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Comparative Histological And Physiological Study Between Rabbits And Sheep In Some Regions Of Digestive Tract

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بسم الله الرحمن الرحيم

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ

أَنتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العلي العظيم

سورة البقرة (الآية ٣٢)

Supervisor 's Certificate

We certify that this thesis entitled " Comparative Histological And Physiological Study Between Sheep And Rabbits In Some Regions Of Digestive Tract

"has been prepared under my supervision at the College of Science, University of Misan; as a partial fulfillment of the requirements for the degree of Master of Biology

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Recommendation of Head of Biology Department

In view of the available recommendations; I forward this thesis to debate by the examining committee.

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College of Science/Misan University Date: / /2020

Dedication

To our absent sun…

To our long –awaited hope...

To our savior from darkness of falsehood to the light of truth …

To our refuge when life strikes us…

To our prayers in every prayer that Allah makes us from his supporters …

To the rest of Allah is in his land…imam (AL-Mahdi)

Every eye for you, please meet you, and there is a loud voice and there…

When do you see us?

When will we see you?

Hawraa Jabbar

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Hawraa Jabbar

Summary

Summary

The current study was conducted to comparative the histomorphological and histochemical for each of esophagus, stomach and small intestine between sheep and rabbit, and measuring some physiological parameters for these animal. To obtain this aim, specimens were collected from Misan local market and slaughterhouse respectively. The study included 20 animals, ten male sheep and ten male rabbits, their weight were from 35-45 kg and 1.5-2.5 kg respectively. The study period was from (4/11/2019) to 4/7/2020). Used for histological studies of two types of stains, hematoxylin and eosin, and special stains (Periodic acid Schiff stains).

Histomorphological study of the esophagus, showed differences in epithelium type of mucosa lining the esophagus between animals, 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 of esophagus does not possess glands. The muscular layer of both was composed of striated muscle, both animals were containing outer layer of loose connective tissue called the adventitia. All esophagus layers in sheep showed more thickness than the in rabbits.

The histochemical study showed that the reaction to PAS stain was similar between the animals and in different places. Only stratum corneum of the sheep esophagus epithelium and surface layer of the rabbit esophagus epithelium demonstrated strong reaction to PAS. In contrast, the rest of the layers of the mucosa and muscular layers were moderate reactions with PAS

stain in all regions of sheep and rabbit esophagus. Sub mucosa and adventitia showed weakly reaction with PAS stain in both animals.

On the other hand, histomorphological study of stomach, showed similarity in epithelium type of mucosa lining the stomach in sheep and rabbits, the epithelium lining was composed from simple columnar, while gastric glands difference between sheep and rabbits, where was the cardiac glands in sheep are simple tubular but in rabbit simple, coiled and branched tubular, but fundus glands shown in sheep simple and straight tubular, in rabbits showed simple, straight and branched tubular. While Pylorus glands in sheep simple , branched tubular and coiled, in rabbit was simple tubular.

Sub mucosa layer in stomach does not possess glands. In stomach, the muscular layer of both was composed of smooth muscle fiber in all stomach regions .Outer layer of stomach compose of loose connective tissue called the serosa. While the thickness of the layers in the stomach was different between the animals.

In addition to that showing mucosa layer of cardiac region in sheep and rabbit strong reaction with PAS, polysaccharides distribution was concentrated in surface cells and body glands. In fundus region of sheep and rabbit the PAS stain showing strong reaction with surface cells of the mucosa layer, while the PAS stain showing weakly reaction in parietal cells and chief cells. In pylorus region, mucosa layer in sheep and rabbit gave strong reaction with PAS, distribution neutral polysaccharides almost equal in all parts pylorus glands reaction with PAS.

In small intestine, the mucosa layer is a simple columnar epithelium, the villi projections different in shape and size, in duodenum sheep long and

thin which finger shape, but in duodenum rabbit were broad and leafy shape ,the center (lacteal) of villi form of loose connective tissue. Lamina propria: formed in both animals from of loose connective tissue, and intestinal glands or called crypts of lieberkuhn consisting of columnar cells, goblet cells and Paneth's cells.

These glands well developed appeared simple tubular. On the other hand, Brunner's glands in sub mucosa layer gave strong reaction with PAS in sheep duodenum which mucous glands, while in rabbit was Brunner's glands mixed glands (serous cells and mucous cells), while sub mucosa layer in jejunum gave reaction weakly with PAS in sheep and rabbit ,but Peyer's patches in sub mucosa of ileum showed moderated reaction with PAS in both animals.

From the physiological aspect, results of present investigation showed that the values of gastrin hormone did not difference significantly ($p>0.05$) in sheep and rabbits. Results of present investigation showed that the values of Pepsinogen 1and Pepsinogen 11 did not difference significantly ($p>0.05$) between animals. In addition to, there are significantly ($p<0.05$) height in serum α –amylase and lipase level in rabbit in comparison to in sheep. All results were analyzed by (T-test). In conclusion, this study showed that sheep and rabbits have similarities and differences in the esophagus, stomach, small intestine; that is, the layers of these organs have different thicknesses and respond differently to PAS.

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List of Abbreviations

Periodic acid Schiff	PAS
Haematoxylin & Eosin	H&E
Alcian blue	AB
cholecystokinin	CCK
Pepsinogen 1	PG A
Pepsinogen 11	PG C
gastrin cell	G cell
enterochromaffin-like	ECL
somatostatin cell	D cell
gastrointestinal tract	GIT
enteric nervous system	ENS
Hydrochloric acid	HCL
horseradish peroxidase	HRP
Enzyme-linked Immunosorbent Assays	ELISA
Chemiluminescence immunoassay analyzers	CLIA
grams	g
Micrometer	µm
Figure	Fig.
kilogram	kg
milligram	mg
millilitre	ml
millimolar	mM
picogram	pg
standard deviation	SD
Unit per litre	(U/L)
litre	L
Alpha	α
ninogram	ng

Chapter One

Introduction

1. Introduction

Evolution between animals causes many changes so that it can adapt to its environments. Each animal species has unique characters that help them survive and can consume different types of feed (Luca *et al*., 2010). Comparative studies between animals very important and especially focus on and their structures more important, they show degree the similarity and different them between from where functionality and composition, as well as to better understand the evolutionary process as a whole (Kardong, 2006). On the other hand, give the comparative investigations of the digestive tract, such as the level of development of all segment is directly related to the living environment, for the understanding of the relations between feeding habits and metabolic needs (Pinheiro *et al*., 2009; Kotze *et al*., 2010).

Sheep and rabbit 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 fibrolytic ability in either their foregut i.e., the rumen of ruminants and the pseudo-ruminants or their hindgut i.e., the cecum and colon of non-ruminant herbivores, for fiber digestion (Kingston-Smith *et al.,* 2012; Furness *et al*., 2015). In addition, animals are classified into various types, on the basis of their habitats, as in Land Animals they live in homes and dairy farms such as sheep, cattle, and camel, the second type of land animals is wild animals, they are called wild because they are not domesticated by human beings as rabbit (Qureshi *et al*., 2012).

Rabbits considered are economically importance animals as they advantage of their meat and furring and are utilized as pets, and they are

importance at scientific and medical experience (Hristov *et al*., 2006). Whereas, sheep have able to use the lingo-cellulosic materials and converts them to animal products of high nutritional values such us meat, milk, wool/fur, hide and manure, in the same vein, sheep intestine can be uses to make "catgut", which is still in use forinternal human surgical sutures and strings for musical instruments (Agrawal *et al*., 2014).

The wall of the digestive tract exhibit four layers that shows a basic histologic organization the layers are the mucosa, sub mucosa, muscularis externa, and serosa or adventitia, due of the different functions of the digestive organs in the digestive process, the morphology of these layers exhibits variation (Eroschenko, 2008).

The gastrointestinal secretion in vertebrates contains number of mucosubstances that can vary according to cell type, functional status, anatomical region, pathological condition, sex, age and species and mucosubstances detect by many of techniques (Choi *et al*., 2003; Schumacher *et al*., 2004).

In addition to, endocrine cells in the gastrointestinal tract play an important role where gastrin and other gastrointestinal hormones regulate the functions of the gastrointestinal tract such as secretion of intestinal, fundic glands, nutrient and absorption (Solcia *et al*., 2000; Schubert. 2008).

On the other hand, secretion of the pancreatic gland to the intestinal tract are response to eating of food the pancreatic juice contain enzymes necessary to digest proteins, carbohydrates and fats (Nzalak, 2010). Enzymes are produce by living cells to cause specific biochemical reactions to catalyzed the catabolic reactions by which substrates are digested into substrates' chemical compounds, these simple compounds

are use in turn for cells growth and α-amylase and lipase are considered important enzymes for this process (Al-Abedi *et al*., 2020).

1.1. Aim of the study :

1. Comparative histomorphological, histomorphometric and histochemical study for each of esophagus, stomach and small intestine between sheep and rabbits.
2. Assessing the serum gastrin hormone, the serum enzymes a-amylase and lipase and the serum enzymes pepsinogen 1 and pepsinogen 11 in sheep and rabbits.

Chapter Two

Literature Review

2.1. Classification of Sheep and Rabbits.

Generally, Sheep belongs to the Bovidae family (Nowak, 1999). Reece, (2009) stated that the order Artiodactyla include cattle, goats, sheep, giraffes, bison, moose, elk, yaks, water buffalo, deer, camels, alpacas, llamas, antelope, blackbuck, pronghorn and nilgai, taxonomically, the suborder Ruminantia includes all those species except the camels, llamas and alpacas, animals that regurgitate and remasticate their food are called ruminants.

The sheep is scientific classified according to the following:

Kingdom - Animalia

Phylum - Chordata

Class - Mammalia

Order - Artiodactyla

Family - Bovidae

Genus - Ovis

Species - aries

Ovis aries - Domesticated sheep (Raney,1968).

The rabbit (Orycotolagus cuniculus) belongs to the family Leporidae (rabbits, hares) of the order Lagomorpha. Once classified as a rodent, the rabbit was given a separate order because of dentition differences, chiefly the incisors. Lagomorphs have 2 pairs of upper incisors (they are born with 3 upper pairs but lose the outer pair early). The 2nd pair of upper incisors is smaller and is located immediately behind the 1st (Brewer and Cruise, 1994).

The rabbit is scientific classified according to the following:

Kingdom - Animalia

Phylum – Chordata

Class – Mammalia

Order – Lagomorph

Family- Leporidae

Genus –Oryctolagus

Species- Cuniculus (Mojari and Saluqi, 2013)

2.2. Nutrition.

Plant tissues contain about 75% carbohydrates, providing the primary source of energy of ruminant, the carbohydrates in plant tissues are primarily polysaccharides , hemicellulose, cellulose, pectins, fructans and starches, with few amounts of other compounds (Cerrilla and Martínez , 2003).

There are phenotypic and dramatic physiological differences found between ruminant and non-ruminant mammalian species, for example, volatile fatty acids produce as by-products of the microbial fermentation in the rumen are use as the major source of energy in ruminants as oppose to glucose absorb from the small intestine in non-ruminants and because of this difference in nutrient usage, ruminants are less sensitive to insulin than non-ruminants (Bao *et al*., 2013). Russell and Mantovani (2002) detected that the anatomical adaptation of the digestive system in ruminants allow them to use cellulose as the energy source.

Linton and Greenaway (2007) documented that animals able potentially vary a numbers of behavioural and physiological characteristics in order to adjust to differences in the quality of available food, these includes the selection type of food items, food intake, mechanical fragmentation of food, the complement of digestive enzymes produce, the retention time of digesta in the gut, and the anatomy of the alimentary.

The digestive tract is a dynamic organ that responds largely to immediate changes in the quantity and quality of food, with the arrival of digestion, the intestinal reception section becomes mechanical (churning, peristalsis) and chemically active (excretion), intestinal mucosa may respond to daily renewable nutrition primarily by enlarging individual endothelial cells, thereby increasing the mass of the digestive system (Kardong, 2006).

Al-Haaik (2016) detected that the relation between intestinal morphophysiology and the kind of nutrition and quantity of feed effect on histological features of the small intestine and the histological developmental changes could occur during the age progress of the animal.

On the other hand, dietary fiber level and sources affects the morphology of the gastrointestinal tract (GIT) mucosa such as villous height, number of the goblet cells and crypt depth where these changes indirect influence grow animals which affect the proliferation of intestinal cells (Yu and Chiou, 1997; Desantis *et al*., 2011).

The motility patterns in the forestomachs seen adaptation depending on the feed type (Münnich *et al*., 2008). Sponheimer *et al*., (2003) they studied digestive efficiency of ruminants, and hindgut fermenters and they confirmed that the quality feed has effect to rate energy. Kingston-Smith *et al*.,(2012) detected the ability of ruminal microorganism to produces the enzymes necessary for fermentation process allow ruminants to efficiently obtain the energy contained in forages. However, the ruminal fermentation process is not completely efficient because it produce some final products e.g methane gas as shown in (Figure 2- 1) (Burns. 2008; Akers and Denbow . 2008).

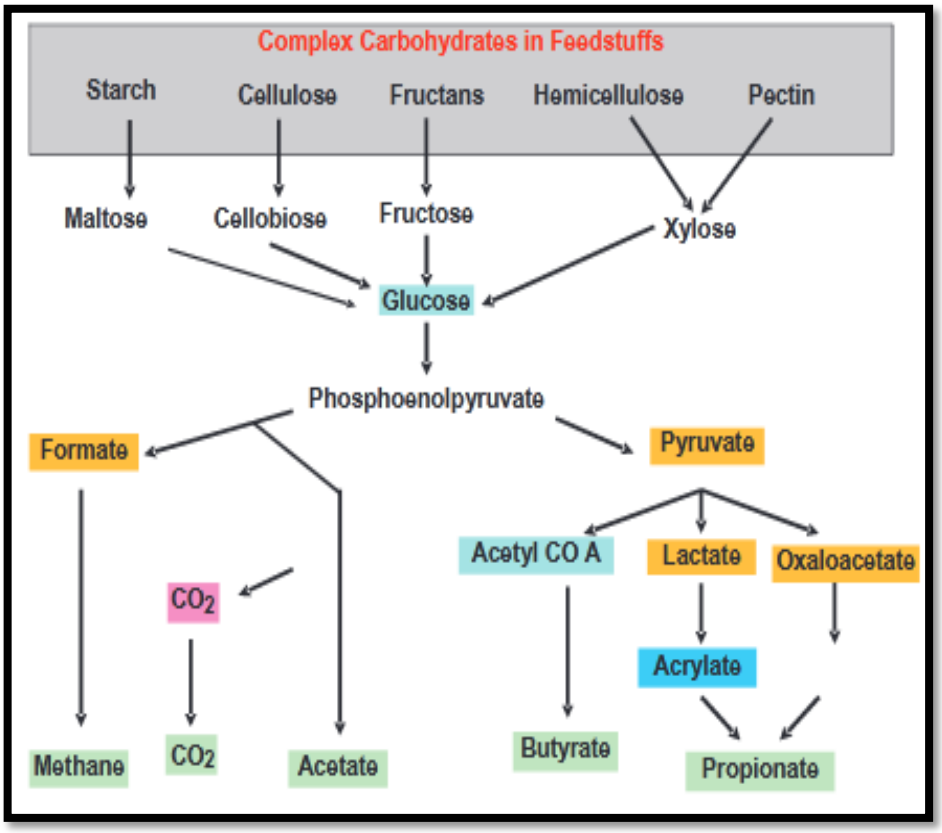


Figure (2- 1) Rumenal carbohydrate fermentation. Complex carbohydrates are fermented by microorganisms within the rumen(Akers and Denbow, 2008)

Generally, foregut fermenters is the main site of digest a retention and therefore of microbial fermentation as shown in (figure 2-2) (A),while hindgut fermenters can be divided into either colon fermenters or caecum fermenters as shown in (figure 2-2) (B) (Hume, 2002). Furthermore, rabbits are true non ruminant herbivores and consider as hind gut fermenters also they have a large cecum that can hold up to 40% of the intestinal contents and enables them to eat a primarily fibrous diet (Sakaguchi. 2003).

