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Page 142 - 149

Histological and Histochemical Study of Jejunum of Adult Male Camels

Camelus dromedarius

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Abstract

Background: The study included histological and Histochemical description of middle part of small intestine, the jejunum of adult male camels and because less researches about it. The camels are important animals at economic and environmental point of view side and less the researches and studies. *Objective*: The current study focuses on the applied importance of the digestive tract of camels especially the jejunum part of small intestine and it shows how eating habits and the environment influence. *Methodology*: 12 sample of jejunum were collected from healthy male camels, collected from the Sha'la massacre and the al-Dewar massacre in Baghdad city. Weights of adult male camels Range about 400-450 k.g. it's age about 24-36 monthly. The specimens were putted inside plastics keepers contain 10% Neutral Buffer Formalin (NBF) for 72 hrs. for fixation. The standard histological techniques were used for tissue sections (5µm) and later the sections were stained using the routine and special stain Hematoxylin and Eosin (H&E), Masson's trichome, Periodic Acid- Schiff (PAS), and examined via a light microscope. *Results:* the study included routine stain and it's represented by Hematoxylin and Eosin for general structure of jejunum, The present study discovered villi and structure, were extended from mucosa toward the out for increase the distance of surface of digestion and so appeared crypts of Lieberkühn within lamina propria, with distribution of goblet cells on villi surface. The jejunum contained aggregation of lymphatic nodules named Peyer's patches began from lamina propria to end submucosa. The special stain showed collagen fibers within submucosa and appeared adipose tissue in addition to inner layer of muscle fibers bundles and outer muscle bundles with presented the serosa. As well as positive reaction of goblet cells during staining of slides by Periodic Acid- Schiff. *Conclusions*: The study showed amount connective and adipose tissue which appeared by Masson's trichrome within tunica submucosa which it's range important evidences for supported and energy sources of the camels.

Key words: Histological, Special stain, Jejunum, Environment, Camels.

الملخص

خلفية البحث : شملت الدراسة الفحص النسيجي والكيميائي النسيجي للجزء الأوسط من الأمعاء الدقيقة، وهو ما يُعرف بالصائم عند ذكور الإبل البالغة. الهدف: تركز هذه الدراسة بشكل خاص على أهمية الجهاز الهضمي للإبل، وتحديدًا الصائم (جزء من الأمعاء الدقيقة)، وتوضح كيف تؤثر العادات الغذائية والبيئة على ذلك. (المنهجية) طريقة العمل : سيتم جمع حوالي 12 عينة (من الصائم) من الإبل الغير المصابة بمجزرة الشعلة ومجزرة الدوار في مدينة بغداد. يتراوح وزن الإبل للذكور البالغة بين 400 و450 كيلوغرامًا، وتتراوح أعمارها بين 24 و36 شهرًا. وُضعت العينات في حافظات بلاستيكية تحتوي على 10% من الفورمالين المختبري لمدة 72 ساعة للتثبيت. استُخدمت التقنيات النسيجية القياسية لتحضير مقاطع الأنسجة (5 ميكرومتر)، ثم صُبغت العينات باستخدام الصبغات الروتينية وصبغة ماسون، وصبغة حمض البيروديك-شيف لفحصها المجهر الضوئي. النتائج: شملت الدراسة التلوين الروتيني، و في صبغة الهيماتوكسيلين والإيوسين، لإظهار البنية العامة للصائم. وقد كشفت الدراسة عن بنية الزغابات، الممتدة من الغشاء المخاطي نحو الخارج لزيادة مساحة سطح الهضم، وظهور خبايا ليبركون ضمن الصفيحة المخصوصة، مع تفاعل سلبي للخلايا الكأسية على سطح الزغابات. احتوى الصائم على تجمعات من العُقيدات اللمفاوية تُسمى بقع باير، تمتد من الصفيحة الخاصة إلى نهاية الطبقة تحت المخاطية. أظهر التلوين الخاص ألياف الكولاجين ضمن الطبقة تحت المخاطية، بالإضافة إلى وجود نسيج دهني، وطبقة داخلية من حزم الألياف العضلية، وحزم عضلية خارجية مع الطبقة المصلية. كما لوحظ تفاعل إيجابي للخلايا الكأسية أثناء تلوين الشرائح بحمض بيروديك-شيف. الاستنتاجات: أظهرت الدراسة وجود كمية من النسيج الضام والدهني، والتي ظهرت عند تلوينها بصبغة ماسون ثلاثية الألوان، ضمن الطبقة تحت المخاطية، مما يُعد دليلاً هاماً على مصادر الطاقة والغذاء لدى الإبل

الكلمات المفتاحية: الأنسجة، التلوين الخاص، الصائم، البيئة، الإبل.

