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ESTUDO COMPARATIVO DAS ESTRUTURAS HISTOMORFOLÓGICAS E HISTOQUÍMICAS NO ESÔFAGO DE OVELHAS (*Ovis aries*) E COELHOS (*Oryctolagus Cuniculus*)

COMPARATIVE HISTOCHEMICAL AND HISTOMORPHOMETRICAL STUDY OF ESOPHAGUS STRUCTURES IN SHEEP (*Ovis aries*) AND RABBITS (*Oryctolagus Cuniculus*)

دراسة مقارنه كيمياء نسيجيه وقياسات نسيجية لتركيب المريء في الاغنام (*Ovis aries*) والارانب (*Oryctolagus Cuniculus*)

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RESUMO

A evolução entre os animais provoca muitas mudanças para que eles possam se adaptar ao seu ambiente. Cada espécie possui características únicas que os ajudam a sobreviver e podem consumir diferentes tipos de alimentos. Ovelhas e coelhos são animais economicamente importantes e usados em muitos aspectos da medicina veterinária. Este estudo teve como objetivo comparar as características histomorfométricas e histoquímicas do esôfago de vinte machos adultos de Ovelhas (n = 10) e Coelhos (n = 10). As amostras foram coletadas no abatedouro e mercado de Misan e foram usadas para estudos histológicos de dois tipos de coloração, hematoxilina e eosina, e colorações especiais (coloração de ácido periódico + reativo de Schiff). O estudo histológico mostrou diferenças no tipo de epitélio da mucosa que reveste o esôfago entre ovelhas e coelhos. O revestimento do epitélio era composto por um epitélio escamoso estratificado queratinizado em ovelhas, enquanto no coelho era composto por um epitélio escamoso estratificado não-queratinizado. Em ambos os animais, a camada submucosa não possui glândulas. A camada muscular de ambos era composta por músculo estriado nas partes cervical, torácica e abdômen do esôfago. Ambos os animais continham camada externa de tecido conjuntivo frouxo chamada adventícia. Todas as camadas em ovelhas mostraram mais espessura do que em coelhos. O estudo histoquímico mostrou que a reação à coloração de Schiff com ácido periódico foi semelhante entre os animais e em locais diferentes. Apenas células do estrato córneo de ovelhas da mucosa e células escamosas da mucosa de coelho demonstraram forte reação a essa coloração. Em contraste, o resto das células da mucosa e das camadas musculares foram reações moderadas com a coloração de Schiff com ácido periódico em todas as regiões do esôfago de ovelhas e coelhos. A submucosa e a adventícia mostraram reação fraca com a coloração de Schiff com ácido periódico em ambos os animais. Em conclusão, este estudo mostrou que ovelhas e coelhos apresentam semelhanças e diferenças no esôfago, ou seja, as camadas deste órgão têm espessuras diferentes e respondem de forma diferente à coloração de Schiff com ácido periódico.

Palavras-chave: *Esôfago; Coelho; Ovelhas; Histomorfologia; Histoquímica.*

ABSTRACT

Evolution between animals causes many changes so that it can adapt to its environments. Each species has unique features that help them survive and can consume different types of food. Sheep and rabbits are economically important animals and used in many aspects of veterinary medicine.This study aimed to compare the histomorphometric and histochemical features of the esophagus of twenty sheep (n=10) and rabbit (n=10) adult males. The samples were collected from slaughterhouse and market Misan and were used for histological studies of two types of stains, hematoxylin and eosin, and special stains (Periodic acid Schiff stains). Histological study showed differences in the type epithelium of mucosa lining the esophagus between sheep and rabbits. The epithelium lining was composed of a keratinized stratified squamous epithelium in sheep while in rabbit was composed of a non-keratinized stratified squamous. In both animals, the submucosa layer does not possess glands. The muscular layer of both was composed of striated muscle in the cervical, thoracic, and abdomen parts of the esophagus. Both animals were containing an outer layer of loose connective tissue called the adventitia. All layers in sheep showed more thickness than in rabbits. The histochemical study showed that the reaction to Periodic acid Schiff stain was similar between the animals and in different places. Only stratum corneum cells of the sheep mucosa and squamous cells of the rabbit mucosa demonstrated a strong reaction to this stain. In contrast, the rest of the cells of the mucosa and muscular layers were moderate reactions with Periodic acid Schiff stain in all regions sheep and rabbit esophagus. Submucosa and adventitia showed weakly reaction with Periodic acid Schiff's stain in both animals. In conclusion, this study showed that sheep and rabbits have similarities and differences in the esophagus; that is, the layers of this organ has different thicknesses and respond differently to Periodic acid Schiff stain.

Keywords: *Esophagus; Rabbit; Sheep; Histomorphology; Histochemical.*

الملخص

يسبب التطور بين الحيوانات العديد من التغييرات حتى تتمكن من التكيف مع بيئاتها. لكل نوع ميزات فريدة تساعده على البقاء ويستهلك أنواعًا مختلفة من الطعام. تعتبر الأغنام والأرانب حيوانات مهمة اقتصاديًا وتستخدم في العديد من جوانب الطب البيطري. هدفت هذه الدراسة إلى مقارنة الخصائص القياسية النسيجية والكيمياء النسيجية لمريء عشرين حيوان الاغنام (العدد = 10) والأرانب (العدد = 10) ذكور بالغين. جمعت العينات من مسلخ والأسواق ميسان واستخدمت في الدراسات النسيجية نوعين من الصبغات، الهيماتوكسيلين والأيوزين، والصبغة الخاصة (صبغه حمض شيف الدورية). أظهرت الدراسة النسيجية وجود اختلافات في نوع ظهارة الغشاء المخاطي المبطنة للمريء بين الأغنام والأرانب. تتكون بطانة الظهارة من ظهارة حرشفية طبقية مقترنة في الأغنام بينما في الأرانب تتكون من طبقة حرشفية طبقية غير مقترنة. في كلا الحيوانين، لا تمتلك الطبقة تحت المخاطية غددًا. تتكون الطبقة العضلية لكليهما من عضلات مخططة في لأجزاء العنقية والصدرية والبطنية من المريء. كان كلا الحيوانين يحتويان على طبقة خارجية من النسيج الضام الرخو تسمى البرانية. أظهرت جميع طبقات الأغنام سماكة أكبر من تلك الموجودة في الأرانب. أظهرت الدراسة الكيميائية النسيجية أن التفاعل مع صبغة دورية حامض شيف كان متشابهًا بين الحيوانات وفي أماكن مختلفة. أظهرت خلايا الطبقة القرنية فقط من الغشاء المخاطي للأغنام والخلايا الحرشفية من الغشاء المخاطي للأرنب تفاعلًا قويًا لهذه الصبغة. في المقابل، كانت بقية خلايا الغشاء المخاطي والطبقات العضلية تفاعلات معتدلة مع صبغة حمض شيف الدورية في جميع مناطق مريء الأغنام والأرانب. أظهرت الطبقة تحت المخاطية والبرانية تفاعلًا ضعيفًا مع صبغه حمض شيف الدورية في كلا الحيوانين. في الختام، أظهرت هذه الدراسة أن الأغنام والأرانب لها أوجه تشابه واختلاف في المريء؛ أي أن طبقات هذا العضو لها سمك مختلف وتستجيب بشكل مختلف لصبغة شيف الدورية.

الكلمات المفتاحية: المريء، الأرنب، الاغنام، القياسات النسيجية، الكيمياء النسيجية.

1. INTRODUCTION:

Evolution between animals causes many changes so that it can adapt to its environments. Each species has unique characters that help them survive and can consume different types of food. Rabbits are considered economically significant animals as they have the advantage of their meat and furring, are used as pets, and they are substantial at scientific and medical experiences (Hristov *et al*., 2006).

Sheep, on the other hand, have been able to use lignocellulosic materials and convert them to animal products of high nutritional value, such as meat, milk, wool/fur, hide, and manure. In the same vein, the intestine of sheep may be used to make catguts, which are still used for internal human surgical sutures and strings for musical instruments (Agrawal *et al*., 2014).

They are herbivorous mammals, but rumen and hindgut represent two different fermentation organs (Mi *et al*., 2018). They depend on a symbiotic relationship with a community of microbes, primarily bacteria with fibrinolytic ability in either their foregut (which the rumen of ruminants and the pseudo-ruminants) or their hindgut (which the cecum and colon of non-ruminant herbivores), for fiber digestion (Crowley *et al*., 2017; Furness *et al*., 2015; Kingston-Smith *et al.,* 2013).