Interestingly, one of the most original features of the rabbit feeding behaviour is the caecotrophy which involve an excretion and an immediate consumption of specific faeces named soft faeces or "caecotrophes", Consequently, daily intake behaviour of the rabbit is constituted of two meals: caecotrophes and feeds (Bels. 2006).

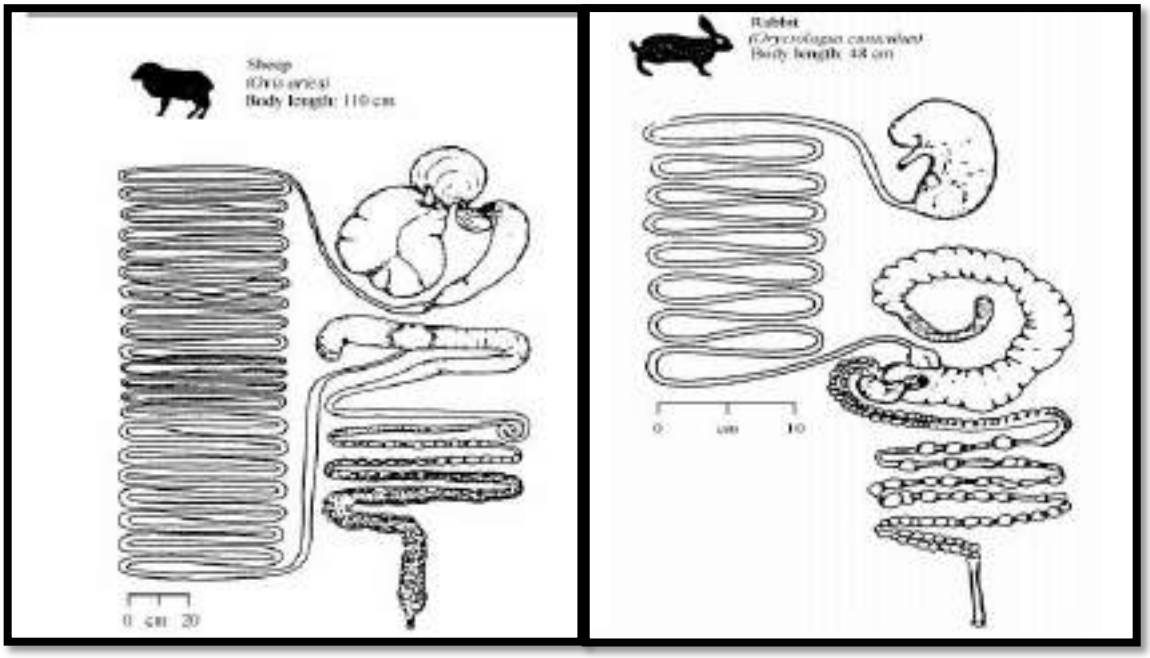


Figure (2-2) (A) the gastrointestinal tract of foregut fermenters in sheep (Hume . 2002).

Figurer (2-2)(B) the gastrointestinal tract of hindgut fermenters in rabbit (Hume . 2002).

2.3. Digestive tract

The digestive tract include the esophagus, stomach, small intestine, and large intestine, the major function of digestive tract is digest of the food when it pass along it, in this process, nutrients and water are absorb, and waste materials are prepared for eliminate from the body each section of the digestive tract has its unique histological features, which are closely associated with the function of that part of the tract, organs of the digestive tract are each hollow, they are compose of four general tunic layers include mucosa, sub mucosa, muscularis externa, and adventitia or serosa (Cui *et al*., 2011).

2.3.1.Esophagus

The esophagus was a narrow muscular folded tube (Kadhim, 2019). It is part of digestive system, in terms of function, the main function of the esophagus is responsible for transfer food and fluid from oral cavity to the stomach, it is the only part of digestive system which does not have metabolic, digest and absorb functions. Esophagus divided to three portions: cervical, thoracic and abdominal (Kumar *et al*., 2009).

Histologically, it has four layers as shown in all digestive system (Frye and Aughey, 2001). The mucosa is consists of three layers a stratified squamous epithelium, a lamina propria, and a lamina muscularis. Esophagus keratinization is difference between species, some species are keratinized and others non-keratinized. In more details, carnivores esophagus characterized by non-keratinized of stratified squamous epithelium tissue but the ruminants esophagus shown different degree of keratinization (Eurell and Frappier, 2006). In addition to that (Igbokwe and Obinna, 2016) observed in *rope squirrel* esophagus that epithelium lined by non-keratinized stratified squamous epithelium.

At some vertebrates, the esophagus mucosa is line with ciliated cells that controlled the flow of lubricated mucus around the food, the ciliated epithelium may helped gather small crumbs of the meal and moved these along to the stomach (Kardong.2006). Previously, Ahmed *et al*., (2009) observed that the epithelium of the esophagus of *Varanus niloticus* cover by ciliated columnar epithelium.

The epithelium and lamina propria are separate by the basal lamina. The lamina propria is consisting from connective tissue, which contain lymphocytes and vascular structure, there are many of dermal papillae that appear as finger-like extensions, the lamina propria appears interdigitated with the epithelium, the muscularis mucosa located between lamina propria and sub mucosa (Hussein *et al*., 2016).Whereas, Ali *et al*., (2008) observed that no muscularis mucosa in Giant African rat esophagus.

In rabbit, Mahmood *et al*., (2017) reported that the sub-mucosa has dense regular connective tissue. Whereas, Calamar *et al*., (2014) observed that the sub-mucosa in esophagus of Chinchilla laniger is layer of loose connective tissue containing collagen fibres, fibroblasts and numerous blood vessels with large lumens (capillaries, arterioles and venules). There are mucus-secreting glands are visible, they are less abundant in humans and more numerous in certain animal species such as dogs and pigs (Shiina *et al*., 2005). Islam *et al*., (2008) reported the presence of sub mucosal glands only in proximal region of esophagus in Black Bengal goat. In dog, Rus *et al*., (2016) state that the glands' excretory channels are cover by a stratified cuboidal epithelium, they passage muscularis mucosae, then lamina propria and opens at the surface of the esophagheal mucosa.

The esophageal muscular layer consists of two layers, in ruminants and dogs, the entire muscular tunic consists of skeletal muscles (Eurell and Frappier, 2006). The mice cannot vomit, they tend to implicate the specific edge or striped muscle deficiency in rat esophagus (Musser and Carleton, 2005).

The adventitia locate at the outer layer of cervical and thoracic region, it was compose of loose connective tissue (Hussein *et al*., 2016).

On the other hand, Arellano *et al*., (1999) observed that the appear region of the esophagus of the *Engraulis anchoita*, the mucous cells of the epithelium contains sulpho glycoproteins, because they were weakly stained with Periodic Acid Schiff (PAS). Ahmed *et al*., (2009) detected that the esophagus in *Varanus niloticus* contain mucous secret cells which stain positive with Periodic Acid Schiff (PAS) and alcian blue (AB), indicating that they secrete neutral and acidic mucus substances.

From the physiological aspect, the esophagus is a highly specialized device designed to push foods from the mouth into the stomach, due to its location in the digestive system, it may be exposed to a variety of harmful stimuli, the mucosal barrier is an important factor in protecting the esophagus from damage, the presence of the mucous barrier in the natural esophagus is contested with reports of its presence, the need to protect the mucous surface is acceptable and the presence of mucin from saliva or glands and esophageal ducts is undisputed (Dixon *et al*., 1999).

2.3.2.Stomach

The stomach is an enlargement portion of the digestive tube specialized for the enzymatic and hydrolytic breakdown of food into digestible nutrients, the muscular wall help in mixing the ingesta, the stomach is lined by glandular mucosa in carnivores, while herbivores have, in addition to a glandular regions, a non-glandular region lined by stratified squamous epithelium (Nzalak, 2010).
There are noticeable differences between species in the gastric chamber, manifested in the difference between monogastric and ruminant stomach (Jennings *et al*., 2017).

In addition, Colville and Bassert, (2008) stated that ruminant animals like cattle, goats and sheep are often referred incorrectly as having four stomachs, they actually have only one true stomach the abomasum and three forestomachs the rumen, reticulum and omasum. In the same context, the forestomachs are compartments of varies sizes and functions, forestomach (proventriculus) whose tunica mucosa is lined by a squamous, keratinized stratified epithelium (García *et al*., 2012).While, the tunica mucosa of the abomasum, by contrast, has a simple, glandular, epithelium like to that found in the stomach of monogastric species (Masot *et al*., 2007).

Davies and Davies (2003) description that the rabbit's stomach has thin walled, sac-like organ, it accounts for 15% of the size of the digestive system, a highly developed cardiac sphincter prevents true vomiting.

Whereas, Bal *et al*., (2007) observed in hamster, rats, mouse, and gerbil that a distinct stratum granulosum in the keratinized stratified squamous epithelium of forestomach.

The non-glandular stomach of *Macropusfuliginosus* was line by keratinized squamous epithelium and was lamina propria of dense connective tissue and prominent lamina muscularis (Shoeib *et al*., 2015).

The stratified squamous epithelium in the non-glandular region turns into a simple vertical epithelium in the glandular region in *Babyrousa babyrussa* (Leus *et al*., 2004).

Previously, Byanet *et al*., (2008) observed three areas of the grass cutter (cardia, fundus, and pylorus) and also noted in the stomach wall contains the same structural layers in all areas regions and these are similar to those in parts of the digestive system.

The rabbit stomach wall consists of four layers of the mucosa tunica, tunica sub mucosa, tunica muscle and serous tunica. Moreover, the stomach lined with a surface lining mucous cells appear as tall simple columnar epithelium that extend through gastric pits, where the gastric glands are open (Khalel. 2012).

The stomach epithelium is invaginated to forms the gastric pit at the bottom of which the gastric glands arises as one or two simple tubules, there are found four cell types, the parietal cells, the zymogene cells, the neck mucous cells, and argentaffine cells, at the fundic glands, was predominantly composed of parietal cells and the zymogene cells, and connective tissue from the lamina propria mucosa between these glands (Frye and Aughey, 2001; Ergun *et al*., 2003).

In sheep stomach, the lamina muscularis mucosa comprise 2-3 rows of longitudinally oriented fine smooth muscles, as noted the sub-mucosa was made up of loose irregular connective tissue with collagen, reticular, elastic fibers, fine blood capillaries and a few isolated nerve bundles (Amit and Pawan, 2017).

While, Adib and Sheibani, (2006) stated that the gastric glands were locate in sub mucosa of fore stomach and lamina propria of posterior part of the stomach in Caspian pony.

The tunica muscularis consists of three layers from smooth muscles an inner oblique, middle circular and outer longitudinal, the myenteric plexuses are locate between the middle and outer muscle layers, while the outermost layer, tunica serosa is compose of mesothelium overlying a layer of loose connective tissue (Cui *et al*., 2011).

In grass cutter, Obadiah *et al*., (2011) descripted that the gastric pits of cardiac region of stomach were deep and lined with simple columnar epithelium and further observed the lamina propria contain simple or branched tubular gastric glands.

In sheep, Amit and Pawan, (2017) reported that the fundic gland region of abomasum was lined with simple columnar epithelium with three main cells types, chief cells with dense chromatin, pyramidal shaped parietal cells intersperses between the chief cells and isolates argentaffin cells located towards the basal portion of the glands. While, Sujana (2017) stated that the fundic mucosa in pigs contain on cells endocrine D, G and Enterochromaffin cells.

Ahmed *et al*., (2009) reported that the glandular portion of stomach in *Varanus niloticus* contains either one type or two types of cells; dark serous "oxyntico-peptic" and clear mucous cells.

In rabbit, the pyloric region of stomach was covered by low columnar to cuboidal epithelium, lamina propria consisted of gastric glands which arose as simple tubules at the bottom of gastric pits, gastric glands comprise of parietal, zymogen, mucous neck and argentaffin cells, further observed the muscularis mucosa in pyloric region was considerably

thicker than in the fundus and composed of two layers of smooth muscle fibers, tunica sub mucosa was consists of loose connective tissue and tunica muscularis was arranged as inner circular and external longitudinal layers covered with external loose connective tissue (Mahdi, 2013).

In addition to, the cardiac and pyloric regions in the human stomach can be distinguish from other regions on the dependent of the histology of their glands, while the mucosa in the cardiac region resembles that of the lower end of esophagus, the gland being compound tubular with many goblet cells, a few parietal cells are also present, either Pyloric glands are simple branched tubular glands, and they extend deeper into the mucosa than other types, they have many goblet cells and relatively few parietal cells (Nzalak. 2010).

On the other hand, Diaz *et al*., (2003) detected that in the cells stomach of *Engraulis anchiota* the epithelial lining were stained with PAS reaction which was more intense than in the cells of the gastric glands, in the different gastric zones, at the apex of the columnar cells which make up the superficial epithelium, large quantities of both acidic and neutral mucosubstance are synthesized, the acidic mucosubstance are shown to be chiefly of the sialylated type.

Huang. (2011) observed that the mucus cells of cardiac glands in human stomach showed positive and negative reactions for PAS and Alcian blue stains respectively. While, Ahmed *et al*., (2009) observed in the *Varanus niloticus*, that the gastric surface epithelium showed histological features in both fundic and pyloric regions and exhibited strong staining with PAS while appeared Oxyntic opeptic cells stain negatively to both PAS.

From the physiological aspect, cells parietal in stomach secrete acid (HCL) and intrinsic factor, and chief cells (secrete pepsinogen), addition to the mucous cells, secretion was mucous (AL-Mahmodi. 2014). Moreover, endocrine cells located in pyloric glands produce at least seven hormones, the major hormone, gastrin, is secreted by G cells found most abundantly in the gastric pits of the pyloric antrum, gastrin stimulates secretion of both parietal and chief cells, and causes contractions of the gastric wall (Akers and Denbow, 2013).

2.3.3.Small Intestine

The small intestine is a very long, tubular organ**,** connects the stomach to the large intestine and can be divided into Duodenum, Jejunum and Ileum based on anatomy and function, the duodenum is a tiny fraction of the small intestine, it is the site of most of the breakdown of the food passing through it, the duodenum is line with duodenal sub mucosal glands, which secrete an alkaline mucus that supports the intestinal enzymes and aids in the absorption of nutrients (Cunningham and Klein, 2007).

The pancreatic duct, which introduces bile and pancreatic juice into the small intestine, is directly connected to the descending duodenum, pancreatic juice contains enzymes that help break down food, while bile aids the digestion and absorption of fats (Cunningham and Klein, 2007).

Histological, the small intestine wall has four concentric layers: mucosa, submucosa, muscularis and serosa, and this structure has also been observe in other mammalian species (Gadelha-Alves *et al*., 2008).

The small intestinal portions appear histological differences at the small intestinal portions, the duodenum contains (Brunner's glands) in the submucosa and has the longest villi of the entire three regions also it has

the highest numbers of goblet cells, but Plica circularis is absent, the jejunum is next to duodenum and has glands or lymphoid nodules in the mucosa, the ileum is the last region of the small intestine, it has permanent aggregates of lymphoid nodules in the submucosa and has the shortest villi, with the least number of goblet cells (Nzalak. 2010).

However, the epithelium of the small intestine is made up of enterocytes, entero endocrine cells, goblet, Paneth cells, and Microfoldcells (M cells). Paneth cells and Microfoldcells (M cells) located in Lieberkühn crypts (Ouellette.1999).

Previously, The stem cells give rise to the four types of cells (enterocytes, enteroendocrine cells, goblet, Paneth cells, and Microfold cells (M cells) of intestine epithelium (Snippert *et al*., 2010). In mice, stem cells were divides slowly in which approximately once every 24 hours (Potten *et al.*, 1990).

The surface epithelium of small intestine is covers by the villi which are already protruded into the intestinal lumen and it also lines the crypts which are extended to the connective tissue (Mohamed *et al*., 2019).

Kadadi. (2012),descripted that the villi are finger like projections forms of a core of reticular tissue covers by surface epithelium, these structures about 0.5-1.5 mm long, are outgrowths of mucosa and are leaf shaped in duodenum and the connective tissue core consists of numerous blood vessels or capillaries and a central lymphatic vessel called a lacteal.