Introduction

The camels can live in a desert and adaptation with difficult climate and high temperature, so arid environments. The camels ate the diet consists mainly of dry food, thorny plants, so the camels were resisted limited poor water [1]. Camels considerable from pseudo-ruminant, Morphology of small intestine characteristics a narrow and long so that reflected on adapted with the harshness of life, the jejunum acerated on the water [2]. Histology, the camels included long villi to help increase the surface of

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 142 - 149

absorption which it's extended finger prominences appeared on tunica mucosa with distribution the goblet cells, generally the small intestine it's have simple columnar epithelia with lymphoid tissue within lamina propria [3].The small intestine of camels are different at other animals, the buffalo arranged in long coiled loops so that increase the absorptive surface area according to nature of food diet which different on camels and adaptation to allow efficient digestion of coarse fibrous diets [4]. Camels recognized long villi with deep crypts and aggregation of lymphatic tissue represent Peyer's patches through bowel, it's play immune role [5]. Goblet cells are located on villi and secretory units of intestinal glands will become a rich neutral mucin and responsible on the protection of mucosal layer from strict of food during eating and work on lubricant and prevent the damage [6,7]. The periodic acid-Schiff (PAS) responsible on neutral glycoproteins which can be identified by staining the histological sections by this stain, it's appeared positive reaction with PAS stain carry magenta color [8]. Whereas appeared acidic mucins are more resistant to degradation by infection factors for examples the bacterial and carried host. Show higher viscosity and acidity when we compared acidity with neutral mucins [9]. In present study aimed to light focus application of the histological and histochemical features of jejunum in the one-humped camel were verified.

Methodology:

Collection of the samples

12 specimens (2cm) of jejunum were collected from healthy male camels collected for current study to show the general structure of the jejunum and additional to specific features. These samples were collected from the of Sha'la Slaughterhouse and the al-Dewar Slaughterhouse in Baghdad city. Weights of adult male camels were about range **400-450 k.g**., it's age was about **24-36** monthly.

Tissue fixation and histological processing

The specimens were immersed inside plastic the cans for fixation by 10% Neutral buffer formalin (NBF). For bout (**72 hrs).** After fixation, the specimens of jejunum were prepared for histological study. Before fixative, the samples were gently cleaning by flushed with 0.9% sodium chloride (normal saline) to get rid the intestinal contents. Then directly placed in a suitable volume of fixing fluid. The specimens were cutting to pieces in middle part by a sharp scalpel transversally. [10] After fixation about three days, these specimens were washed with running tap water for (**2 to 3 hrs**.) to prepared for histological techniques, and after dehydrated by a series of graded alcohol (**70%, 80%, 90%, 95% and 100%)** for two hours for each concentration, then clearing in xylene were used to take about (**2hrs**.). and then embedding in molten paraffin wax to prepare the blocked specimens, the sections were cutting by rotary microtome machine to (**5µ**) thickness. These sections of paraffin were prepared for work the slides. Then the slides were stained by routine **Hematoxylin and Eosin (H&E)** to display the general histological structure, **Masson's trichrome** for collagen fibers and **Periodic Acid- Schiff** (**PAS**) for appeared reaction of mucin of these the glands.[11].

Results and Discussions

The present study included the histological structure of jejunal wall of one hump camels. Where it's presented light microscope by using of staining Hematoxylin and Eosin (H&E). The jejunum consisted of first layer is called tunica mucosa, included epithelia, lamina propria and muscularis mucosa **(fig. 1a,b).** The study showed simple columnar epithelia with appeared the finger protrudes is called the villi. They participated on increase of surface area of absorption (**fig.1c**). Either lamina propria was showed large aggregation of lymphatic tissues