Besides, there are phenotypic and dramatic physiological differences fund between ruminant and non-ruminant mammalian species. For example, volatile fatty acids produced as by-products of the microbial fermentation in the rumen are used as the primary source of energy in ruminants, as oppose to glucose absorbed from the small intestine in non-ruminants because of this of difference in nutrient usage (Bao *et al*., 2013). Furthermore, one of the most original features of the rabbit feeding behavior is the caecotrophy, which involves an excretion and immediate consumption of specific feces named soft feces or (caecotrophes). Consequently, the daily intake behavior of the rabbit is constituted of two meals, caecotrophes, and feeds (Bels, 2006).

Animals are classified into various types based on their habitats. Land Animals, such as sheep, cattle, and camel, live in homes and dairy farms. The second type of land animals is called wild animals. They are called wild because human beings do not domesticate them as a rabbit, for instance (Qureshi *et al*., 2012).

Generally, the esophagus is one of the first parts of the digestive system. In terms of function, the primary function of the esophagus is to transfer food and fluid from the oral cavity to the stomach. It is the only part of the digestive system which does not have metabolic, digest, and absorb functions. Kumar *et al*. (2009), reported that the esophagus is divided into cervical, thoracic, and abdominal. Histologically, it has four tunics layers (mucosa, submucosa, muscular, and adventitia), as seen in the digestive system (Aughey and Fry, 2001).

The mucosa layer consists of three layers; the epithelium, the lamina propria, and the muscularis mucosa. The epithelium and lamina propria are separate by the basal lamina (Hussein *et al*., 2016). However, the degree of keratinization of the esophagus depends on the animal's food (Alsafy and El-Gendy, 2012). Previously, Ahmed *et al*. (2009), observed that the epithelium of the esophagus of *Varanus niloticus* cover by ciliated columnar epithelium and mucous secreting goblet. Whereas, the submucosa is a thick layer of loose connective tissue containing collagen fibers, fibroblasts, and numerous blood vessels with large lumens (capillaries, arterioles, and venules) (Calamar *et al*., 2014).

The esophageal muscular consists of two layers of muscle; in ruminants and dogs, the entire muscular tunic consists of skeletal muscles (Eurell and Frappier, 2006). The adventitia locates at the outer layer of the cervical and thoracic region. It was composed of loose connective tissue (Cui *et al*., 2011). Gastrointestinal secretion in vertebrates contains several mucous substances that can vary according to cell type, functional status, anatomical region, pathological condition, sex, age, and species mucosubstances detect by many techniques (Choi *et al*., 2003; Schumacher *et al*., 2004). Many researchers have also been publishing about the microscopic structure for the esophagus in different species (Gupta and Sharma, 1991; Ali *et al*., 2008; Islam *et al*., 2008; Hameed *et al.*, 2018).

This study aimed to compare the histomorphometric and histochemical similarities of the esophagus of sheep (herbivorous ruminant) and rabbit (herbivorous and coprophagous).

2. MATERIALS AND METHODS:

2.1. Surgical Procedures

This study was carried out in the department of biology Sciences at the University of Misan. A total of ten sheep, adult males, were collected from local slaughterhouses. The esophageal samples were taken from different regions (the cervical, thoracic, and abdomen). A total of ten three months old, adult males rabbits weighting 1.5 - 2.5 kg were collected from Misan city. The experiments on rabbits followed the guidelines provided by the University's Animal Ethics Committee. The rabbits were raised under standard procedures and euthanized following the animal euthanization protocol. A physical examination was performed to all animals to guarantee they ere all in the right health conditions before euthanasia. The euthanizing procedures were done by placing 2 mL of chloroform ($CHCl_3$) on cotton and then set on the animal's nose, according to Blackshaw *et al*., 1988. By using appropriate tools like scissors, tweezers, and scalpels, regional gross dissection was performed of each specimen. The abdomen of the rabbits was incised, and the esophagus extracted. After, segments of 1 cm from each esophagus were taken and from different parts (cervical, thoracic, and abdominal).

2.2. Histological examination

All esophagus samples of rabbits and sheep were fixed in 10% neutral buffered formalin promptly. After fixation for 72 hours, all samples were processed with a series of ascending ethanol concentrations (70% for 2h, 80% for 30min, 96% for 2h repeat three times, and the absolute 99% for 9h) then finally put them in absolute ethanol 99% for one hour to dehydrate. Then, all samples were cleared with xylene for one hour and embedded in paraffin wax to make paraffin blocks. Finally, sections were cut at the 7-micrometer thickness and processed with two stains (Luna, 1968). Hematoxylin and Eosin and Periodic Acid-Schiff (PAS) stains were used to stain all tissue sections for histomorphometry identification and carbohydrates determinations, respectively (Luna, 1968).

2.3. Micromorphometric measurements

Ten slides were made for each part of the esophagus (cervical, thoracic and abdominal). To detect the thickness of mucosa, submucosa, muscular, and serosa, an optical microscope was employed with the exact ophthalmic scale (ocular micrometer) after the exact ophthalmic scale was matched with the theatrical scale using the magnification force (Galigher and Kozloff, 1964).

2.4. Statistical analysis

The values were expressed as mean ± SD (standard deviation). The statistical analysis of the data was performed to know the significant differences using the t-test at $P<0.05$ of probability (Al-Rawi and Khalaf Allah, 2000).

3. RESULTS AND DISCUSSION:

3.1. Histological study

In sheep and rabbits, all esophagus regions (cervical, thoracic, and abdominal) were their walls composed of four layers (Tunics): Mucosa, submucosa, muscular, and adventitia layer or serosa (Figures 1 and 2). Mucosa contains epithelium, lamina propria, and muscular mucosa; the findings showed variations in the form of mucosa epithelium lining the esophagus between sheep and rabbits. The epithelium lining consisted of a keratinized stratified squamous epithelium in sheep (Figure 3), while a non-keratinized stratified squamous epithelium was present in rabbit (Figure 4).

The epithelium layer of the ovine esophagus consisted of four cells. Stratum basale has a cuboidal or low columnar form and basophilic cytoplasm, while the last three strata (spinosum, stratum granulosum, and stratum corneum) have varied forms and are full of keratin (Figure 3). On the other hand, the epithelium of the esophagus of the rabbit is formed of three cells. Stratum basal are cuboidal or low columnar and are located in the below of the stratified epithelium; cells in the intermediate layers of the epithelium are polyhedral and surface flattened; squamous cells lack keratin. The presence of keratin in the sheep that covers the stratified squamous epithelium may also be because these animals consume raw food. The presence of keratin on the surface of the epithelium supports its protection and this agreement with the Meyer and Schnapper study (2014), who suggest that keratinization of the epithelium plays an essential role in mechanical stabilization.

Malik and his team (2018) reported that lamina epithelium in sheep esophagus consists of keratinized stratified squamous epithelium with four regions, stratum corneum, stratum granulosum, stratum spinosum, and stratum basale. Besides, Mahmood *et al*. (2017) and (Boonzaier, 2012) reported that the epithelium lining of the esophagus is the non-keratinized stratified squamous epithelium. Eroschenko (2008) stated that the non-keratinized stratified squamous epithelium layer of the esophagus consisted of three cells, squamous cells, polyhedral cells, and stratum basale. Furthermore, Ranjan and Das (2016) observed that the epithelium lining of the rabbit esophagus consists of keratinized stratified squamous epithelium. In both animals, the lamina propria were formed from loose connective tissue contain elastic and collagen fibers, fibrocytes, and blood vessel. There were many of dermal papillae that appeared as finger-like extensions. The lamina propria was identified and was thicker in sheep than the rabbit (Figures 5 and 6).

The muscular mucosa consisted of smooth muscle fiber arranged longitudinally, and it was more thickness in sheep than in the rabbit (Figures 1 and 2). The muscular mucosa was located between lamina propria and submucosa, and it was identifiable along the length of the esophagus. This finding disagrees with the study of Selim *et al*. (2017), which observed that in the lactating rabbit, the muscular mucosa layer was absent, and this difference may be due age.