Also, among the villi are small openings of the simple tube glands called the intestinal glands (crypts of Lieberkuhn), all of the crypts and villi expands the surface of the mucous membranes of the small intestine, in humans, e.g, the villi lead to a 5-6-fold enlargement of the absorption surface of the small intestine (Leonhardt, 1990).

Whereas, Al-Shamary *et al*., (2017) stated that the crypts of Lieberkühn are simple tubular glands called intestinal glands that were extend from the muscularis mucosa till the bases of the villi, they were lined by a simple columnar epithelium.

Moreover, beneath epithelium, there is loose connective tissue in the form of lamina propria mucosae, the deepest part of the mucosa is named muscularis mucosa which consists of smooth muscle fibers with various thickness at different parts of the intestine separated the mucosa from the underlying submucosa (Rao and Wang, 2010).

There are various factors that are expected to control epithelial growth in the small intestine, these may be genetic pre-programming, or growth factors in caulking breast milk, ingested food, or bile or pancreas secretion or hormones in the cavity lumen, the intestinal growth pattern in the fetus and after childbirth is performed by bilateral crypts fission, which has been documented in neonatal mice and in human infants (Cummins and Thompson, 2002).

Calamar *et al* ., (2014) reported that the sub-mucosa tunic is formed of loose connective tissue and provides support for the vascular and nerve network.

The Brunner's glands are branched tubuloalveolar glands, located in sub mucosa, they existed at each mammalian species (AL-Baghdady *et al*., 2012). The ducts of the Brunner's glands penetrate the muscularis mucosa and ascend through the lamina propria, to empty to the base of intestinal glands (Kadhim *e t al*., 2012). Hassan and Moussa, (2015) observed in *Capra hircus* that sub mucosa was devoid of glands in all three small intestine regions.

The muscularis layer of small intestine composes of two layers of smooth muscle cells internal-circular and external-longitudinal (Calamar *et al* ., 2014). The intestine has no serosa, the layer external to the tunica muscularis would be referred to as the adventitia (Nzalak. 2010).

From the physiological aspect**,** digestion of the small intestine and its absorption in rabbits is similar to that found in other species, bicarbonate ions are excreted in the duodenum to neutralize the acidity of the chemistry that passes through the stomach, most carbohydrate and simple protein digestion occurs in duodenum and jejunal, and product of this digestion (monosaccharides and amino acids) are absorbed across the the jejunal brush border (Davies and Davies , 2003).

In rabbit, include digestion and absorption of the cecotroph materials e.g amino acids, vitamins, volatile fatty acids and digested microbial organisms, the digestion of cecotroph microbial protein is aid by the addition of lysozyme the cecotrophs as they passes through the large intestine, lysis of the microbes within the cecotrophs also release microbial enzymes, notably amylase, which enhances the rabbit's own digestive processes, the ileum also plays an important role in regulating and recycling the electrolytes secreted by the stomach and proximal small intestine by reabsorbing bicarbonate ions (Davies and Davies, 2003).

The glands in small intestine secretes a mucous alkaline fluid in response to parasympathetic stimulation, the secretion has been shown to be viscous, high in bicarbonate, this fluid helps to neutralize the acid chyme that enters the duodenum from the pyloric part of stomach, these features suggest a protective role against the acid chime, these glands are most numerous and largest near the pylorus, and form an almost complete layer in the superior part and proximal half of the descending duodenum,

thereafter they gradually diminish in number and disappear at the duodenojejunal junction (Kadadi. 2012).

Peyer's patches characterized by the presence of numerous lymphatic follicles and inter follicular T cell regions, indeed, antigens are transport from lumen across the epithelium of intestine to stimulates the pre B cells presents in the follicles of peyer's patches which subsequently proliferate and migrate to distant sites (Reboldi and Cyster, 2016).

Ahmed *et al*., (2009) noticed that it was difficult to distinguish between the different parts of the small intestine of *Varanus niloticus*, they observed that two basic types of cells were present in the intestinal lining epithelium; columnar absorptive cells and goblet cells that secrete both types of mucinous substances as indicated by positive reaction to both PAS and AB.

Brunner's glands of human duodenum secretes neutral mucosubstances, but in sheep secretes scanty neutral mucin and predominantly acid mucin. Guinea pig Brunner's glands secrete mixture of acid and scanty neutral mucins (Kadadi.2012).

In the adult one humped camels (*Camelusdromedarius*) the glandular cells of these glands were weakly positive for PAS (Kadhim *et al*., 2012).

2.3.4.Enteroendocrine Cells

The endocrine cells play a role in the function of the digestive system, these cells are diffuse in the differs regions of gastrointestinal tract (Beehler-Evans and Micchelli, 2015). The intestinal tract is contain about 15 different types of endocrine cells that releases more than 100 biologically active peptides and hormones (Ahlman and Nilsson, 2001).

Burns and Pachnis, (2009) reported that the stomach and the intestines are rich innervated with nerves and neuronal messengers called the enteric nervous system (ENS), the enteric nervous system is consists of intrinsic and extrinsic afferent and efferent neurons distributed in two major complexes the myenteric and submucosal plexuses.

Moreover, the gastric mucosa endocrine and paracrine cells "gastrin cells, enterochromaffin-like (ECL) cells, somatostatin cells", exocrine cells "parietal cells, chief cells, mucous cells", smooth muscle cells, and stromal cells are regulated by neuronal messengers (Ekblad *et al*., 2000). The pyloric gland area, the hallmark of which is secretion the gastrin, comprises 20% of the antrum (Joseph *et al*., 2003).

2.3.4.1.Gastrin

Gastrin is the peptide hormone released from a specific endocrine-type cells called gastrin cells (G cells) (Timurkaan *et al*., 2009). It is the main acid stimulatory agent during ingestion of a meal (Schubert. 2003).

It is secreted from G cells in the antrum in an endocrine type and stimulates the parietal cells directly to produce gastric acid or indirectly through the release of histamine from the ECL cells, gastrin binds to the (Cholecystokinin B receptor) CCK2R located both on the parietal as well as (ECL) cells, and activates a signaling cascade involving

phospholipase C and release of intracellular calcium (Tømmerås *et al*., 2002).

Geibel *et al*., (1995) believed that intracellular concentrations of cAMP have to reach a particular threshold for gastrin to be able to directly stimulate the parietal cells. The primary action of gastrin on the parietal cells seems to involve sensitizing them to other secretagogues through synergistic interactions between signaling pathways, the main mechanism by which gastrin is thought to stimulate gastric acid secretion is through the activation of CCK2Rs on the ECL cells and following stimulation of histamine release (Schmitz *et al*., 2001).

There are some hormones and neurotransmitters stimulates the release of gastrin such as somatostatin inhibit release (Waldum *et al*., 1991). Hydrochloric acid is an important function of the stomach, gastric acid helps protein digestion, absorption of iron, calcium, and vitamin B- as well as prevents bacterial overgrowth and enteric infection (Schubert and Peura, 2008).

Gastrin is generally considered to be a trophic factor for the oxyntic gland area mucosae of the stomach and may also be involved in regulating mucosal growth in small intestine and colon (Morisset. 2005). Generally, the regulated of gastric acid secreted is achieved by the interplay between two major gastric endocrine cells; the G cell and the somatostatin D cell, regulation of these cells occurs by stimulatory or inhibitory paracrine, neural pathways and endocrine, when food enter the stomach, the protein component stimulate G cells situated in the antral region of the stomach to releases the hormone gastrin, which stimulates the (ECL) cells to releases histamine and stimulates parietal cells to secretes acid, as the acidity of the stomach and duodenum increases,

protective feedback pathway are activate to inhibit further acid secretion as shown in figure (2-3) (Hersey and Sachs, (1995); Liu *et al.*, 2005).

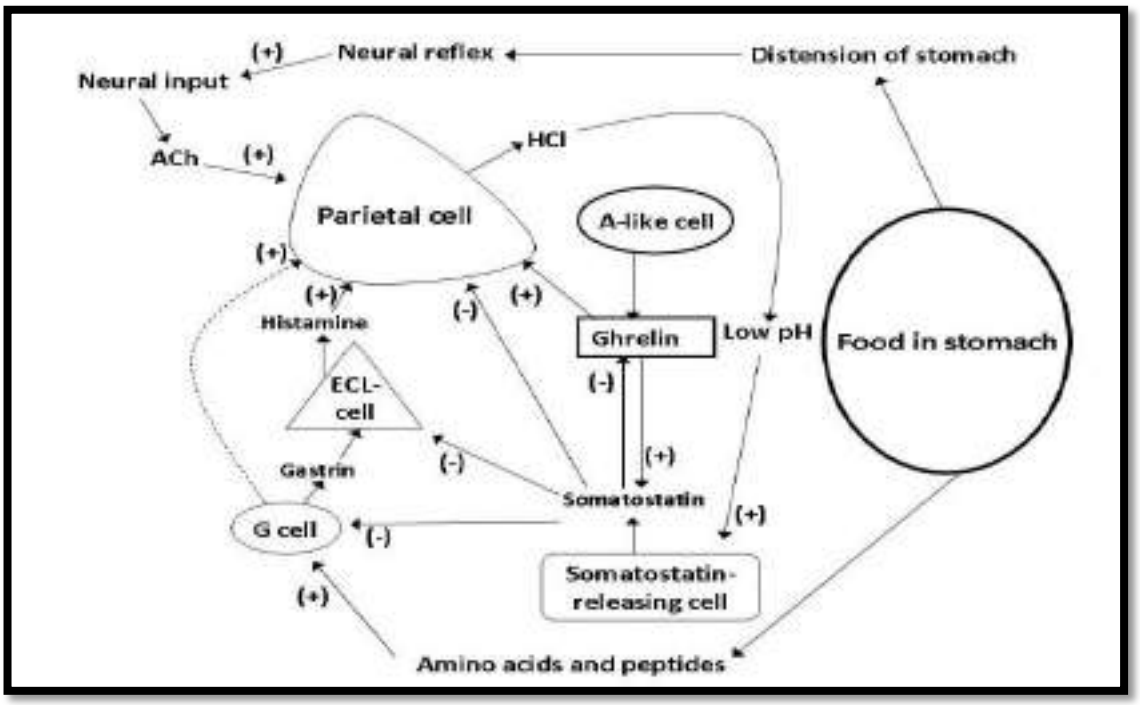


Figure (2-3) Schematic illustration of control of gastric acid secretion Hersey and Sachs, (1995)

2.3.5.Digestive Enzymes.

2.3.5.1.Pepsinogen

Pepsinogen (PG), a proenzyme is an inactive form of pepsin which is the most important proteolytic enzyme of gastric juice (Kataria *et al*., 2008). It is synthesis and secretion are regulated by positive and negative feed-back mechanisms. In the resting state pepsinogens are stored in granules, which inhibit further synthesis. After appropriate physiological or external chemical stimuli, pepsinogens synthesised by the chief cells ,secreted in the stomach lumen where hydrochloric acid, secreted by the parietal cells, converts them into the corresponding active enzyme pepsins. The stimulus-secreting coupling mechanisms of pepsinogens appear to include at least two major pathways: one involving cAMP as a mediator, the other involving modification of intracellular Ca^{2+}concentration. Physiological or external chemical stimuli acting through the intracellular metabolic adenyl cyclase are more effective in inducing pepsinogen synthesis than those acting through intracellular Ca^{2+}. The activation of protein kinase C (PK-C) would appear to be involved in regulatory processes (Gritti *et al*., 2000).

According to the biochemical and immunological characteristics, pepsin could be divided into two subtypes PG I (PGA) and PG II (PGC). PG I was mainly secreted by the primary cells of the fundic glands and mucous neck cells, while PG II was not only secreted by the fundic glands, but also could be the gastric antrum and proximal duodenum Brunner gland secretion. PG, which is called "serological biopsy", is a valuable indicator of gastric mucosa secretion function (Zhang *et al*., 2012).

Low pH allows pepsinogen to cleave itself and form active pepsin. When it reaches the duodenum, though, it assumes an inactive form as the pH rises above 6. Nonetheless, protein digestion continues to take place throughout the small intestines via the effects of pancreatic enzymes: trypsin, chymotrypsin, elastase, and carboxypeptidase. It is worth mentioning that pepsin remains structurally stable until at least a pH of 8. Therefore, it can always be reactivated as long as pH remains below 8 (Bardhan *et al*., 2012). pepsinogen is converted to pepsin by a loss of the N terminal sequence consisting of a variable number of amino acids (Kageyama and Takahashi, 1980). Pepsin is a digestive enzyme present in all vertebrates; however minor structural differences occur between species (Peter *et al*., 2019).

Pepsin is an endopeptidase that breaks down dietary proteins reaching the stomach into amino acids. It is function by digesting peptide bonds, the predominant chemical bonds found in proteins. In response to various stimuli, small basophilic cells in the deeper layers of gastric glands, known as Chief cells, produce pepsinogen. Notably, acetylcholine, gastrin, and low pH directly stimulate chief cells to secrete pepsinogen. Besides enhancing chief cell activity, acetylcholine also stimulates parietal cells to produce hydrochloric acid (HCl) via their proton pumps. (Samloff .1989).

It is capable of hydrolyzing peptide bonds of most proteins, mucin being one important exception. Pepsin splits bonds involving phenylalanine, tyrosine, and leucine most readily but can hydrolyze almost all other peptide bonds (Gritti *et al*., 2000).

2.3.5.2. Pancreatic Enzymes.

The pancreatic acinar cells synthesis a variety of digestive enzymes. In transit through the secretory pathway, these enzymes are separated from constitutively secreted proteins and packaged into zymogen granules, which are localised in the apical pole of the cell, stimulation of the cell by secret agogues such as acetylcholine and cholecystokinin, acting at receptors on the basolateral plasma membrane, causes the generation of an intracellular Ca^{2+} signal, this signal, in turn, triggers the fusion of the zymogen granules with the apical plasma membrane, leading to the polarised secretion of the enzymes (Wäsle and Edwardson, 2002).

The acinar cell of the exocrine pancreas has the greatest rate of protein synthesis of any mammalian organ, the acinar cell has a highly developed endoplasmic reticulum (ER) system combined with mechanisms to modify and transport newly synthesized proteins through the secretory pathway as show in (Figure 2- 4) (Case. 1978).

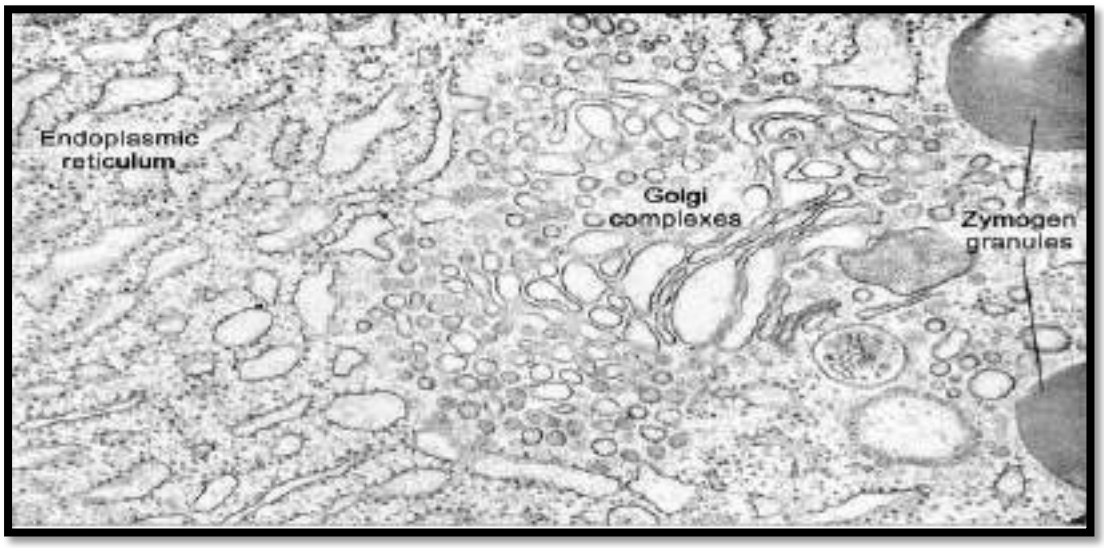


Figure (2-4) Electron micrograph of the pancreatic acinar cell. This electron micrograph shows the key cellular structures involved in synthesis, processing and storage of digestive enzymes (Case. 1978).

