B)
Crossman	1	2	2	3	2	4

The staining intensity is denoted by a numerical scale, where 4 represents a very high intensity, 3 represents a vigorous intensity, 2 signifies a moderate intensity, 1 represents a weak intensity, and 0 denotes a negative intensity. In this context, R represents the color red, B represents the color blue, and P represents the color purple.

-els within the crypts. Conversely, the concentration of neutral mucopolysaccharides peaked near the tips of the villi.

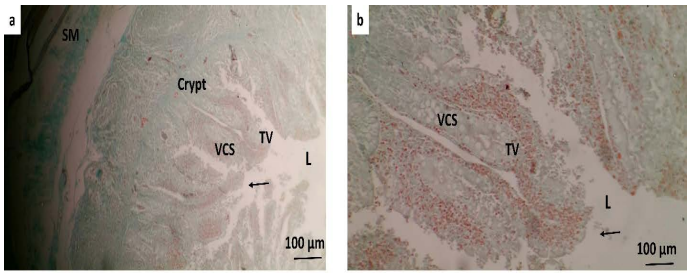

Figure 3: Histological stain: (a) Jejunum, (b) Ileum. PAS/ AB stain X 20.

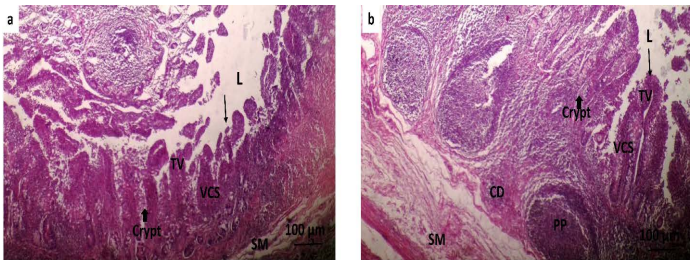

Figure 4: Histological stain: (a) Jejunum, (b) Ileum. Crossman stain X 20.

DISCUSSION

This study examined the carbohydrate composition of goblet cells and the structure of the mucosa in the jejunum and ileum of the dromedary camel. Crossmon's adaption of Mallory's trichrome technique enabled the histometric investigation of the intestinal mucosa. By examining the cross sections, we may make informed estimations regarding the composition of the mucosal structure, the dimensions of the villi (length and diameter), and the depth of the crypts. The small intestine is responsible for various processes, such as digestion and the assimilation of nutrients and water.

Enzymes present in the small intestine facilitate the process of food decomposition into its constituent parts (Sensoy ,2021).Prior studies on the camel's small intestine have revealed four distinct layers: the mucosa, the submucosa, the muscularis, and the serosa. The lamina mucosa constituted a component of the tunica mucosa, among two other layers. A simple columnar epithelium enveloped it ((Junqueira and Mescher ,2013 and Suhaib *et al.*,2023) Villi are elongated projections that line the inner membrane of the small intestine and enhance the surface area to absorb nutrients from food (El-Bahr,2017).The presence of villi in the small intestine enhances the probability of a food particle encountering a digestive enzyme and being absorbed through the epithelium into the bloodstream (Denbow,2015) Secretory epithelial cells produce crypts. The length of the villi in the jejunum was substantially greater than that of the villi in both the jejunum and the ileum. The ileum exhibited the largest villus diameter, whereas the jejunum possessed the deepest crypts. The findings indicated that the jejunum served as the main location for absorption.

The mucous gel layer in the intestine is vital in providing structural support, aiding the passage of luminal material, and preventing the mucosa from drying (Taherali *et al.*, 2018).The enterocyte brush boundary of duodenal lambs contains neutral mucosubstances and carboxyl-rich, non-sulfated glycoconjugates. On the other hand, sulphomucin is absent in mature sheep's duodenal glands and goblet cells 18. The cattle's duodenum, jejunum, and ileum exhibit more goblet cells that produce acidic mucin than goblet cells that produce neutral mucin 19. Acidic mucins exhibit comparable efficacy to sulfated mucins in inhibiting the transmission of pathogens (Machado-Neto *et al.*, 2013).

They exhibit greater resistance to breakdown by proteases produced by the host and glycosidases produced by bacteria. The stomach mucosa secretes more neutral mucins than the intestinal epithelium, which secretes acidic mucins (Deplancke and Gaskinsm 2001). The current study revealed a decrease in the concentration of acidic mucosubstances as one moves from the jejunum to the ileum. The small intestine of camels was discovered to possess the most elevated concentration of carboxylic acidic mucosubstances. The findings of the present investigation suggest that the jejunum, where gastric contents are transferred to the intestines, possesses a stronger immune response against microorganisms due to the bactericidal effects of

acidic mucosubstances. The concentration of neutral mucosubstances was higher in the ileum, whereas glycogen and sulfated acidic mucosubstances were reduced in intestinal goblet cells.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

NOVELTY STATEMENT

The findings of the present investigation suggest that the jejunum, where gastric contents are transferred to the intestines, possesses a stronger immune response against microorganisms due to the bactericidal effects of acidic mucosubstances.

AUTHORS CONTRIBUTION

All authors contributed equally.

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