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 142 - 149

which associated with jejunal wall, it's knew Peyer patches **(fig.1a)**, this structure found within lamina propria and submucosa of intestine, these lymphoid nodules, it's the important part of enteroimmuno system, Usually, Peyer's patches more common found within ileum, but this study was observed within jejunal wall. Aggregations were more developmental relatively because the camels were adapted with deserted environment and continuous exposure of infectious factors in addition to dusts and thorny plants. Lamina propria layer included crypts of Lieberkühn (intestinal glands), it's located in connective tissue of lamina and these glands were simple tubular, it's extended from surface to depth of lamina (**fig.1b).** Prior researches, which it's have on histological structures of the camel's small intestine which included four distinct layers: the mucosa which contained (epithelia, lamina propria and muscularis), the submucosa was connective tissues, the muscularis, and the serosa. The epithelia was seem a simple columnar, which it's was included lamina mucosa constituted a component of the tunica mucosa, which enveloped by simple columnar epithelia it [12,13]. Villi characteristics elongated fingers of the projections, it's extended from line the mucous membrane of the small intestine, and play important a role to accelerant the surface area to absorb key elements at food [14]. The villi presences in the small intestine effects from exposure the possibility of a food materials encountering a digestive enzyme and being absorbed across through epithelium into the blood [15]. [16], showed camel's mucosa through small intestine lined by simple columnar epithelia for absorption of food particles with distributed goblet cells on it. There are many relatively with bowels -associated lymphoid tissue (GALT), these included isolated or aggregation of lymphoid tissues constituent forming Peyer's patches. Nodular lymphatic aggregations can ability to response immune sensory through the gut. And play main a role in resistant luminal antigens and bacteria. Peyer's Patches (PPs) can be induction of immune tolerance or defense against pathogens [17]. The camels recognized long villi with deep crypts and aggregation of lymphatic tissue represent Peyer's patches through bowel, it's play immune role [5]. Camel's villi would begin decrease towards the ileum [19]. The jejunum is a distinguished portion of mesenteric canals characteristics presence of goblets cells, villi, simple tubular glands, and aggregation of Payer's patches, within histological structures which exposure to modification gradually. It's same to dedendum in histological structure high concentration goblet cells.[20]. The small intestine characteristics appeared lymphoid follicles in intestinal mucosa called Peyer's patches [21].The generally The epithelium of the small intestine is represented by villi, it's evaginations by tiny crypts which ending large structures called villi, that invaginations lead to crypts of Lieberkühn . these glands contained many secretory cells for example; paneth cells at their bases, enterocytes (columnar absorptive cells), goblet cells, enteroendocrine cells also the stem cells [22]. [23] which said most mammalian, the small intestine consist of epithelium rested on basement membrane on-epithelial stromal tissue. Our study appeared statistics parameters for histological structures of jejunal wall in Tab.1. The study showed the dimensional parameters, where recorded epithelia height about (24.15±0.75µm), villus length was (603.24±6.60µm), depth of crypts (98.8±2.89µm), ratio /length villi to depth of crypt recorded 6.10±2.40 µm , Thickness of Lamina propria and muscularis /tunica mucosa was 198.49±2.28µm, Thickness of submucosa tunica was (120.14±1.09 µm), Thickness of tunica muscularis, about recorded (178.84±1.36 µm), Thickness of tunica serosa was (21.09±0.63 µm). The study showed digestive capacity by ratio length of villi to crypts

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 142 - 149

increase more 6 times from villi length with crypt depth.

Table (1): Micromorphometric measurements of jejunal wall in adult Camels

Epithelium height (µm) (Mean±SE)	Villus length (µm) (Mean±SE)	Depth of crypts (µm) (Mean±SE)	Ratio /length villi to depth of crypt (µm) (Mean±SE)	Thickness of Lamina propria and muscularis /tunica mucosa (µm) (Mean±SE)	Thickness of tunica submucosa (µm) (Mean±SE)	Thickness of tunica muscularis (Mean±SE)	Thickness of tunica serosa (Mean±SE)
24.15±0.75	603.24±6.60	98.8±2.89	6.10±2.40	198.49±2.28	120.14±1.09	178.84±1.36	21.09±0.63