The enzymes that are released from the pancreas are excreted ineffectively, and when enter the digestive tract, they become effective and help digest food (Ismail. 2008). There are four types of enzymes that are excreted by the pancreas, they are lipolytic enzymes, Protein-dissolving enzymes such as trypsin and chymotrypsin enzymes, enzymes that degrade nucleic acids Pancreatic nucleases and enzymes analyzing carbohydrates, the most important of which are α -amylase (Ismail. 2008).

In ruminant digestion processes occurring in duodenum are possible due to enzymes secreted by pancreas cells and intestinal lumen. In contrast to monogastric animals, in ruminants the secretion of pancreatic juice and bile is permanent because of the constant flow of digesta to the duodenum, without separating period between feeding (Croom Jr *et al*., 1992).

Lipases (triacylglycerol acylhydrolase) are a group of water soluble enzymes, which exhibit the ability of acting at the interface between aqueous and organic phases. They primarily catalyze the hydrolysis of ester bonds in water insoluble lipid substrates. However, some lipases are also able to catalyze the processes of esterification, interesterification, transesterification, acidolysis, aminolysis and may show enantioselective properties (Stoytcheva *et al*., 2012).

Pancreatic lipase is a key enzyme for lipid breakdown to absorb fatty acids, it's of the exocrine enzymes of pancreatic juice, catalyzes the hydrolysis of emulsified esters of glycerol and long-chain fatty acids. Short-chain fatty acids can be directly absorbed into the blood, while long-chain fatty acids and monoglycerides combine with bile salts to form water soluble micelles as show in (Figure 2- 5) (Shin *et al*., 2003; Akers and Denbow, 2008).

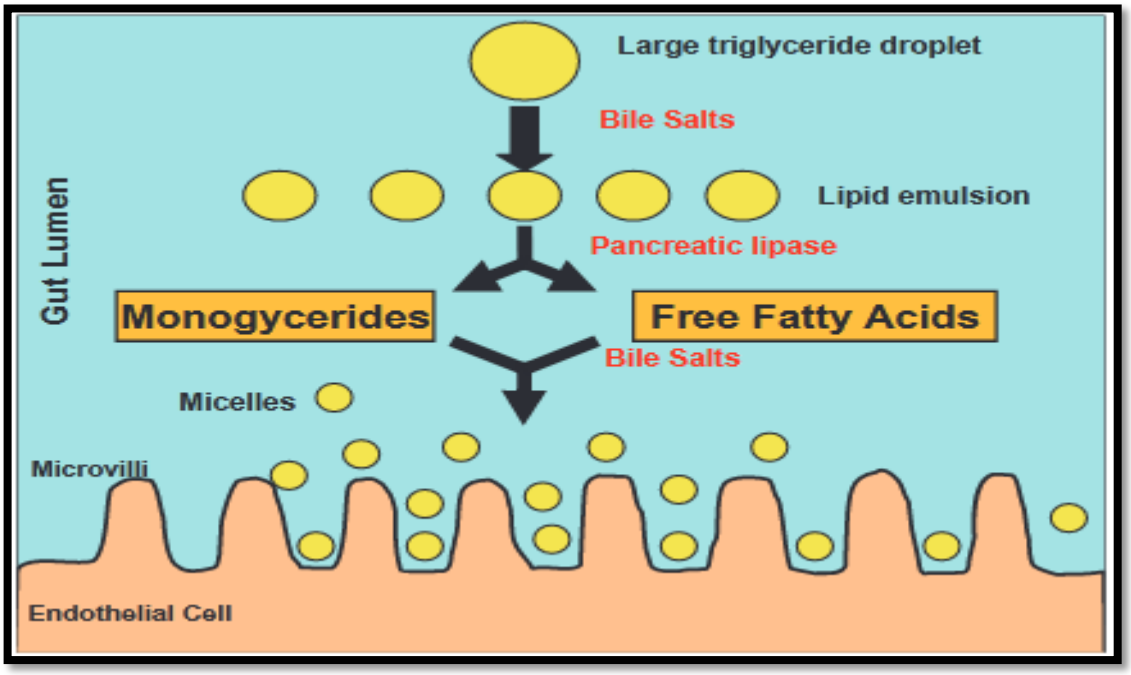


Figure (2- 5) showing Lipid digestion in the small intestine. Lumenal digestion of lipids results in the production of monoglycerides and free fatty acids (Akers and Denbow, 2008) .

There are two types of amylase enzymes, salivary amylase initiates digestion in the mouth and may account for a significant portion of starch and glycogen digestion because it is transported with the meal into the stomach and small intestine, where it continues to have activity, optimal enzyme activity occurs at neutral pH, during a meal, gastric pH can approach neutrality despite gastric acid secretion because of the buffering from molecules in the meal as well as alkaline secretions from the salivary glands and gastric mucus. Salivary amylase can contribute up to 50% of starch and glycogen digestion while pancreatic amylase contributes the remainder (Wright *et al*., 2003).

The action of both salivary and pancreatic amylase is to hydrolyze 1,4-glycoside linkages at every other junction between carbon 1 and oxygen. The products of amylase digestion are maltose and maltotriose (2- and 3-α-1,4-linked molecules, respectively) and α-dextrins containing 1,6-

glycosidic linkages because 1,6-glycosidic linkages in starch cannot be hydrolyzed by amylase as show in (Figure 2- 6) (Wright *et al*., 2003; Akers and Denbow, 2008).

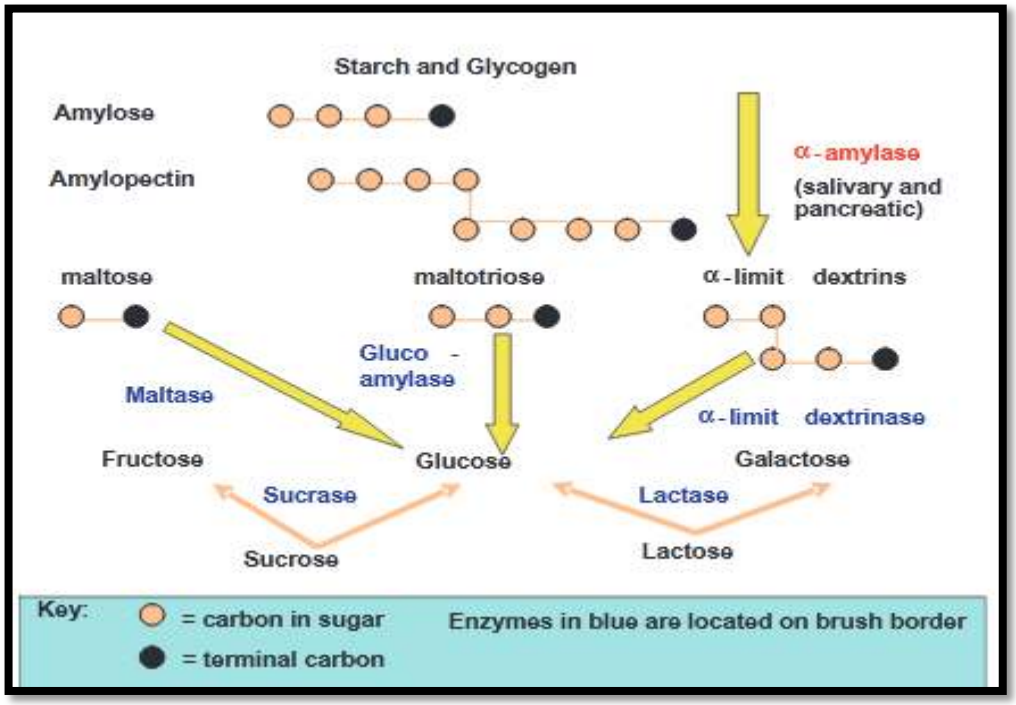


Figure (2- 6) showing Carbohydrate digestion in the small intestine, digestion of carbohydrates results in the production of maltose, maltotriose and α-dextrins (Akers and Denbow, 2008)

Digestion of starch to glucose requires the action of several enzymes produced by the salivary glands, the rumen microorganisms , the pancreas and small intestine. Amylase secreted by the nasolabial glands is found at relatively high levels in the saliva of some ruminants, such as the buffalo (Cerrilla and Martínez, 2003). Alpha-amylase is secreted by the pancreas, while isomaltase, maltase-glucoamylase, trehalase and lactase are secreted by the intestinal mucosa (Harmon, 1993). Alpha-amylase, beta-amylase, R-enzyme, pullulanase, iso-amylase or alpha-limit dextrinase are produced by the rumen microorganisms (Cerrilla and Martínez, 2003).

Chapter Three

Materials and Methods

3. Materials and Methods

3.1. Collection Of Samples

The present study was carried out in the department of biology, College of Sciences University Misan. The study period was from (2019/11/4 to 2020/7/4).The study included 20 adult animals, ten male sheep and ten male rabbits, their weight were from 35-45 kg for sheep and 1.5-2.5 kg for rabbits. The animals collected from local market and slaughterhouses Misan. A physical examination was performed to all animals to guarantee they were all in the right health.

3.1.1. Blood Samples : collected from sheep After the slaughter process and from the heart of rabbits by syringe. Blood was placed into a gel tube, and centrifuged (5000r/m. for 5 minutes) to separate the serum. Serum was stored at -20 ° C for determination of gastrin , PG1 (PG A), PG 11(PG C), amylase and lipase concentrations in the blood.

3.1.2. Surgical Procedures

Before euthanasia rabbits were raised under standard procedures following the animal euthanization protocol. Euthanasia by placed 2 ml of chloroform (CHCl3) on cotton and then placed it on the animal's nose (Blackshaw *et al*., 1988). Then using appropriate tools as scissors, tweezers and scalpels of dissection, then the rabbit's abdomen was incision along the was carefully, and extract (the esophagus, stomach, small intestine) after their wash well with saline solution. While sheep sample taken from slaughterhouses after that, each organ was divided into three sections and took 1 cm from each sections and table (3-1) explain digestive tract organs used in this study.

Table (3-1). The digestive organs their areas and stains used in current study

samples	sheep	rabbit	stains
esophagus	Cervical, thoracic, abdominal	Cervical, thoracic, abdominal	H&E and PAS
stomach	Abomasum (cardiac, fundus, pylorus	(cardiac, fundus, pylorus)	H&E and PAS
Small intestine	Duodenum, jejunum, ileum	Duodenum, jejunum, ileum	H&E and PAS

3.2.Chemicals

The chemicals used in the current study and explain which country and their company

Table (3.2):List of chemicals, and suppliers.

Chemicals	company	Country
Albumin for eggs		Iraq
Canada Balsam	Roth	Germany
Chloroform	Sigma	Switzerland
Charcolal Activated	BDH	England
Ethanol (absolute 100%)	BDH	England
Formalian	BDH	England
Fuchsin Basic	Dakocytomation	Denmark
Glacial acetic acid	BDH	England
Haematoxylin & Eosin	BDH	England
Hydrochloric Acid	Sigma	USA
Normal Saline Solution	Fresenius Kabi	Germany
Paraffin wax	Merck	Germany
Peridic acid	Dakocytomation	Denmark

Sodium metabisulphte	BDH	England
xylene	BDH	England

3.3.Equipments And Their suppliers.

Table (3.3): List of equipments and their suppliers.

Instrument	company	Country
Centrifuge	Janetzki	Germany
Chemiluminescence immunoassay analyzers(CLIA)	mindray	China
Cobas c111	Roche	Germany
Digital Camera	Sony	Chine
Distillator unit	Tglassco	India
Enzyme-linked Immunosorbent Assays (ELISA)		USA
Hot plate	Tglassco	India
Incubator	Binder	USA
knife of microtome	LG	USA
Light Microscope	Olympus	Japan
Microtome	Leitz	Germany
Micro pipetes		Germany
Oven	Binder	Germany
paraffin dispenser		Chine
Refrigerator	LG	USA
Surgical Set	Hebson	India
Water bath	Tafesa-Hannover	Germany

3.4. Laboratory Kit

Table (3.4):List of the kits that used in this study with producing companies and countries

Kit	Company	Origin
Pepsinogen 1	mindray	China
Pepsinogen 11	mindray	China
Lipase	Roche Cobas	Germany
Amylase	Roche Cobas	Germany
Gastrin Sheep	MyBioSource	Canada
Gastrin Rabbit	MyBioSource	Canada

3.4.1. Contents Of Elisa kits

Table (3.5):Gastrin rabbit kit components.

materials	specification	quantity
Microtiter plate	48 wells	stripwell
Enzyme conjugate	3.0 ml	1 vial
Standard A	0 pg/ml	1 vial
Standard B	50 pg/ml	1 vial
Standard C	100 pg/ml	1 vial
Standard D	250pg/ml	1 vial
Standard E	500pg/ml	1 vial
Standard F	1000pg/ml	1 vial
Standard A	6 ml	1 vial
Standard B	6 ml	1 vial
Stop solution	6 ml	1 vial
Wash solution (100x)	10 ml	1 vial
Balance solution	3ml	1 vial
Instruction	1	

Table (3.6):Gastrin sheep kit components.

components	**Quantity**
Standard solution (1600g/L)	0.5ml x1
Pre-coated Elisa plate	12*4 well strips x1
Standard diluent	3 ml x1
Streptavidin-HRP	3 ml x1
Stop solution	3 ml x1
substrate solution A	3 ml x1
substrate solution B	3 ml x1
Wash buffer concentrate (25x)	20 ml x1
Biotinylated sheep GAST antibody	1ml x1
User instruction	1

3.5. Experimental Design

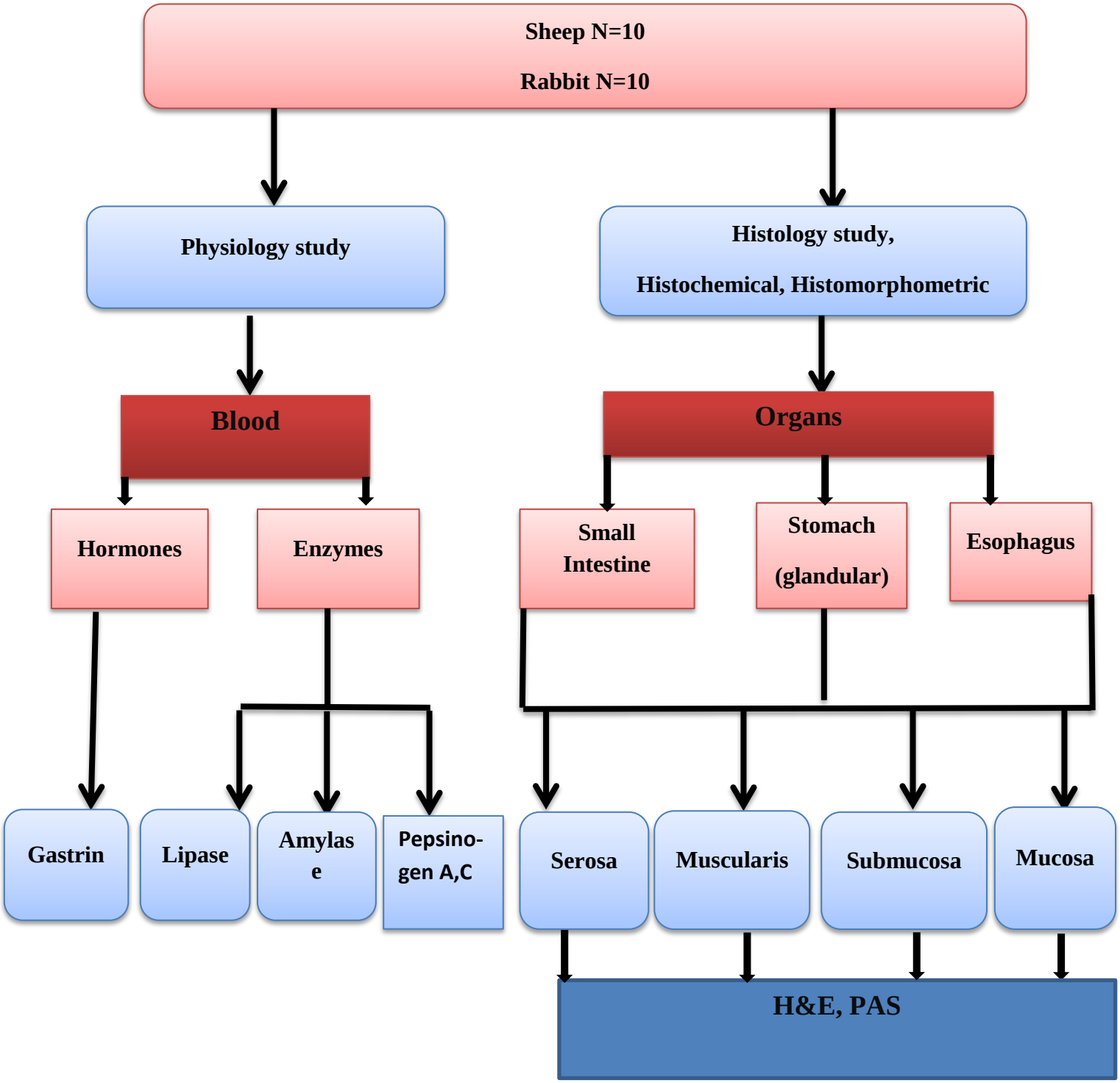


Diagram:Experimental Design

3.7.Methods

3.7.1. Histological Study

All tissue samples that isolated from each sheep and rabbits were processed according to (Luna,1968) for light microscopic study as in follow:

1-fixiation:

10% formalin solution were used to stabilize the samples for 48 h.