In both sheep and rabbits, the submucosa layer has loose connective tissue composed interwoven collagen fiber, elastic fiber, fibrocytes, lymphocytes, and blood vessels with the presence of the adipose connective tissue in sheep denser of the rabbit. Also, no submucosal glands were observed throughout the length of the esophagus for both animals. These results are similar to the study of Hameed *et al*. (2018). According to Pawan *et al*., 2009, the presence or absence of esophageal glands was dependent on gruff feed, especially vegetable fodder (Pawan *et al*., 2009). On the other hand, this study disagrees with the results of Naghani and Andi (2012) which reported the presence of great submucosal glands throughout the length of the esophagus in a one-humped camel and this difference in results might be due type food and species. However, Gupta and Sharma (1991) detected that there is a seromucous tubuloalveolar gland in the initial portion of the esophagus in buffalo calves. Mahmood *et al*. (2017) observed glands in rabbits' esophagus. Regarding the information about the absence or presence of glands at the esophagus, the literature is contradictory and scarce. The glands are more numerous in certain animal species such as dogs and pigs and less abundant at humans (Shiina *et al*., 2005).

In both animals, the muscular layer was

composed of two layers: the outer longitudinal layer and inner circular layer. Collagen and reticular fibers separated the two muscle layers from each other. Moreover, both sheep and rabbit tunica muscular was composed of striated muscle throughout the cervical, thoracic, and abdominal region. Banks (1986) suggested that striated skeletal might allow regurgitation to chew and also allow to push any foreign body toward the rumen faster. However, complete striated muscles had been reported in buffalo calves esophagus (Gupta and Sharma,1991) and ruminants (Banks,1986). Ranjan and Das (2016) wrote that tunic muscular in all rabbit esophagus regions are formed of striated muscle, and this finding matches with the results of this study. In the abdominal area, the adventitia layer consisted of loose connective tissue (Figures 9 and 10) slowly transformed into tunica serosa, which consists of loose connective tissue and a mesothelium layer. It matches with the study of Hussein *et al.* (2016).

3.2. Histomorphometric study

The thickness of sheep mucosa in the cervical (629.91±109.97µm), thoracic (657.90±56.93µm), and abdominal (657.90±56.93 µm) sections were significantly ($p<0.05$) larger in comparison to the cervical, thoracic, and abdominal sections of the rabbits' esophagus where the values found were (289.29±110.63µm), (251.96±21.44µm) and (312.62±45.61µm), respectively (Table 1). This find might be related to the fact that the rabbit has epithelium of type non-keratinized stratified squamous epithelium.

The thickness of sheep submucosa in the cervical (891.20±269.11µm), thoracic (639.23±121.06µm), and abdominal (513.26±83.75µm) sections were significantly ($p<0.05$) larger in comparison to the same sections of the rabbits' esophagus (429.27±227.84µm), (319.62±85.22µm) and (228.63±47.68µm), respectively (Figures 11, 12 and 17). This find might be related to the physiological situation related to the blood supply, nervous and lymphatic system and the difference of species or back submucosa thickness of sheep once it has a great amount of adipose tissue.

In sheep, the thickness of the submucosa in the thoracic region was (639.32±121.06µm) (Table 1). This result agrees with Malik *et al*. (2018) which observed the thickness of submucosa in the thoracic region was (645.5 ±46.93)µm. In the same context, the thickness of the submucosa of the rabbit in the thoracic region was (319.62±85.22µm) (Table 1). This find, however, disagrees with the study of Kadhim (2019), which observed that the thick submucosa in this region of *(Herpestidae edwardsii)* was (131±17.7µm). This difference might be related to the nature of the nutrition intake of the animals.

The thickness of the muscular sheep layer in the cervical (1572.41±97.27µm), thoracic (1530.42±117.00µm), and abdominal (1250.45±255.39µm) sections were significantly ($p<0.05$) larger in comparison to the same sections of the rabbits' esophagus (552.92±59.27µm), (613.57±60.28µm) and (424.60±70.24µm), respectively (Figures 7, 8 and Table 1). This find might be related to a difference in the use of esophageal muscles; that is, to swallow food from the mouth to the top and again to return food from rumen to the mouth for rumination; and a third time to push food from the mouth to the stomach.

There were non-significant ($p>0.05$) differences between the thickness of the serosa in sheep and rabbit in the cervical and thoracic part. On the other hand, the thickness of the sheep abdominal part was significantly ($p<0.05$) larger (163.31±19.04µm) in comparison to the same section in the rabbits (65.32±18.40µm) (Table 1).

3.3. Histochemical study

The results showed that the stratum corneum cells of stratified squamous keratinized of the mucosa layer had a strong reaction with PAS (Figure 13). In contrast, the rest of the cells of the mucosa layer had a moderate response with PAS in all regions of the sheep esophagus (Figures 15, 17, and 19). In the rabbit, it showed a strong reaction with PAS of the squamous cells of the mucosa layer (Figure 14). The rest of the cells of the mucosa layer had a moderate response with PAS in all regions of the rabbit esophagus (Figures 16, 18, and 20). This find indicates that the presence of carbohydrates includes cytoplasm in these cells.

The results of this study are in agreement with Malik *et al*. (2018), which stated that the stratum corneum cells in sheep esophagus showed a strong reaction with PAS. Selim *et al*. (2017) observed that the response with PAS was a strong reaction with the inner layer of mucosa and moderate reaction with lamina propria in the esophagus rabbit. Ranjan and Das (2016) observed in rabbit esophageal that the epithelium and basement membrane performed a moderate reaction with PAS. Igbokwe and Obinna (2016) observed in rope squirrel esophagus that the mucosal layer reacted moderately with PAS. In both animals, the submucosa showed weakly reaction with PAS in each region's esophagus in the cervical (Figures 15 and 16), thoracic (Figures 17 and 18), and abdominal (Figures 19 and 20). This might be because of the absence of glands. In the same context, Nzalak *et al*. (2010) reported the esophagus of the African giant rat does not have glands in the submucosa layer. The mucous produced by the salivary glands might be helping in protecting the mucosal surface of the esophagus from sharp objects since the mucous barrier was also an essential factor in the protection of the esophagus from damage.

In sheep and rabbits, the muscular external layer reacted moderately with PAS in cervical, thoracic, and abdominal esophagus regions (Figures 19 and 20). This might be because of the presence of glycogen in the muscle-skeletal but in quantities not high. Listrat and his team (2016) detected that muscle-skeletal contain 1% of glycogen. The results of this study disagree with Selim *et al*. (2017) which observed a low reaction with PAS of the muscular layer. However, the serosa showed a weak reaction with PAS in the cervical, thoracic, and abdominal regions in both sheep and rabbits.

4. CONCLUSIONS:

In sheep and rabbits, the esophagus is composed of four layers: mucosa, submucosa, muscular, and adventitia layer or serosa. The epithelium layer of the ovine esophagus consisted of four cells, and the epithelium lining consisted of a keratinized stratified squamous epithelium. In comparison, the epithelium of the rabbit esophagus is formed of three cells, and a non-keratinized stratified squamous epithelium was present. In both animals, no submucosal glands were observed throughout the length of the esophagus. The mucosa, submucosa, and the muscular layer thickness was significantly larger for sheep in comparison to rabbits. There were non-significant differences between the thickness of serosa in sheep and rabbit. The stratum corneum cells showed a strong reaction with PAS. A strong response with PAS was also observed in the squamous cells of the mucosa layer in rabbits. The rest of the cells of the mucosa layer showed a moderate reaction with PAS in all regions, both to the rabbit and sheep esophagus.

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Table 1. *Mean thickness of mucosa, submucosa, muscularis, and adventitia in Cervical, Thoracic, and Abdominal regions of the esophagus of the sheep and rabbit.*

Thickness	Thick mucosa		Thick submucosa		Thick muscularis		Thick serosa	
Esophagus	Sheep Mean ±SD	Rabbit Mean ±SD	Sheep Mean ±SD	Rabbit Mean ±SD	Sheep Mean ±SD	Rabbit Mean ±SD	Sheep Mean ±SD	Rabbit Mean ±SD
Cervical region	629.91^{a} ± 109.97	289.29^{b} ± 110.63	891.20^{a} ± 269.11	429.27^{b} ± 227.84	1572.41^{a} ± 97.27	552.92^{b} ± 59.27	100.29^{a} ± 15.72	93.32^{a} ± 26.93
Thoracic region	657.90^{a} ± 56.93	251.96^{b} ± 21.44	639.23^{a} ± 121.06	319.62^{b} ± 85.22	1530.42^{a} ± 117.00	613.57^{b} ± 60.28	100.29^{a} ± 15.72	83.98^{a} ± 25.07
Abdominal region	657.90^{a} ± 56.93	312.62^{b} ± 45.61	513.26^{a} ± 83.75	228.63^{b} ± 47.68	1250.45^{a} ± 255.39	424.60^{b} ± 70.24	163.31^{a} ± 19.04	65.32^{b} ± 18.40

Notes: *value represent (mean± SD); *different letters refer to ($p<0.05$) significant difference between values; *the similar letters refer to non-significant ($p>0.05$) difference between values.