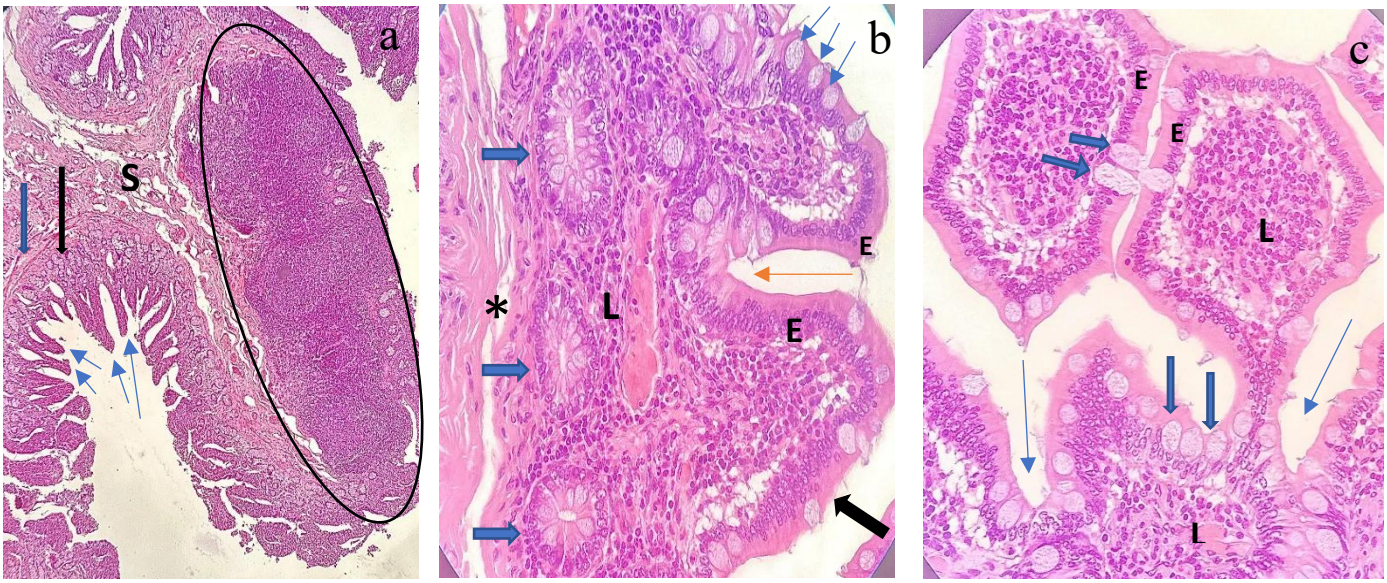

*Fig.1a. Microphotograph shows the wall of jejunum of adult camels. It shows villi (**blue arrow**), lamina propria (**black arrow**), mucosa muscularis (**bold blue arrow**), submucosa (**S**) and Aggregation of Peyer's patches (**circle**). **X40 H&E**, **b:** Microphotograph shows the wall of jejunum of adult camels. It showed villi (**bold black arrow**). It shows simple columnar epithelia (**E**), distribution of goblet cells (**blue arrow**) lamina propria (**L**) and crypt (**orange arrow**), crypts of Lieberkühn (**bold blue arrow**), submucosa (**black star**) .X 100 H&E. c: Microphotograph shows the wall of jejunum of adult camels. It showed villi (**bold black arrow**). It shows simple columnar epithelia (**E**), distribution of goblet cells (**bold blue arrow**) lamina propria (**L**) and crypt (**blue arrow**), X 100 H&E*

The light microscope showed through special stain, appearance of collagen fibers in submucosa layer by Masson's trichrome stain (**fig.2a**). In addition to, the study observed amounts of adipose tissues in same tunica because physiological state of the camels to

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 142 - 149

adapted with deserted environments and which less food and water therefore the adipose tissues were placed to store lipids in different structure from the body and don't only hump of camels (**fig.2a).** the present study showed goblets cells on epithelial surfaces by staining by periodic acid-Schiff (PAS) in (**fig.2b,c)** , where appeared positive reaction for mucin carried magenta color. The previous studies showed the goblet cells are located on villi and secretory units of intestinal glands will become a rich neutral mucin and responsible on the protection of mucosal layer from strict of food during eating and work on lubricant and prevent the damage[6,7]. The periodic acid-Schiff (PAS) responsible on neutral glycoproteins which can be identified by staining the histological sections by this stain, it's appeared positive reaction with PAS stain carry magenta color [8]. Whereas appeared Acidic mucins are more resistant to degradation by infection factors for examples the bacterial and carried host, it's show higher viscosity and acidity when we compared acidity with neutral mucins [9].Goblet cells is located through three segment of bowel on surface of epithelia which are work for the production mucous. Mucin is included glucoproteins and protective function maintaining on villi shape. So the mucin help on lubricantand transport the materials between the contents in the lumen and the epithelial cells [24].The mucin appeared either acidic or neutral and different gradually between mammalians,[25,26]. And mucus is different thick degree when compare with large bowel (colon), [27]. The mucous is secreted from upper portion of goblet cells [28]. The classification of carbohydrate through histochemical techniques staining observed positive reaction of the goblet cells with PAS and appeared that a neutral carbohydrate. which take magenta color [29]. Special stain represented through PAS stain reaction with oxidation of the glycol linkages in polysaccharides by periodic acid which give goblets cells shape take magenta color [30,31].