2-Washing:

The samples were washed with running water for remove the formalin from samples.

3- Dehydration :

Passing the samples with a series of graded concentration of ethyl alcohol (70%, 2h. 80%, 30min. 96%, three changes, 2h each. 100% absolute, 9h. than 100% hour).

4- Clearing:

Samples was treated with Xylene, two changes, one hr. each.

5- Infiltration and Embedding

The specimens were put in a mixture of clearing solution and pure paraffin wax. After that, specimens were put in wax paraffin at melting point (56 °C) twice passing and replaced the wax by new pure wax for two hr. each, next the tissue is oriented and embedded in paraffin blocks. The samples were embedded in melting paraffin used special containers either metal molds (blocks).

6-Sectioning

The paraffin block were cut with a thickness of 5-7 microns using a rotary microtome, the sections were then transferred to a 40° C water bath for section brushes, then picked up on slides coated with albumin and then placed overnight on a hot plate.

7- Staining

Staining Procedure: Hematoxylin & Eosin staining

Use Hematoxylin and Eosin as a routine histological stain has been used to demonstrate the general component of the tissue (Luna, 1968). (Appendix: 1-2)

1. The slides were dewax by xylene for (5) min.
2. Slides dried from xylene and rehydrated in graded series concentration of alcohol (100%, 95%) for about (3) min in each concentration.
3. Washed with water for (1) min.
4. The sections were stained with hematoxylin type Ehrlich stian for (12) min.
5. Then washed with water, all slides.
6. After that stained with eosin stain type Y for (3) min, followed by washing with water .
7. Then dehydrated in graded series concentrations of alcohol (95%, 100%) for (5-6) second in each concentration.
8. The slides were left in xylene for (5) min.
9. The slides were mounted with mounted material D.P.X (Dextrin-Plastizer_ Xylene), covered and leaved it to dry on the warm plate for a night, then examined with light microscope.

3.7.2. Histochemical Study

Staining Procedure :Periodic acid Schiff stain (PAS).

This stain used for demonstration glycogen in each of the studied organs. (Culling *et al.*,1985). (Appendix: 3)

1. The slides were dewax by xylene for (5)min.
2. Slides dried from xylene and rehydrated in graded series concentration of alcohol (100%, 95%) for about (3) min in each concentration .
3. Bring slides to disilled water.
4. Treat with periodic acid for (5) min.
5. Washing slides well with distilled water.
6. Cover with Schiff's reagent for 5-10 min.
7. Wash in running tap water 5-10 min.
8. Counter stain with Herris hematoxylin for approximately 15 sec.
9. Wash in tap water.
10. Rinse in increasing concentration of alcohol (70%,80%,95%,100)
11. Clear in Xylene.
12. Formation of insoluble magenta colored complex denotes positive result.

After staining procedures the sections were examined under the microscope under magnification power (40x,100x, 400x) and photographs were captured.

3.7.3. Histomorphometric Study

Multiple measurements were made to determine the thickness of the layers mucosa , sub mucosa, muscularis, serosa and villi height using the optical microscope 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).

3.8.Physiological Study

3.8.1.Principle of The Assay of Kits

3.8.1.1.Principle of the Assay of Kit Gastrin Rabbit according to (Crowther . 2001).

1- GAST ELISA kit applies the competitive enzyme immunoassay technique utilizing a polyclonal anti-gast antibody an and GAST-HRP conjugate.

2-The assay sample and buffer are incubated together with GAST-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five time.

3-The wells are then incubated with a substrate for HRP enzyme . the product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow.

4-The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the GAST concentration since GAST-HRP conjugate compete for the anti-GAST antibody binding site. Since the number of sites is limited, as more sites are occupied by GAST from the sample, fewer sites are left to bind GAST-HRP conjugate.

5- A standard curve is plotted relating the intensity of color (O.D.) to the concentration of standard. The GAST concentration in each sample is interpolated from this standard curve.

3.8.1.1.1. Reagent Preparation of Kit Gastrin Rabbit.

1- Bring all kit components and samples to room temperature (20-25° C) before use.

2- Sample-please predicat the concentration before assaying if concentration are unknown or not within the deterction range a preliminary experiments is recommended to determine the optimal dilution PBS (pH7.0-7.2) or 0.9% physiological saline can be used as dilution buffer.

3- Wash solution-dilute 10ml of solution concentrate (100x) with 990 ml of deionized or distilled water to prepare 1000ml of wash solution (1x) if creystals have formed in the concentrate warm to room temperature and mix gently until the crystals have completely dissolved the 1x wash solution is stable for 2 weeks at 2-8 ° C.

4- Do not dilute the other components which are ready to-use.

3.8.1.1.2. Assay Procedure Kit Gastrin Rabbit.

1-Secure the desired numbers of coated wells in the holder then add100µl of standards (shake the bottle of each standard gently by hand and Pipettle up and down the solution of standard for 3 times before adding or samples to the appropriate well add 100µl of PBS (7.0-7.2) in blank control well.

2-Dispense 10µl of balance solution into 100µl samples only mix well.

3-Add 50 µl of conjugate to each well .mix well. Mixing well in this step is important cover and incubate the plate for 1 hour at 37° C.

4- Wash the microtiter plate using one the specified methods indicated below :

A-manual washing :remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. fill in each well completely wash 1x wash solution and then aspirate contents of the plate into a sink or proper waste container repeat this procedure five times for a total of five washes .after washing invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears.

B-automated washing wash plate five time with diluted wash solution (350-400µl\well\wash)using an auto washer after washing dry plate above it is recommended that the washer be set for a soaking time 10 seconds and shaking time of 5second between each wash.

5-Add 50µl substrate A and 50µl substrate B to each well including blank control well subsequently cover and incubate for 15-20 min at 37^0C
6-Add 50µl of stop solution to each well incuding blank control. Well mix well.

7-Determine the Optical Density (O.D) at 450nm using amicroplate reader immediately. (Appendix: 4)

3.8.1.2.Principle of the Assay of Kit Gastrin Sheep according to (Crowther .2001)

1-This kit is an Enzyme-Linked Immunosorbbent Assay (ELISA). The plate has been pre-coated with sheep GAST antibody. GAST present in the sample is added and binds to antibodies coated on the wells.

2- Then biotinylated sheep GAST is added and binds to GAST in sample. Then streptavidin-HRP is added and binds to the biotinylated GASP antibody. After incubation unbound streptavidin-HRP is washed away during a washing step.

3-Substrate solution is then added and color develops in proportion to the amount of sheep GAST. The reaction is terminated by addition of acidic stop solution and absorbance at 450 nm.

3.8.1.2.1. Reagent Preparation of Kit Gastrin Sheep.

1-All reagents should be brought room temperature before use.

2- Standard reconstitiute the 120µl of the standard diluent to generate a 800ng/L standard stock solution. Allow the standard to sit for 15 min with gentle agitation prior to making dilution prepare duplicate standard points by serially diluting the standard stock solution (800ng/L) 1:2 with standard diluent to produce 400ng/L, 200ng/L, 100ng/L and 50ng/L solution. Standard diluent serves as zero standard (0 ng/L) any remaining solution should be frozen at -20 ° C and used within one month. Dilution of standard solution suggested are as follows:

800ng/L	Standard N0.5	120 µl Original standard +120 µl Dilution standard
400ng/L	standard N0.4	120 µl standard No.5 +120 µl Dilution standard
200ng/L	standard N0.3	120 µl standard No.4 +120 µl Dilution standard
100ng/L	standard N0.2	120 µl standard No.3 +120 µl Dilution standard
50ng/L	standard N0.1	120 µl standard No.2 +120 µl Dilution standard

Standard concentration	standard No.5	standard No.4	standard No.3	standard No.2	standard No.1
1600ng/L	800ng/L	400ng/L	200ng/L	100ng/L	50ng/L

3-Wash buffer dilute 20ml of wash buffer concentrate 25x into deionized or distilled water to yield 500 ml of 1x wash buffer if crystals have formed in concentrate mix gently unit the crystals have completely dissolved.

3.8.1.2.2. Assay Procedure Gastrin Hormone In Sheep.

1-Prepare all reagents standard solution and samples as instructed. bring all reagents to room temperature before use the assay is performed at room temperature.

2-Determine the number of strips required for the assay insert the strips in the frames for use the unused strips should be stored at 2-8 ° C.

3-Add 50µl standard to standard well.

4-Add 40µl sample to sample wells and then add 10 µL anti-GAST antibody to sample wells then add 50µL streptavidin-HRP to sample wells mix well. Cover the plate with a sealer, incubate 60 min at 37° C.

5-Remove the sealer and wash the plate 5 times with wash buffer .soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 min for each wash. Automated washing, aspirate all wells and wash 5 times with wash biffer, overfilling wells with wash buffer .blot the plate onto paper towels or other absorbent material.

6-Add 50µl substrate solution A to each well and then add 50µL substrate solution B to each well. Incubate plate covered with a new sealer for 10 min at 37° C in the dark.

7-Add 50µl stop solution to each well the color will change into yellow immediately.

8-Determine the optical density (O.D) value of each well immediately using a microplate reader set to 450nm within 10 min after adding the stop solution. (Appendix: 5).

3.8.1.3.The Principle of the Measurement of the Enzyme Pepsinogen 1 according to to (Dorny and Vercruysse,1998).

1-The CL-sereies pepsinogens 1 assay is two-site sandwich assay to determine the level of pepsinogens 1.

2-In the first step, sample, paramagnetic microparticles coated with monoclonal anti-PG 1 antibody (mouse) and monoclonal anti-PG 1 antibody (mouse) –alkiline phosphatase conjugate are added into a reaction cuvette. After incubation, PG 1 present in the sample binds to both anti-PG 1 antibody coated microparticles and anti-PG 1 antibody (mouse) –alkiline phosphatase-labeled conjugate to form a sandwich complex .

3-Microparticles are magnetically captured while other unbound substraances are removed by washing .

4-In the second step, the substrate solution is added to the reaction cuvette.

5-It is catalyzed by anti-PG 1 antibody (mouse) –alkiline phosphatase conjugate in the immunocomplex retained on the microparticles .

6-The resulting chemiluminescent is measured as relative light unite (RLUs) by photomultiplier built inside the system.

7-The amount of PG 1 present in the sample is proportional to the relative light units (RLUs) generated during the reaction . the PG concentration can be determined via a calibration curve.

3.8.1.4.The Principle of the Measurement of the Enzyme Pepsinogen 11 according to (Dorny and Vercruysse,1998).

1-The CL-sereies pepsinogens 11 assay is two-site sandwich assay to determine the level of pepsinogens 11.

2-In the first step, sample, paramagnetic microparticles coated with monoclonal anti-PG 11 antibody (mouse) and monoclonal anti-PG 11 antibody (mouse) –alkiline phosphatase conjugate are added into a reaction cuvette. After incubation, PG 11 present in the sample binds to both anti-PG 11 antibody coated microparticles and anti-PG 11 antibody (mouse) –alkiline phosphatase-labeled conjugate to form a sandwich complex.

3-Microparticles are magnetically captured while other unbound substraances are removed by washing .

4-In the second step, the substrate solution is added to the reaction cuvette.

5-It is catalyzed by anti-PG 11 antibody (mouse) –alkiline phosphatase conjugate in the immunocomplex retained on the microparticles .

6-The resulting chemiluminescent is measured as relative light unite (RLUs) by photomultiplier built inside the system.

7-The amount of PG 11 present in the sample is proportional to the relative light units (RLUs) generated during the reaction . the PG concentration can be determined via a calibration curve.

3.8.1.5.The Principle of the Measurement of the Enzyme Lipase according to (Gargouri *et al.*,1983) .

1-Enzymatic colorimetric assay with 1,2-O-dilauryl-rac-glycero-3-glutaric-acid-(6-methylresorufin) ester as substrate.

2-The chromogenic lipase substrate1,2-O-dilauryl-rac-glycero-3-glutaric-acid-(6-methylresorufin)ester is cleaved by the catalytic action of alkaline lipase solution to form 1,2-O-dilauryl-rac-glycerol and an unstable intermediate, glutaric acid-(6-methylresorufin)ester ,this decomposes spontaneously in alkaline solution to form glutaric acid and methylresorufin, addition of detergent and colipase increases the specificity of the assay for pancreatic lipase.

3-The color intensity of the red dye formed is directly proportional to the lipase activity and can be determined photometrically

1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin)ester

lipase → 1,2-O-dilauryl-rac-glycerol + glutaric acid-(6-methylresorufin)ester

glutaricacid-(6-methylresorufin) ester spontaneous decomposition → glutaricacid+ methylresorufin.

3.8.1.6. The Principle of the Measurement of the Enzyme Amylase according to (Kurrle-Weitenhiller *et al*., 1996)

1-Enzymatic colorimetric assay. Defined oligosaccharides such as 4,6-ethylidene-(G7)p-nitrophenyl-(G1)-α, D-maltoheptaoside (ethylidene-G7PNP) are cleaved under the catalytic action of α-amylases.

2-The G2PNP, G3PNP and G4PNP, fragments so formed are completely hydrolyzed to p-nitrophenol and glucose by α-glucosidase, simplified reaction scheme.

3-The color intensity of the p-nitrophenol formed is direct lyproportional to the α-amylase activity, it is determined by measuring the increase in absorbance

5 ethylidene-G7PNP[a]+5H2O α-amylase →

2ethylidene-G5+2G2PNP+2ethylidene-G4+2G3PNP+ethylidene-G3+G4PNP

2G2PNP+2G3PNP+G4PNP+14H2O5 α-glucosidase → PNP+14G[b]

PNP=p-nitrophenol/ G=Glucose

3.9. Statistical Analysis

The values expressed as mean ± SD. The statistical analysis of data was performed to know the significant differences using analysis of T-test by (SPSS) to show the important statistic and significant differences limited on $P<0.05$ of probability (Al-Rawi and Khalaf Allah, 2000).

Chapter Four

Results

4.Results

4.1.Morphological study

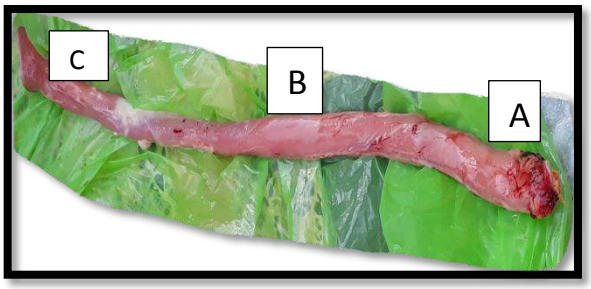


Fig (3-1) Gross photograph showing esophagus in sheep(A) cervical, (B) thoracic, and (C) abdominal regions.