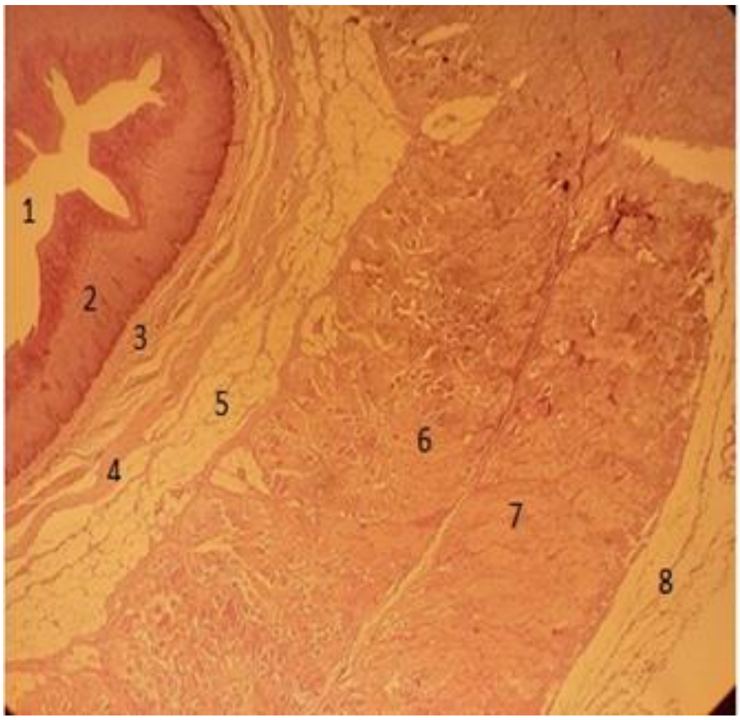

Figure 1. *The esophagus of the sheep. (1) leumin, (2) stratified squamous epithelium layer (keratinized), (3) lamina propria, (4) muscularis mucosa, (5) submucosa muscularis layer, (6) circular muscularis (7) longitudinal, (8) serosa. H&E.40X.*

Figure 2. *The esophagus of the rabbit. (1) leumin, (2) stratified squamous epithelium layer (non-keratinized), (3) lamina propria, (4) muscularis mucosa. (5) submucosa muscularis layer (6) circular muscularis (7) longitudinal, (8) serosa. H&E.40X*

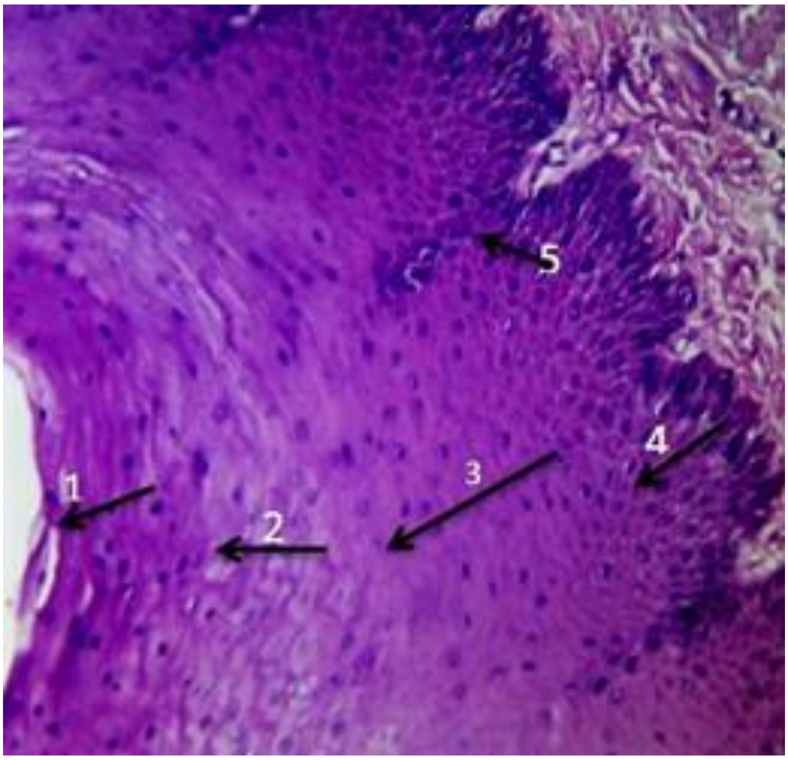

Figure 3. *The esophagus of the sheep showing a stratified squamous epithelium layer (keratinized), including four cells (1) Stratum corneum full keratin, (2) Stratum granulosum, (3) Stratum spinosum, (4) stratum basale, and the (5) dermal papillae. H&E .100X*

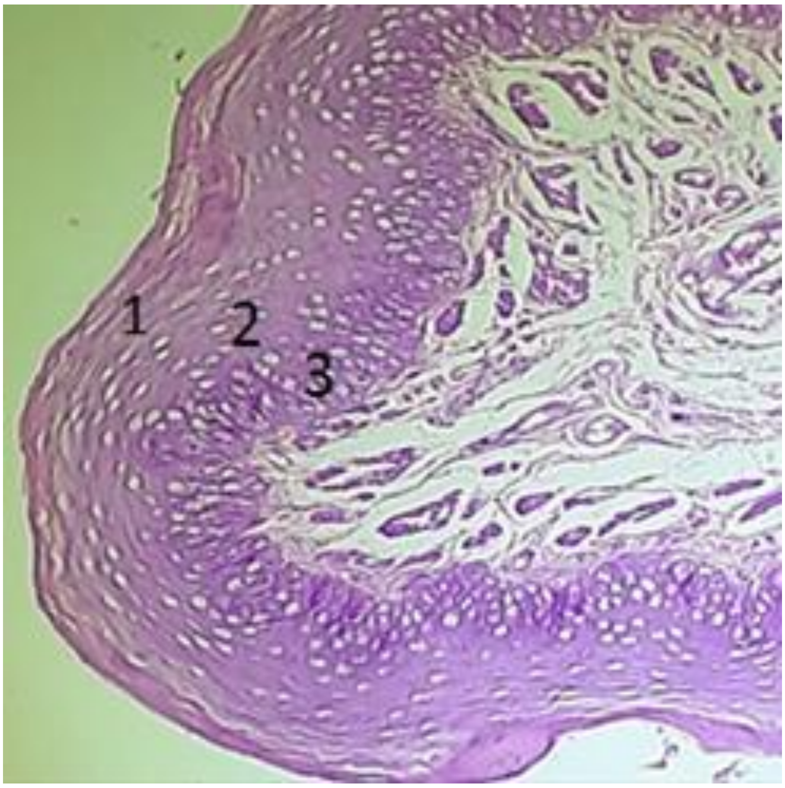

Figure 4. *The esophagus of the rabbit showing a stratified squamous epithelium layer (non-keratinized) consisting of three cells (1) Squamous cells, (2) polyhedral cells and, (3) stratum basale. H&E.100X*