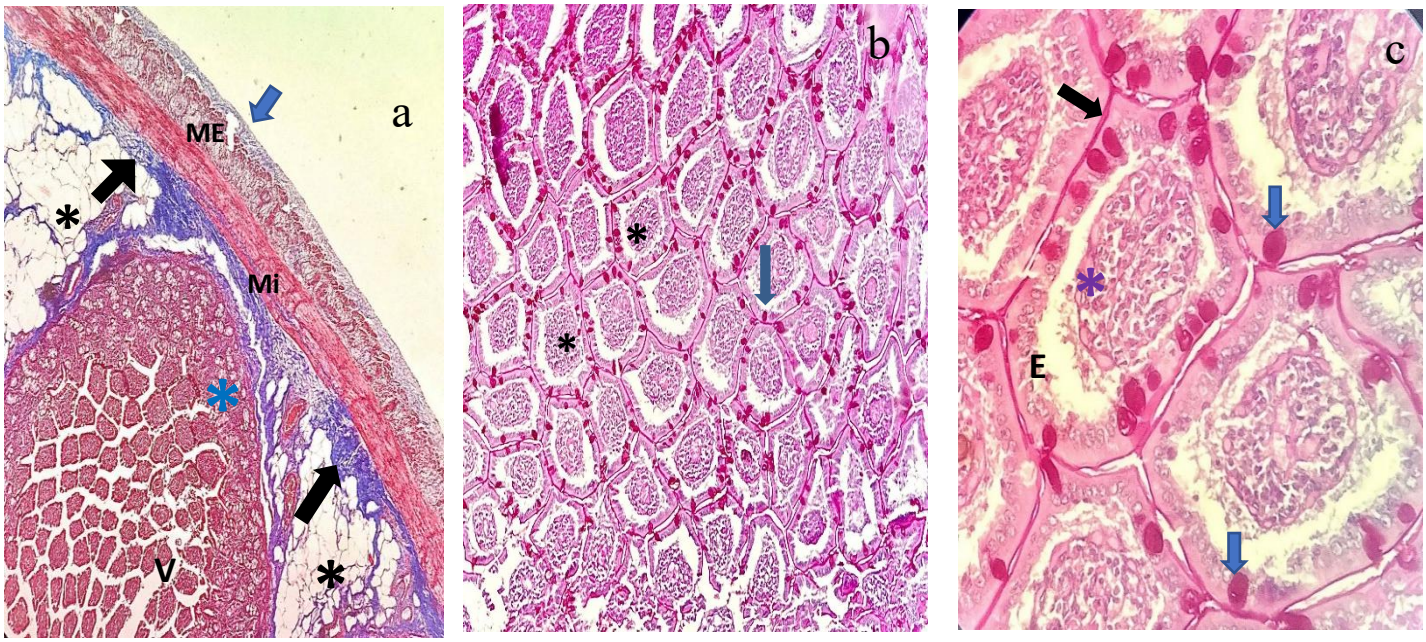

Fig.2a*. Microphotograph shows the wall of jejunum of adult camels. It showed villi of tunica mucosa. It shows lamina propria* ***(Lp)*** *included crypts of Lieberkühn (blue star) , submucosa represent collagen fibers* ***(bold black arrow),*** *adipose tissue* ***(black start)*** *tunica muscularis showed inner muscles bundles*

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 142 - 149

*(Mi) , outer muscle bundles **(Me)** and Serosa (bold blue arrow) **. X40Masson's trichrome. b:** Microphotograph shows the wall of jejunum of adult camels. It showed villi. It shows simple columnar epithelia **(E)**, Basement membrane positive reaction, villi, distribution of positive reaction of goblets cells **(bold blue arrow),** lamina propria **(black star) . X100 PAS. c;** Microphotograph shows the wall of jejunum of adult camels. It showed tunica mucosa. It shows simple columnar epithelia **(E)**, Basement membrane positive reaction **(bold black arrow)**, villi, distribution of positive reaction of goblets cells **(bold blue arrow),** lamina propria **(black star). X400 PA***

Conclusions

The light microscope showed amount connective and adipose tissue which appeared by Masson's trichrome within tunica submucosa which it's important evidences for supported and energy sources of the camels. Camels were different a rest another mammalian because nature of deserted climate and dry diet lead to animals to the adaptation.

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Al-Shatrah Veterinary and Biological Sciences Journal
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Volume 2 , Issue 3 , June 2026
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Al-Shatrah Veterinary and Biological Sciences Journal
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