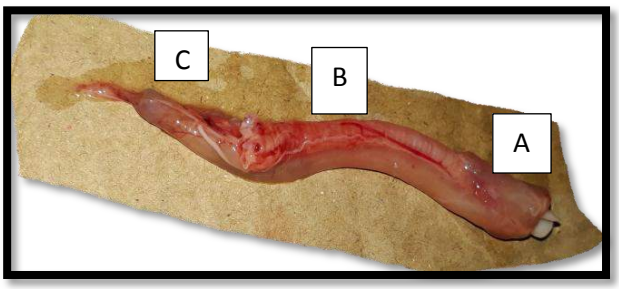


Fig (3-2) Gross photograph showing esophagus in rabbit (A) cervical, (B) thoracic, and (C) abdominal regions

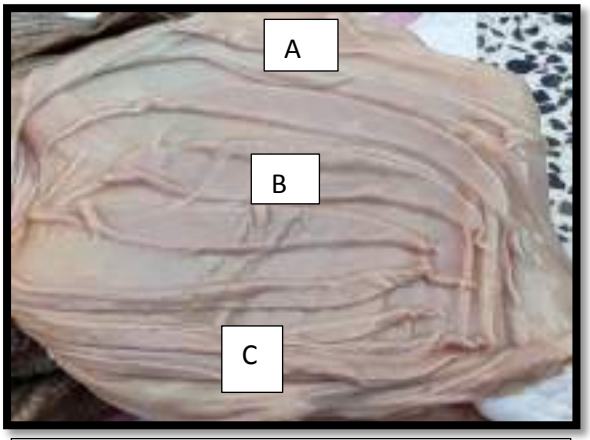


Fig (3-3) Gross photograph showing stomach (abomasum) in sheep (A) cardiac, (B) fundus, (C) pylorus regions.

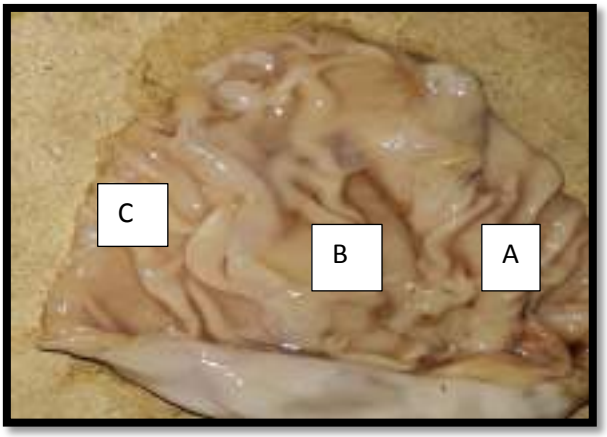


Fig (3-4) Gross photograph showing stomach in rabbit sheep (A) cardiac, (B) fundus, (C) pylorus regions.

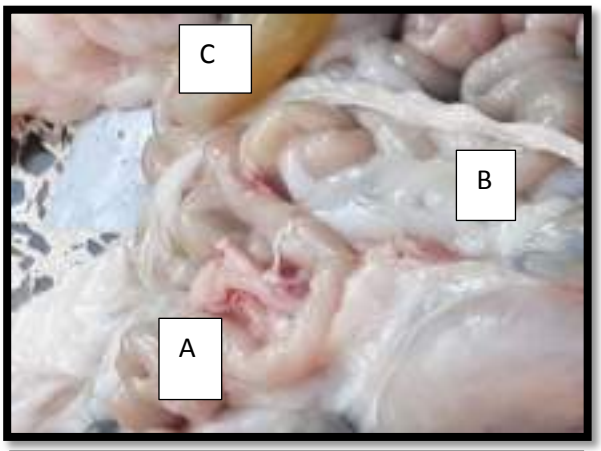


Fig (3-5) Gross photograph showing small intestine in sheep (A) duodenum, (B) jejunum and (C)ileum regions.

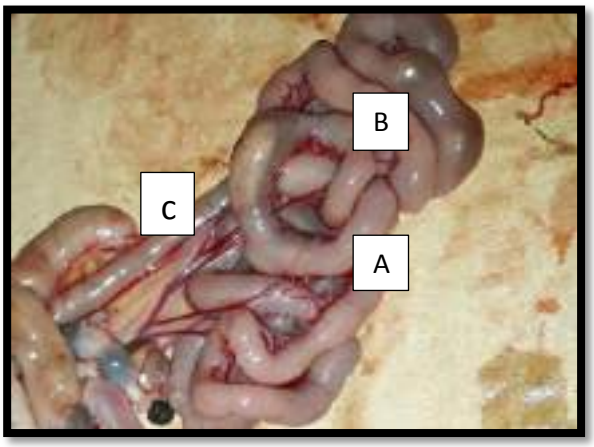


Fig (3-6) Gross photograph showing small intestine in rabbit(A) duodenum, (B) jejunum and (C)ileum regions.

4.2.Esophagus.

4.2.1.Histological study:

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

The epithelium layer of the sheep esophagus was consisted of four layers. Stratum basale has a cuboidal or low columnar form and basiophilic cytoplasm, while the last three strata (spinosum, stratum granulosum and stratum corneum) have varied forms and are full of keratin (Figure 4-3).

On the other hand, the epithelium of the esophagus of the rabbit is formed of three layers. Basale layer is cuboidal or low columnar cells and is located in the under of the stratified epithelium, middle layer of the epithelium is polyhedral cells and the surface layer is flattened squamous cells lack keratin (Figure 4-4).

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 (Figure 4-5 and 4-6).

The muscularis mucosae consisted of smooth muscle fiber arranged longitudinally and it is more thickness in sheep than in rabbit (Figure 4-1and 4-2). The muscularis mucosa was located between lamina propria and sub mucosa and it was identifiable along length of esophagus.

In both sheep and rabbits, the submucosa layer has loose connective tissue composed from interwoven collagen fiber, elastic fiber, fibrocytes, lymphocytes, and blood vessels with the presence of the adipose tissue in sheep thickness of the rabbit. Also, no submucosal glands were observed throughout the length of the esophagus for both animals.

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 muscular layer was composed of striated muscle throughout the cervical, thoracic (Figure 4-7 and 4-8), and abdominal region (Figure 4-11 and 4-12).

Adventitia layer was an external layer that covered the esophagus. It was composed of a loose connective tissue (Figure 4-9 and 4-10), it gradually transformed into serosa layer in abdominal region which it composed of loose connective tissue and a mesothelium layer .

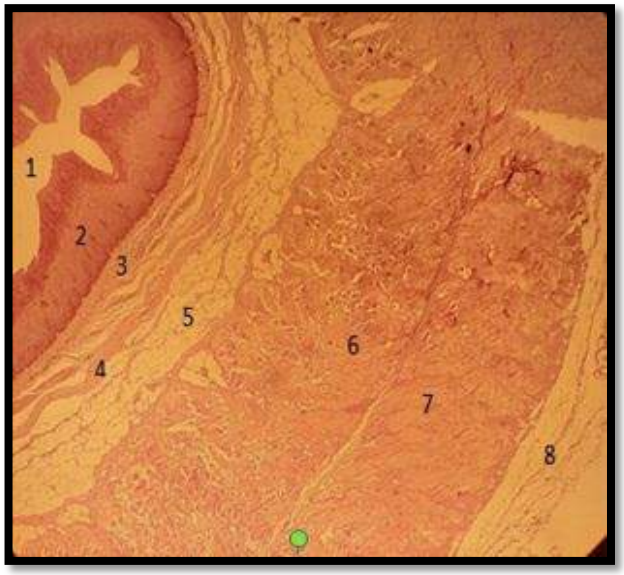


Fig (4-1) The cervical esophagus of the sheep showing (1) lumen, (2) stratified squamous epithelium layer (keratinized) ,(3) lamina properia, (4) muscularis mucosa, (5) sub mucosa. Muscularis layer(6) circular muscularis (7) longtuduinl, (8)serosa. H&E.40X

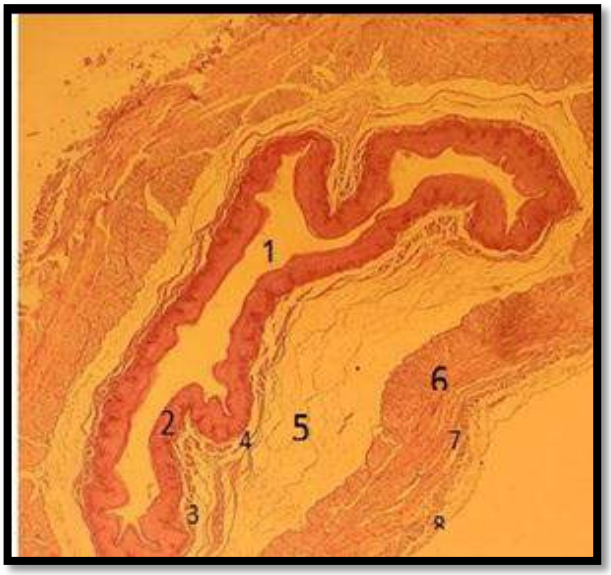


Fig (4-2) The cervical esophagus of the rabbit showing (1) lumen,(2) stratified squamous epithelium layer (non-keratinized) ,(3) lamina properia, (4) muscularis mucosa, (5) sub mucosa. Muscularis layer (6) circular muscularis, (7) longtuduinl, (8) serosa. H&E.40

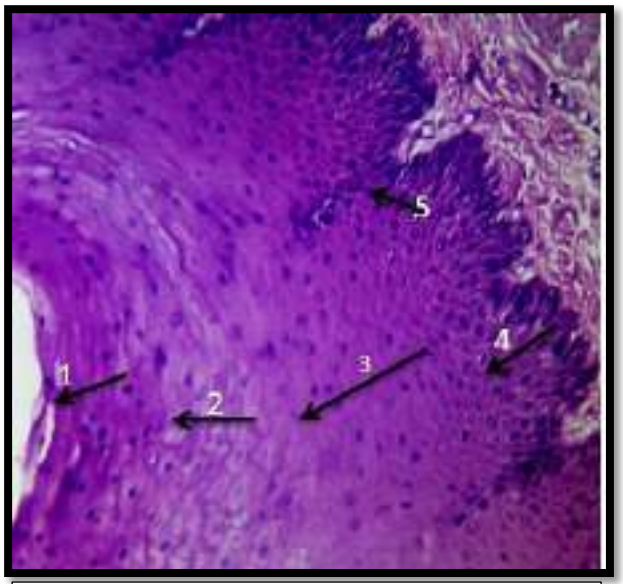


Fig (4-3) The thoracic esophagus of the sheep showing a stratified squamous epithelium layer (keratinized) include four layers (1) Stratum corneum full keratin, (2) Stratum granulosum ,(3) Stratum spinosum,(4) stratum basale, and the(5) dermal papillae .H&E .100X.

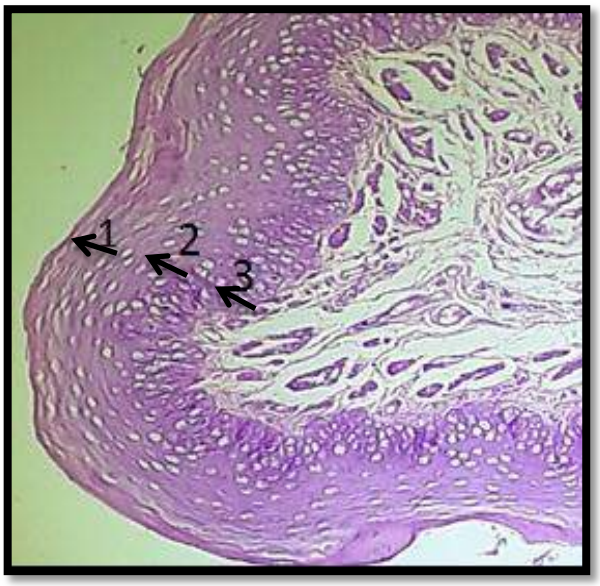


Fig (4-4) The thoracic esophagus of the rabbit showing a stratified squamous epithelium layer (non-keratinized) consist of three layers (1) surface layer, (2) middle layer, and (3) basale layer .H&E .100X.

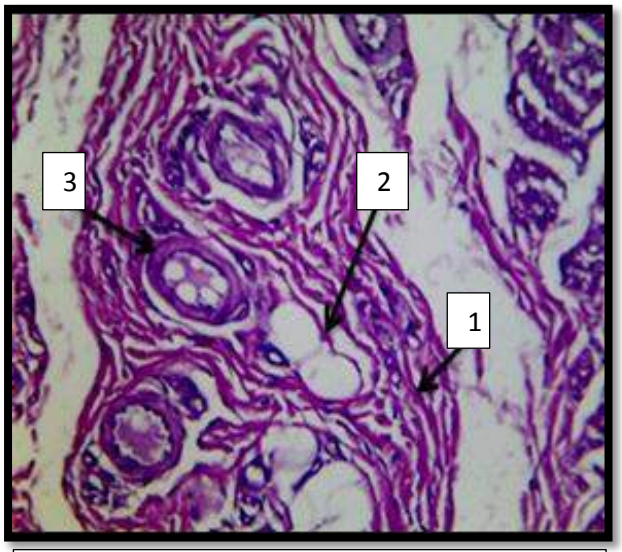


Fig (4-5) The thoracic esophagus of the sheep showing lamina properia consist of loose connective tissue contains (1) fiber,(2) fat cells, (3) blood vessels.100X. H&E

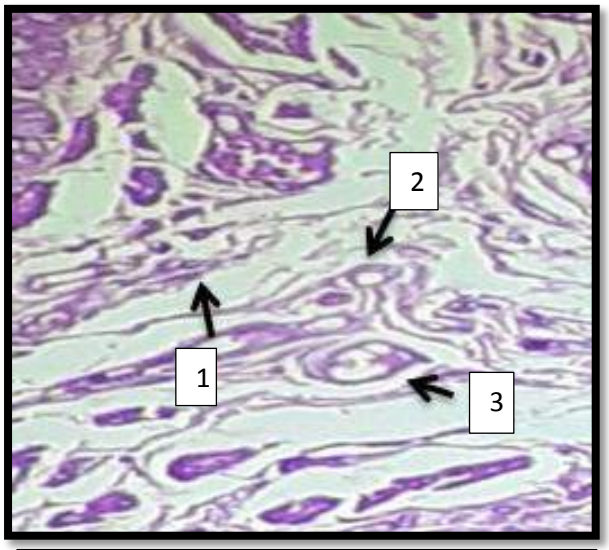


Fig (4-6) The thoracic esophagus of the rabbit showing lamina properia consist of loose connective tissue contain (1) fiber,(2) fat cells, (3) blood vessels.100X. H&E

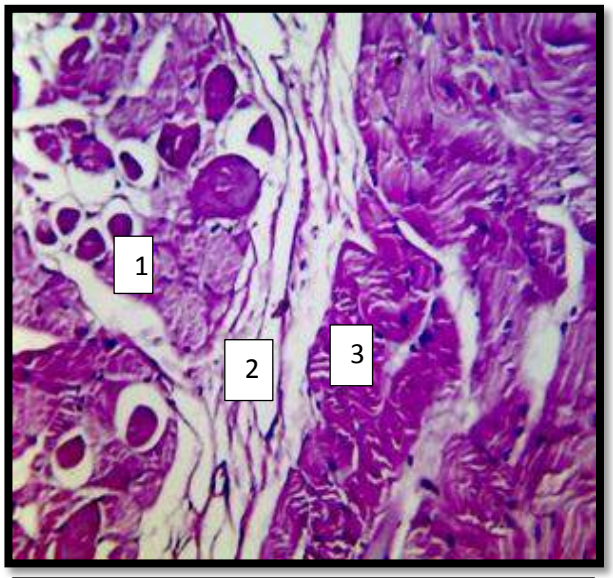


Fig (4-7) The thoracic esophagus region of the sheep showing (skeletal muscle) (1) circular muscularis, (2) connective tissue, (3) longtudunial muscularis. H&E.100x