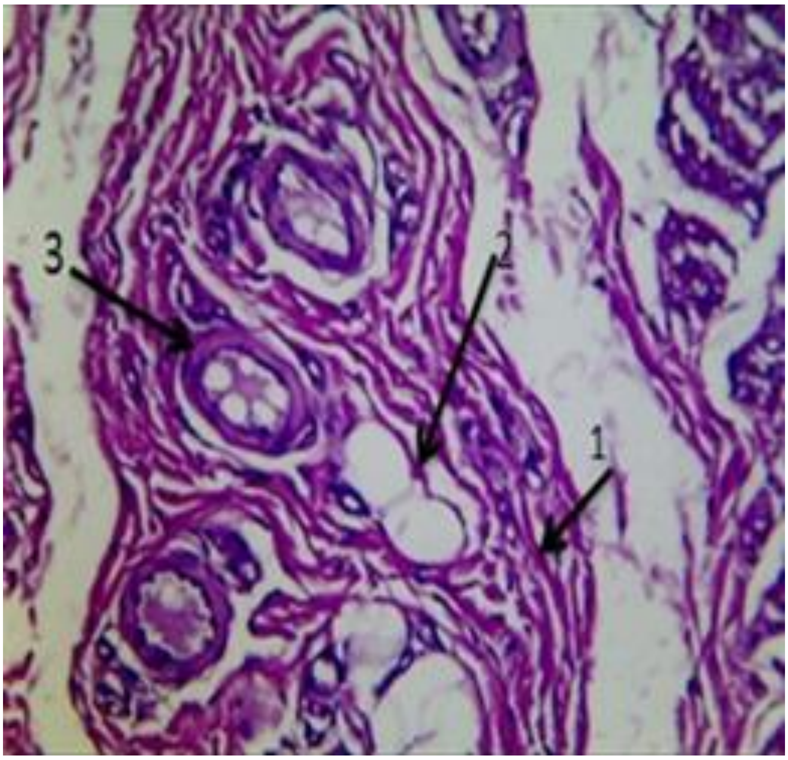

Figure 5. *The esophagus of the sheep showing lamina propria consisting of loose connective tissue contains (1) fiber, (2) fat cells and, (3) blood vessels. H&E 100X.*

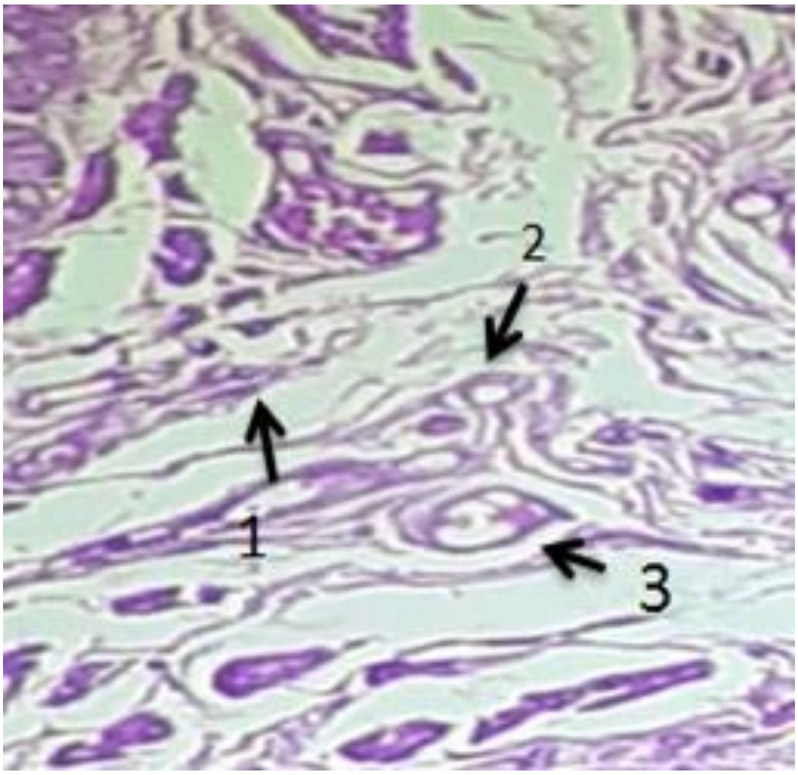

Figure 6. *The esophagus of the rabbit showing lamina propria consist of loose connective tissue containing (1) fiber, (2) fat cells and, (3) blood vessels. H&E 100X.*

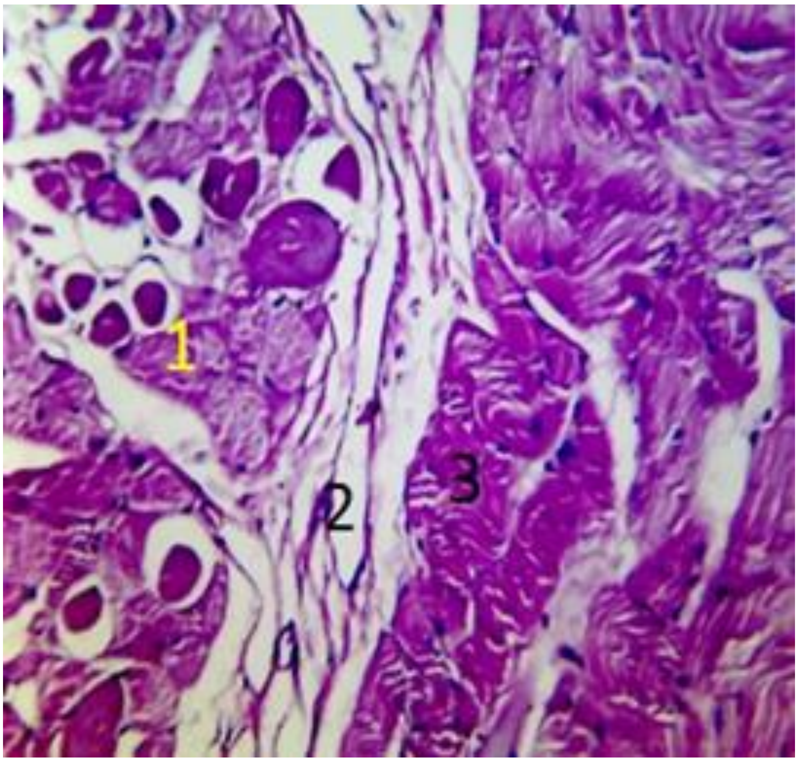

Figure 7. *The cervical esophagus region of the sheep showing (skeletal muscle) (1) circular muscularis, (2) connective tissue, and (3) longitudinal muscularis. H&E.100x*

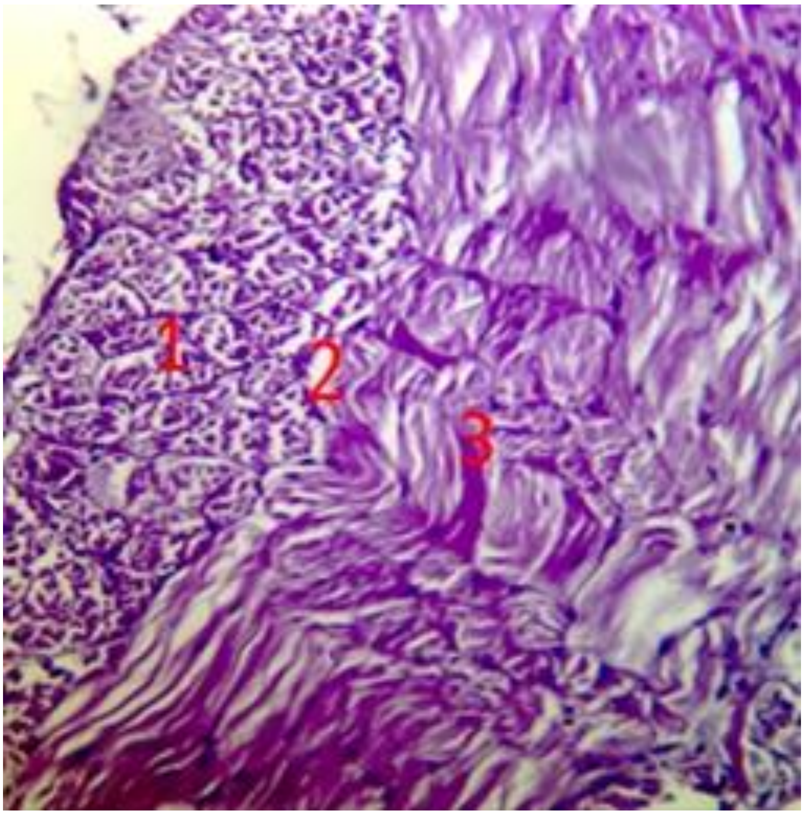

Figure 8. *The cervical esophagus region of the rabbit showing (skeletal muscle). (1) circular muscularis, (2) connective tissue and, (3) longitudinal muscularis. H&E.100x*