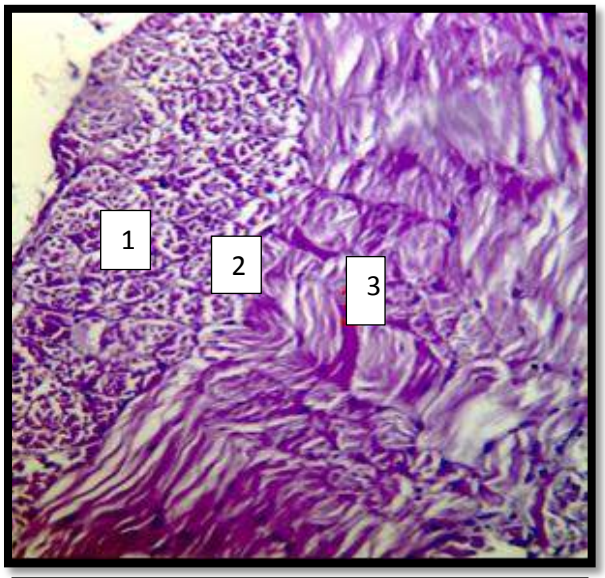


Fig (4-8) The thoracic esophagus region of the rabbit showing (skeletal muscle) (1) circular muscularis, (2) connective tissue,(3) longtudunial muscularis. H&E.100x

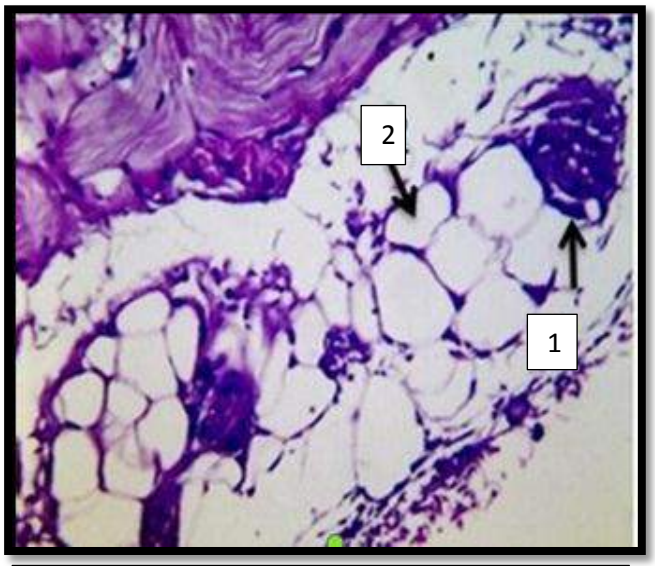


Fig (4-9)The thoracic esophagus of the sheep showing serosa consist of (loose connective tissue) contains (1) blood vessels (2) fat cells. 100X. H&E

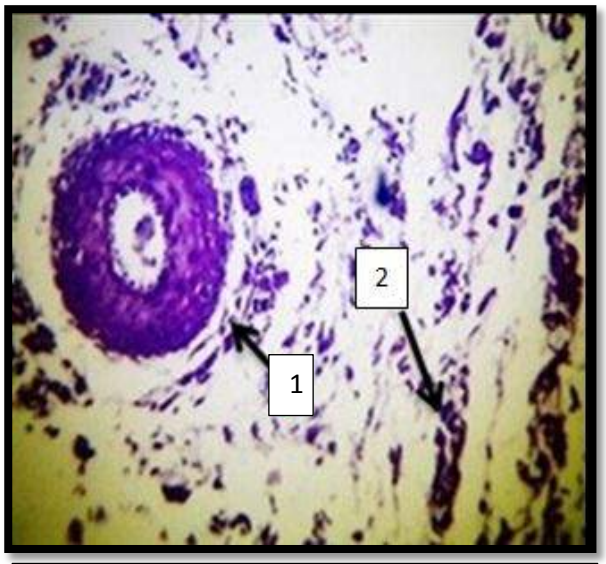


Fig (4-10) The thoracic esophagus of the rabbit showing serosa consist of (loose connective tissue) contains (1) blood vessels,(2) fibers . 100X.H&E

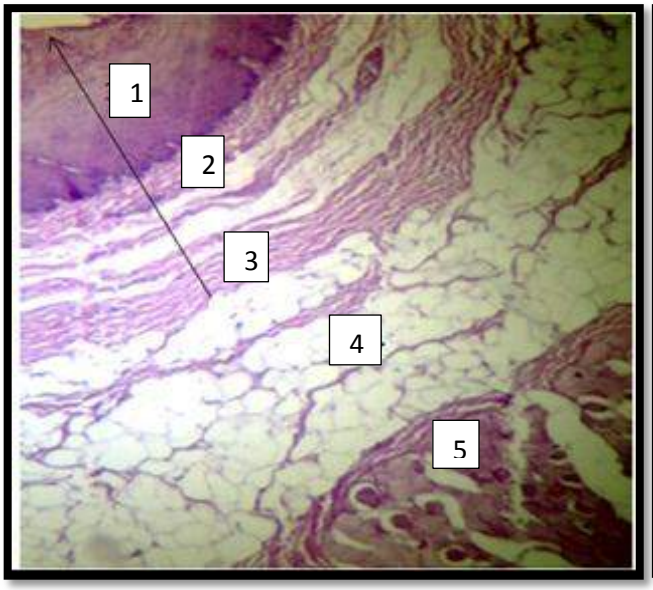


Fig (4-11) The abdominal esophagus region of sheep showing (arrow black) mucosa include (1) stratified squamous epithelium layer (keratinized layer),(2) lamina properia,(3)musculari mucosa, and (4)sub mucosa, (5) muscularis .H&E 100X

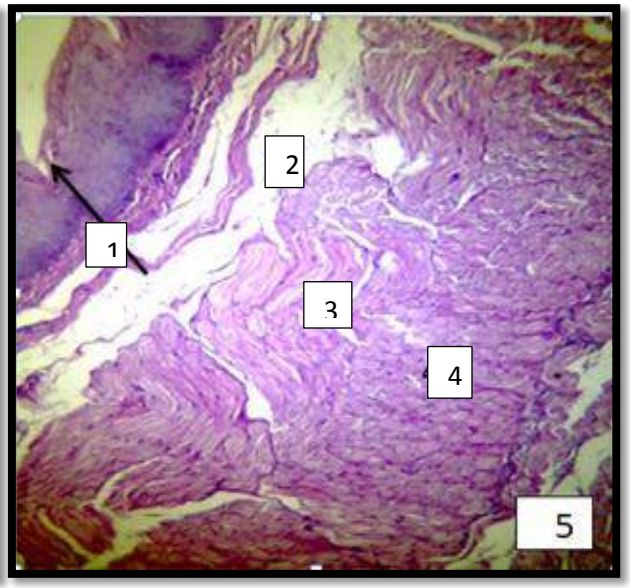


Fig (4-12) The abdominal esophagus region of the rabbit showing(1) mucosa,(2)sub mucosa. skeletal muscle include (3) circular muscularis, (4)longtudunial muscularis, and (5)serosa H&E 100X .

4.2.2.Histomorphometeric 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), (Table 4-1) respectively.

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.

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), (Table 4-1) respectively.

There were non-significant ($p>0.05$) differences between the thickness of the serosa in sheep and rabbit in the cervical and thoracic part(Table 4-1). 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 4-1).

Table (4-1): Mean thickness of mucosa, submucosa, muscularis, and adventitia in Cervical, Thoracic and Abdominal regions of the esophagus of the sheep and rabbit. (n= 10)

Thickness	Mucosa		Sub mucosa		Muscularis		Serosa	
Esophagus	Sheep	Rabbit	Sheep	Rabbit	Sheep	Rabbit	Sheep	Rabbit
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

*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.

4.2.3.Histochemical study:

The results showed that the stratum corneum of stratified squamous keratinized of the mucosa layer had a strong reaction with PAS (Figure 4-13). In contrast, the rest the strata (spinosum, stratum granulosum except stratum corneum) of the epithelium layer had a moderate response with PAS in all regions of the sheep esophagus (Figures 4-15, 4-17, and 4-19). In the rabbit, surface layer of the epithelium layer showed a strong reaction with PAS (Figure 4-14), but the rest layers of epithelium layer was moderate reaction with PAS in all regions rabbit esophagus (Figure 4-16,4-18, and 4-20). In both animals, the submucosa showed weakly reaction with PAS in each region's esophagus in the cervical (Figures 4-15 and 4-16), thoracic (Figures 4-17 and 4-18), and abdominal (Figures4-19 and 4-20).

In sheep and rabbit, the musculuris layer reacted moderately with PAS in cervical, thoracic, and abdominal esophagus regions (Figure 4-19 and 4-20). However, the serosa showing a weakly reaction with PAS in cervical, thoracic, abdominal regions in both sheep and rabbit .

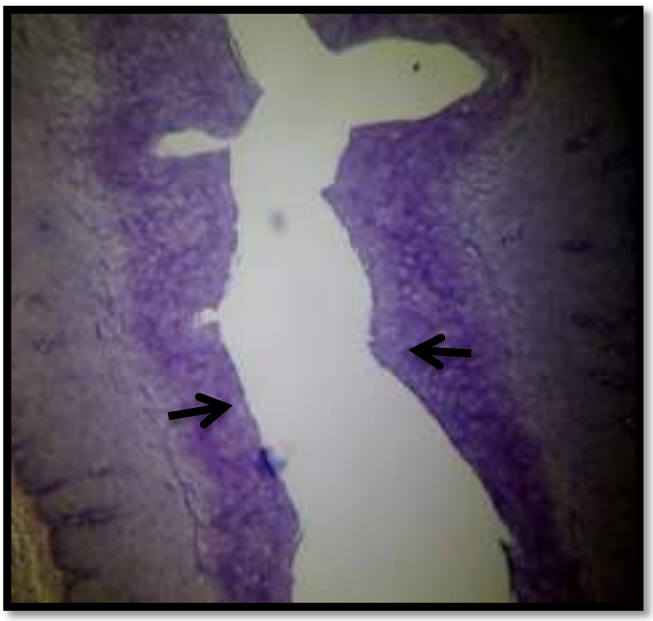


Fig (4-13) The cervical esophagus of the sheep showing (black arrow) Stratum coruneum of keratinized stratified squamous epithelium layer with a strong reaction with PAS100X

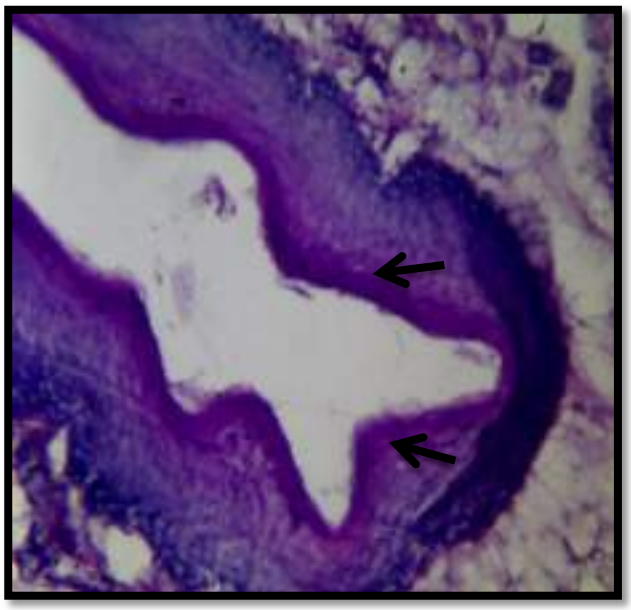


Fig (4-14) The esophagus of the rabbit showing (black arrow) Squamous layer of non- keratinized stratified squamous epithelium layer with strong reaction with PAS 100X.

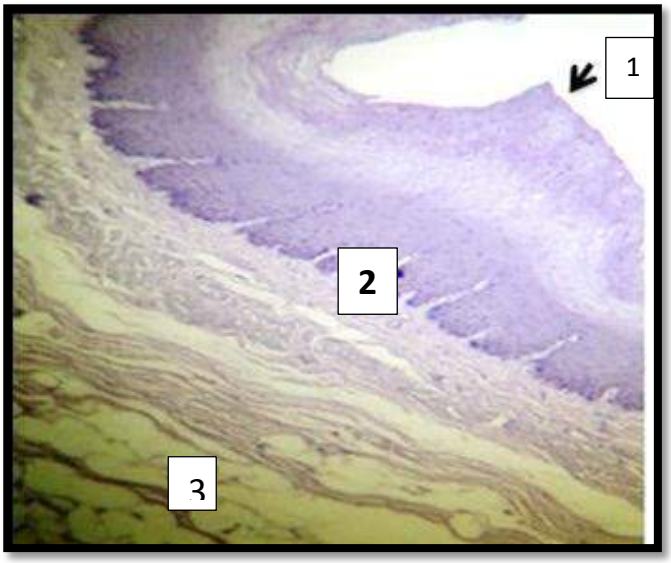


Fig (4-15) The cervical esophagus region of the sheep showing (1)(black arrow) stratum corunum with a strong reaction with PAS . (2) mucosa layer (except for stratum corunum) with a moderate reaction with PAS and (3) sub mucosa with weak reaction with PAS .100X.

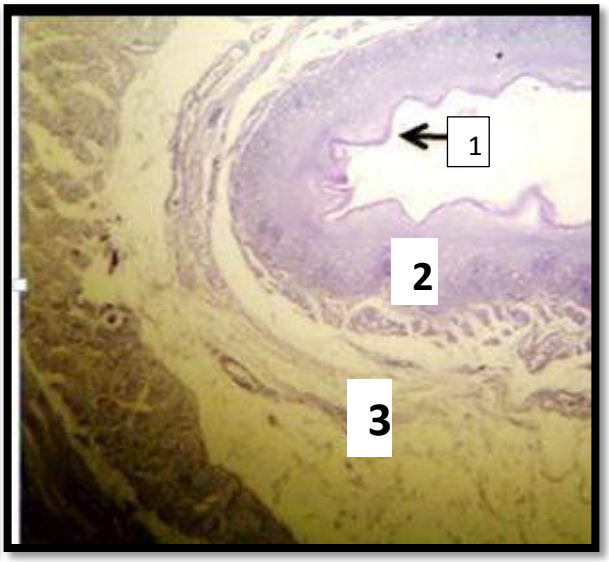


Fig (4-16) The cervical esophagus region of the rabbit showing (1)(black arrow) squamous cells strong reaction with PAS ,(2) mucosa layer (except for surface layer) with a moderate reaction with PAS and (3) sub mucosa layer with a weak reaction with PAS .100X.