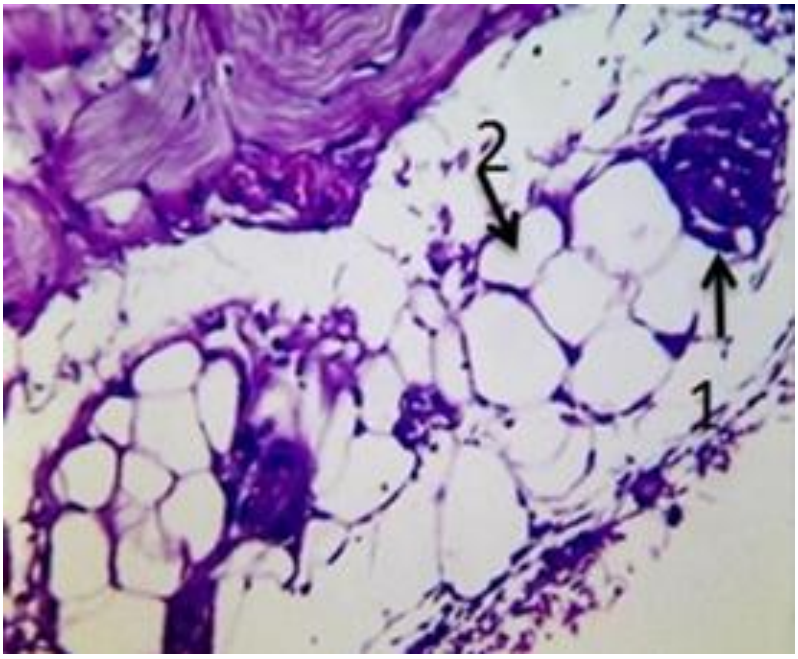

Figure 9. *The esophagus of the sheep showing serosa consisting of (loose connective tissue) and containing (1) blood vessels and (2) fat cells. H&E 100X*

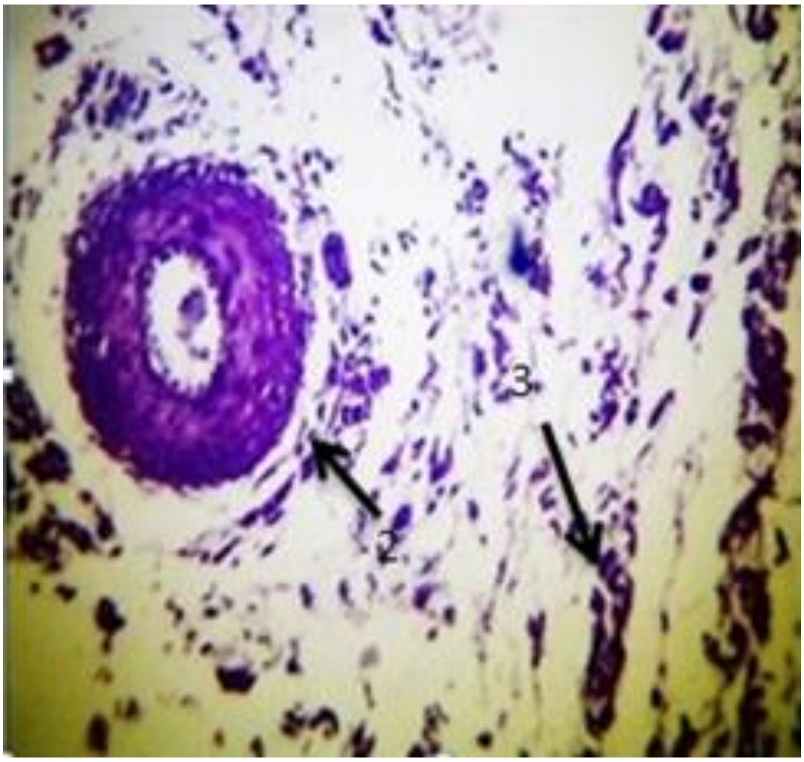

Figure 10. *The esophagus of the rabbit showing serosa consisting of (loose connective tissue) and containing (1) blood vessels and (2) fibers. H&E 100X*

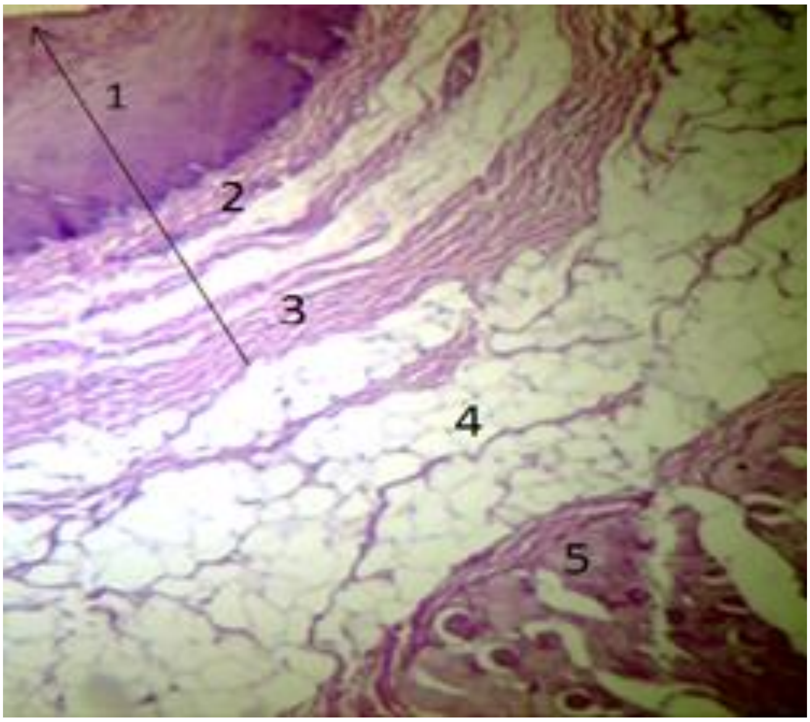

Figure 11*. The abdominal esophagus region of the sheep showing (1) stratified squamous epithelium layer (keratinized layer), (2) lamina propria, (3) muscularis mucosa, (4) submucosa and (5) muscularis .H&E 100X*

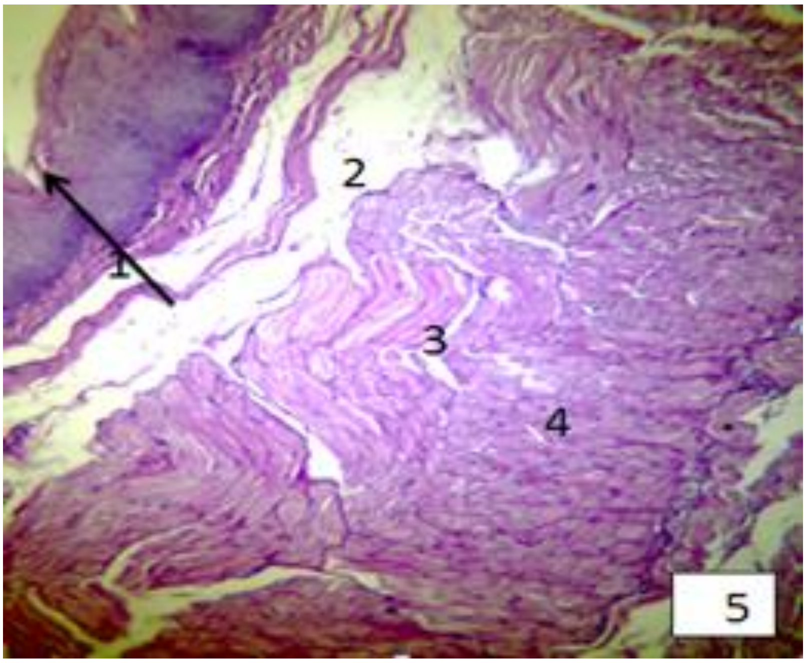

Figure 12*. The abdominal esophagus region of the rabbit showing (1) mucosa, (2) submucosa skeletal muscle including (3) circular muscularis, (4) longitudinal muscularis and (5) serosa. H&E100X*

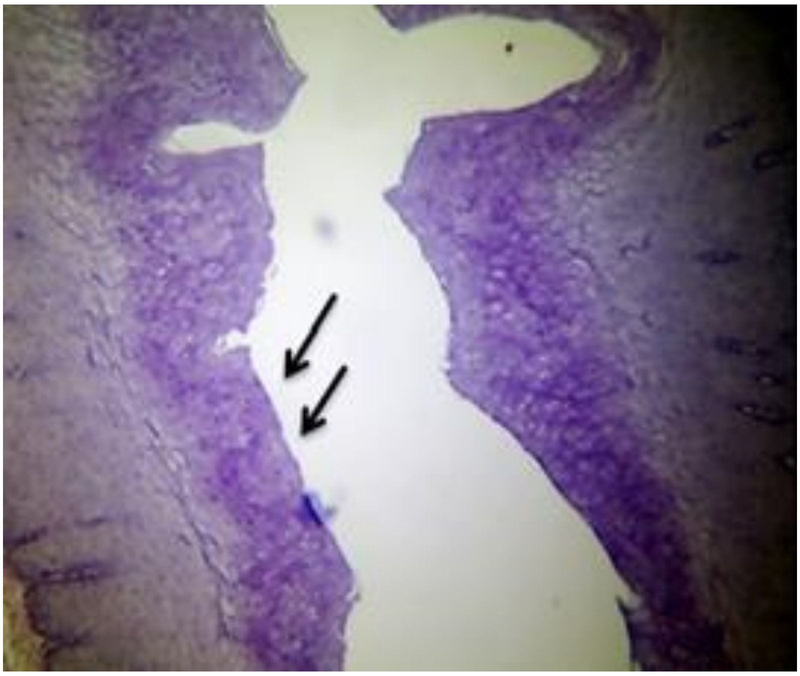

Figure 13. *The esophagus of the sheep showing Stratum corneum cells of keratinized stratified squamous epithelium layer with a strong reaction with PAS 100X.*

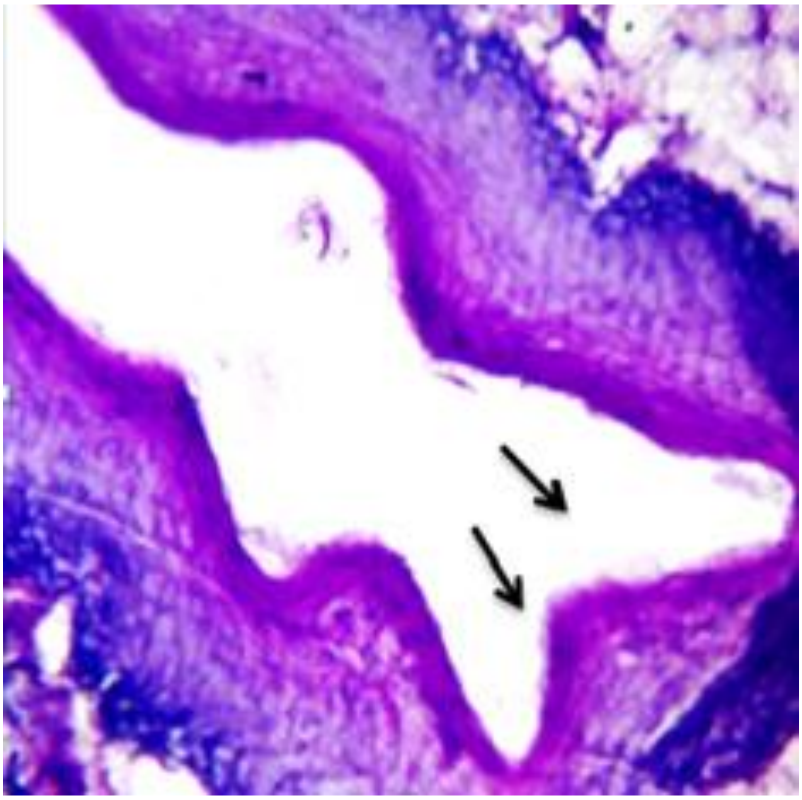

Figure 14. *The esophagus of the rabbit showing Squamous cells of non- keratinized stratified squamous epithelium layer with strong reaction with PAS 100X.*

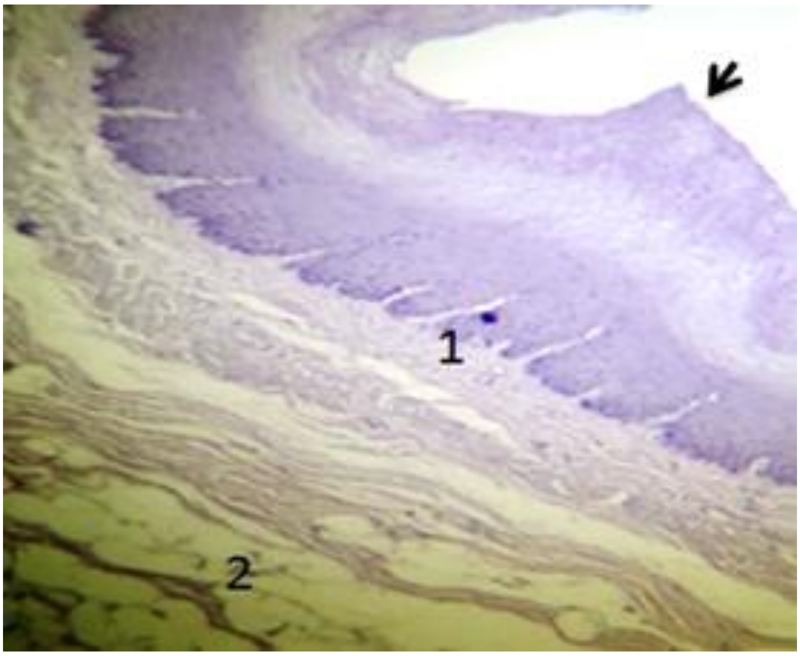

***Figure 15**. The cervical esophagus region of the sheep showing (arrow black) corneum cells with a strong reaction with PAS. (1) mucosa cells (except for corneum cells) with a moderate reaction with PAS and (2) submucosa with a weak reaction with PAS 100X.*

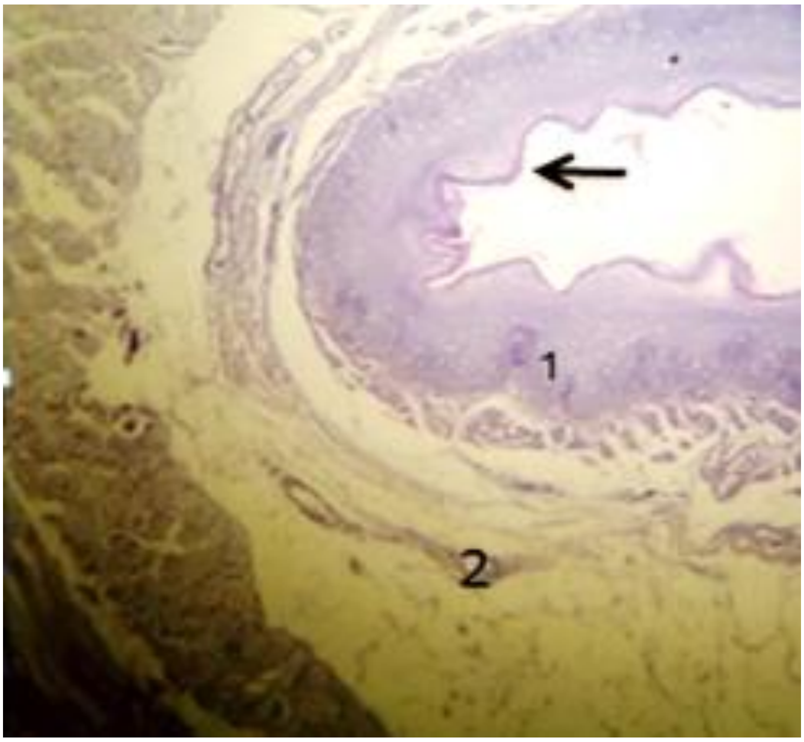

***Figure 16**. The cervical esophagus region of the rabbit showing (arrow black) squamous cells with a strong reaction with PAS, (1) mucosa cells layer (except for squamous cells) with a moderate reaction with PAS and (2) submucosa layer with a weak reaction with PAS 100X.*

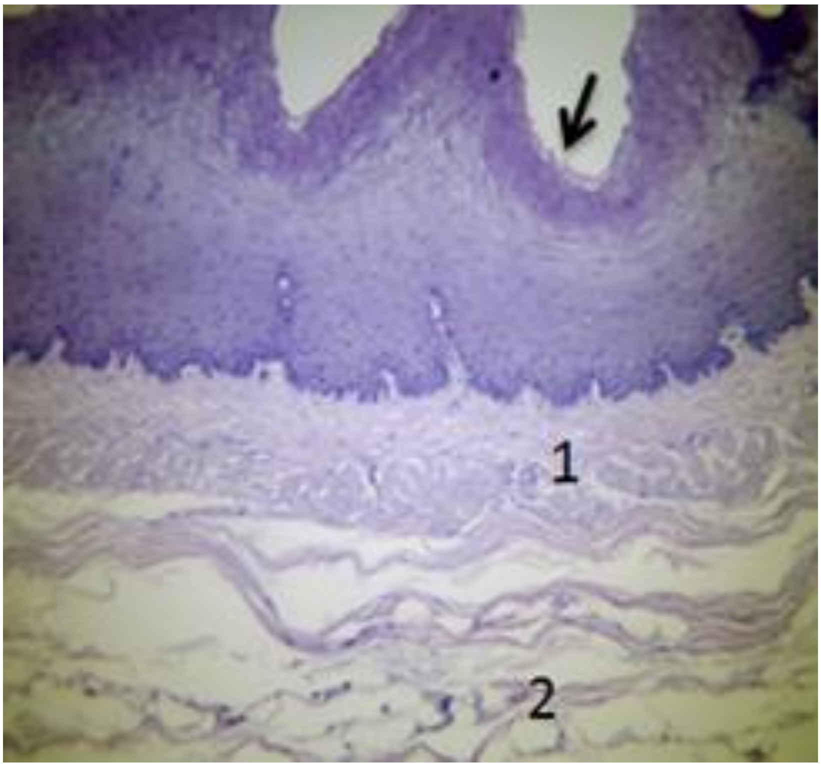

***Figure 17.** The thoracic esophagus region of the sheep showing (arrow black) corneum cells with a strong reaction with PAS, (1) mucosa cells (except for corneum cells) with a moderate reaction with PAS, (2) submucosa with a weak reaction with PAS 100X.*

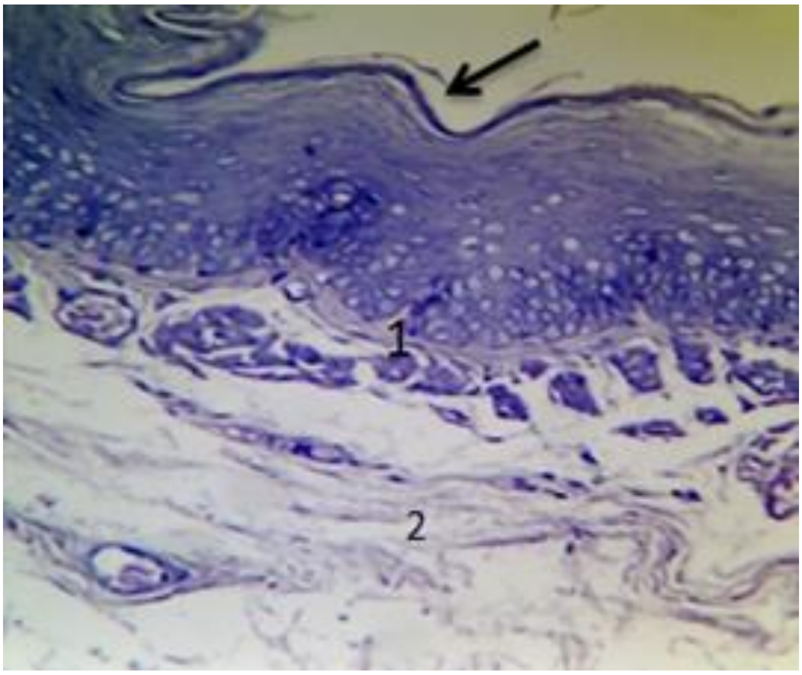

Figure 18. *The thoracic esophagus region of the rabbit showing (arrow black) squamous cells with a strong reaction with PAS, (1) mucosa cells (except for squamous cells) with a moderate reaction with PAS, (2) submucosa with a weak reaction with PAS 100X.*

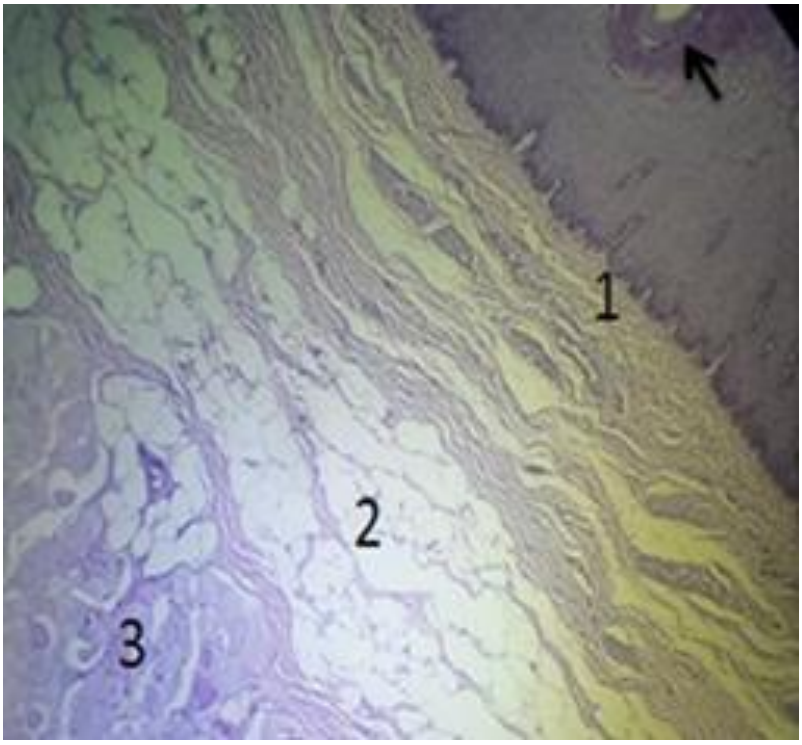

Figure 19. *The abdominal esophagus region of the sheep showing (arrow black) corneum cells with a strong reaction with PAS, (1) mucosa cells (except for corneum cells) with a moderate reaction and (2) submucosa with a weak reaction with PAS, (3) muscularis with a moderate reaction with PAS 100X.*

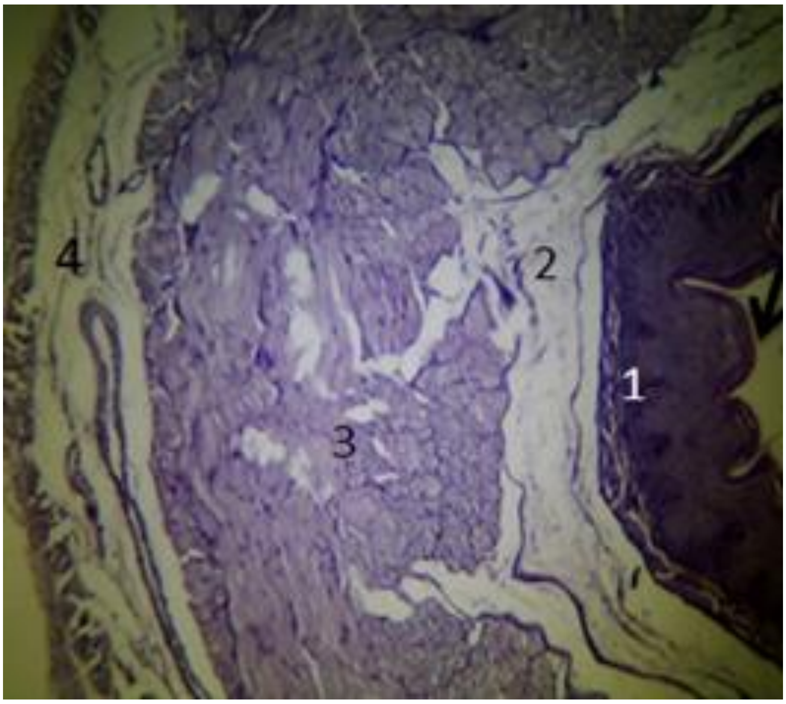

Figure 20. *The abdominal esophagus region of the rabbit showing (arrow black) squamous cells with a strong reaction with PAS, (1) mucosa cells (except for squamous cells) with a moderate reaction, (2) submucosa and serosa with a weak reaction with PAS, (3) muscularis with a moderate reaction with PAS 100X.*