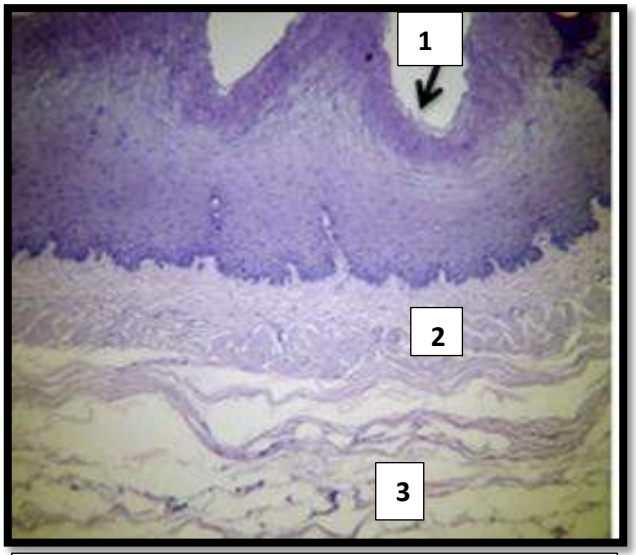


Fig (4-17) The thoracic esophagus region of the sheep showing (1)(arrow black) stratum corunum with a strong reaction with PAS , (2) mucosa layer (except for stratum corunum) with a moderate reaction with PAS , (3) sub mucosa with a weak reaction with PAS .100X

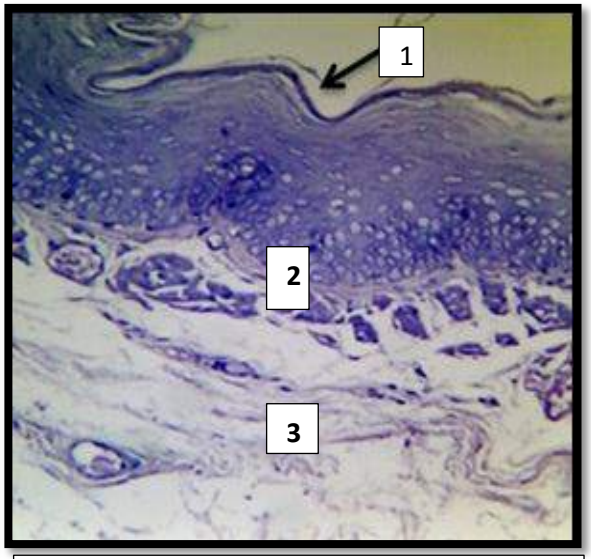


Fig (4-18) The thoracic esophagus region of the rabbit showing (1)(arrow black) surface layer strong reaction with PAS ,(2) mucosa layer (except for surface layer) with a moderate reaction with PAS, (3) sub mucosa with a weak reaction with PAS .100X

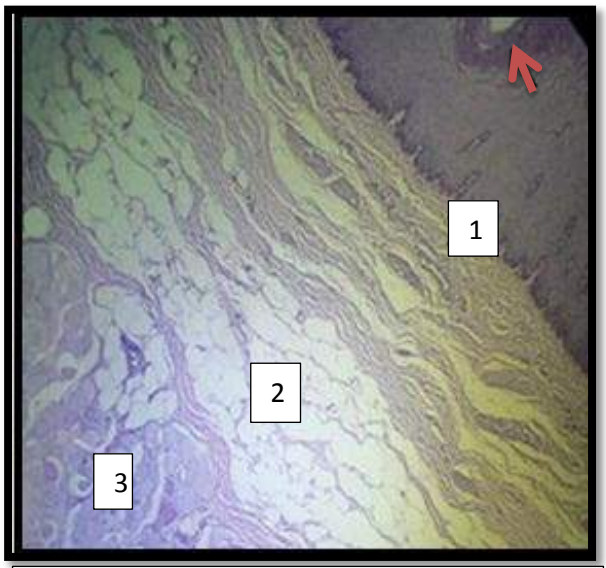


Fig (4-19) The abdominal esophagus region of the sheep showing (red black) stratum corunum with a strong reaction with PAS , (1) mucosa layer (except for stratum corunum) with moderate reaction and (2) sub mucosa with weak reaction with PAS,(3) muscularis with moderate reaction with PAS .100X

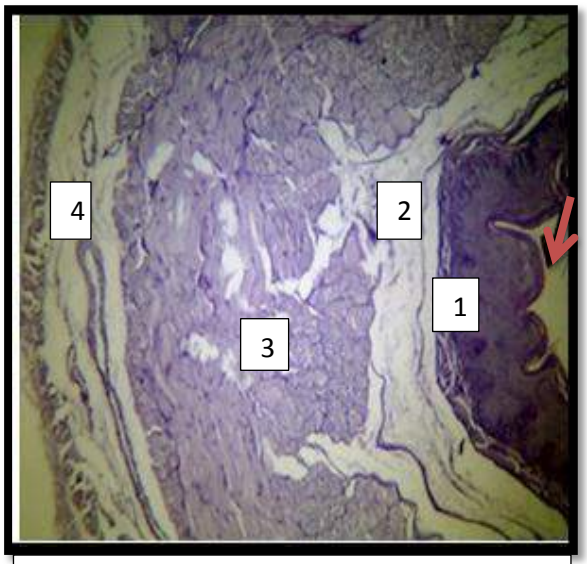


Fig (4-20) The abdominal esophagus region of the rabbit showing (red black) surface layer strong reaction with PAS ,(1) mucosa layer (except for surface layer) with moderate reaction, (2) sub mucosa and serosa gave weakly reaction with PAS,(3) muscularis with moderate reaction with PAS .100X

4.3. Stomach

The stomach in sheep composed from four compartments: rumen, reticulum, omasum, and abomasum. The rumen, reticulum and omasum form the fore-stomach (proventriculus) which non-glandular, who mucosa is lined by a stratified squamous keratinized epithelium. The abomasum (glandular stomach) has a simple columnar epithelium.

4.3.1.Histological study:

The glandular stomach (abomasum) in sheep and rabbit stomach composed of four layers (tunics): mucosa, sub mucosa, muscularis, and serosa (Figure 4-21 and 4-22), the mucosa layer consist of epithelium, lamina propria, and muscularis mucosae in both animals .

The glandular stomach contains folds called rugae are longitudinal in rabbit, while sheep have spiral folds. In sheep and rabbits, the epithelium lining consisted of simple columnar epithelium, the epithelium is invaginated into the lamina propria to form gastric pits (Figure 4-21 and 4-22).

In both animals, the gastric pit deep vary between stomach regions, in sheep and rabbit, the lamina propria were formed from loose connective tissue contain blood vessel, lymphocytes, elastic fibers, collagen fibers and glands.

The muscularis mucosa consisted of smooth muscle fibers arranged longitudinally, it was more thickness in rabbit than in the sheep (Figure 4-21 and 4-22).

In both sheep and rabbits, the sub mucosa layer has loose connective tissue and contains blood vessels, lymphatic vessels, fat cells, and nerve fibers called (Meissner) plexuses (Figure 4-21 and 4- 22). Also, no

submucosal glands were observed throughout (cardiac, fundus and pyloric region) of sheep and rabbits stomach.

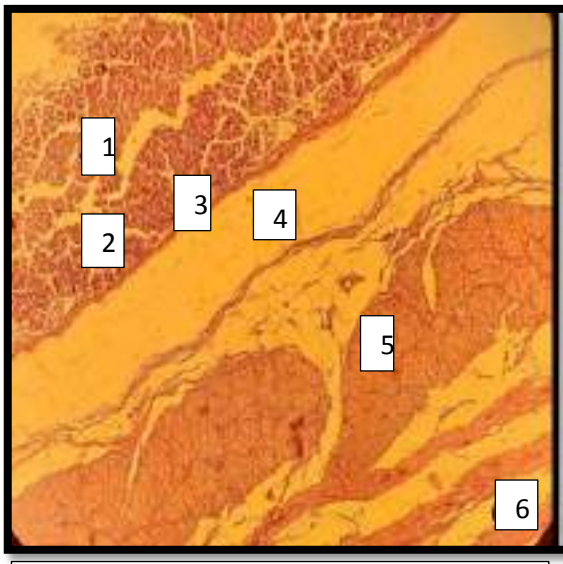
In both animals, the muscularis layer was composed of two layers of smooth muscle fibers, a relatively thick inner circular layer and a thin outer longitudinal layer between them connective tissue. The serosa layer in sheep and rabbit consist of loose connective tissue cover with mesothelium.

Depending on the type of glands in the stomach, the stomach divided to three areas cardiac, fundus and pylorus. The cardiac glands in sheep are simple tubular (Figure 4-23) while in rabbit simple, coiled and branched tubular (Figure 4-24) gastric pits in the cardiac region were deep, and the glands in these the region form of mucous cells and that had shape tall columnar that secrete mucous and lysozymes (Figure 4-25 and 4- 26), some parietal cells have also been observed in this region, which secret hydrochloric acid.

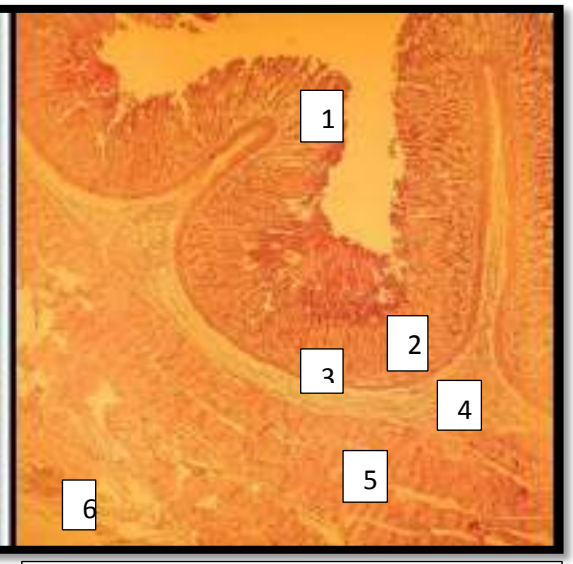
The fundus glands in sheep were, simple and long straight tubular (Figure 4-27) but, in the rabbits it showed simple, straight and branched tubular (Figure 4-28) and these glands made up of cells of the most important call cells parietal cells and that had shape pyramid with eosinophilic cytoplasm and circuit nucleus, which distributed in the fundic glands were filled with strong eosinophilic granules in the cytoplasm (Figure 4-27 and 4-28) too there are the chief cells have circuit shape but basophilic cytoplasm (Figure 4-27 and 4- 28) that secrete pepsinogen.

The pylorus glands in sheep simple, branched tubular and coiled (Figure 4-29), in the rabbits were simple tubular (Figure 4-30), these glands composed of mucus-secreting cells tall columnar also have

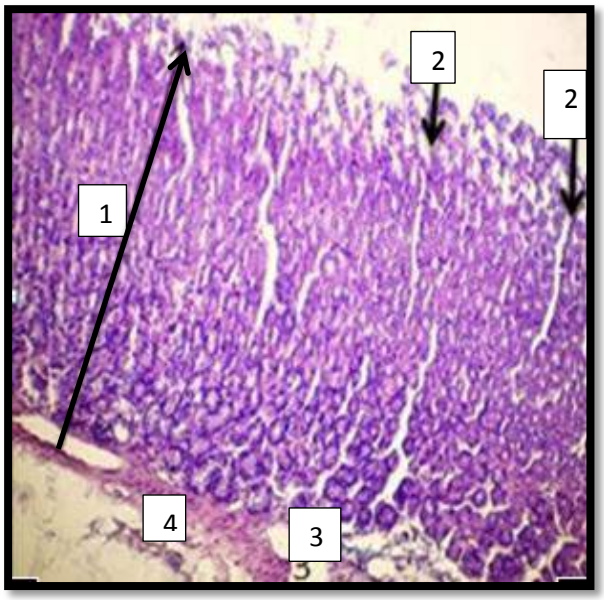
basophilic cytoplasm and contain flattened basally located nuclei, mucus-secreting cells and few of parietal cells.



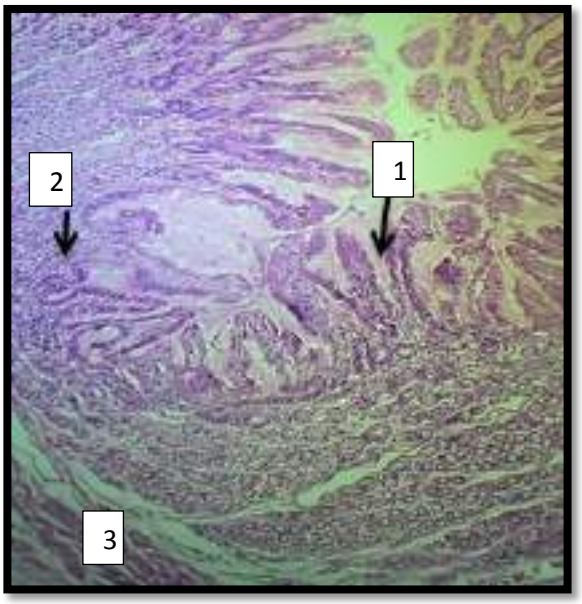
Fig(4-21) The stomach (abomasum part)-cardiac of the sheep showing layers (1) epithilum (simple columner) ,(2) lamina properia, (3) muscolaris mucosa,(4) submucosa,(5) muscularis , (6) serosa .H&E.40X



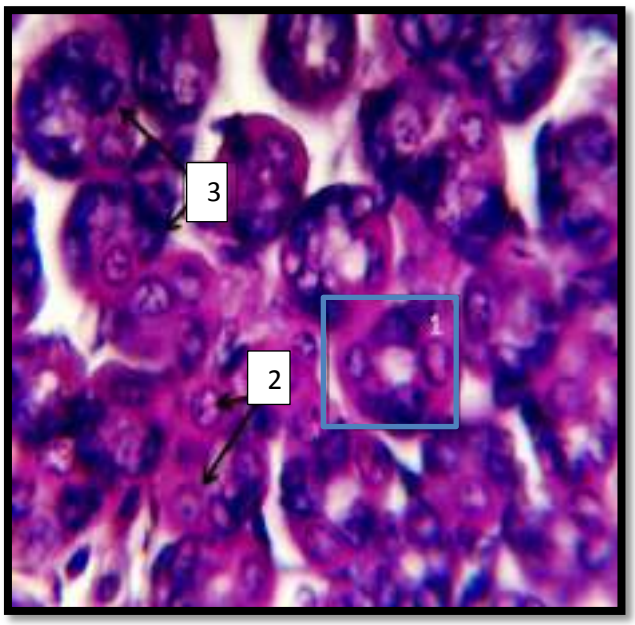
Fig(4-22) The stomach-fundus of the rabbit showing layers (1) epithilum (simple columner) ,(2) lamina properia, (3) muscolaris mucosa,(4) submucosa,(5) muscularis , (6) serosa .H&E.40X



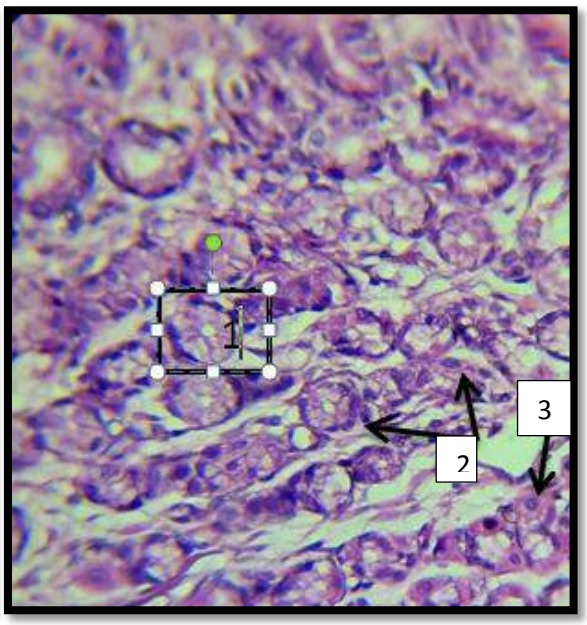
Fig(4-23) The sheep stomach -cardiac region showing (1) mucosa layer ,(2) gastric pit ,(3) lamina properia, (4) muscularis mucosa. H&E.100X



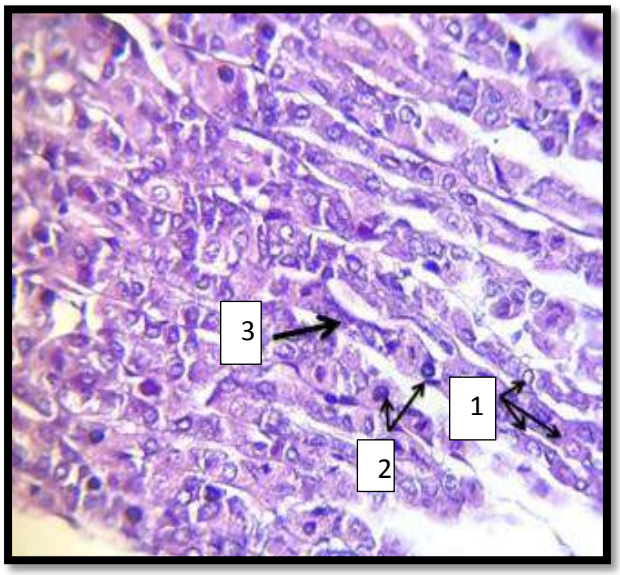
Fig(4-24) The rabbit stomach -cardiac region showing(1) gastric pit,(2) gastric glands, (3) muscularis mucosa .H&E.100X



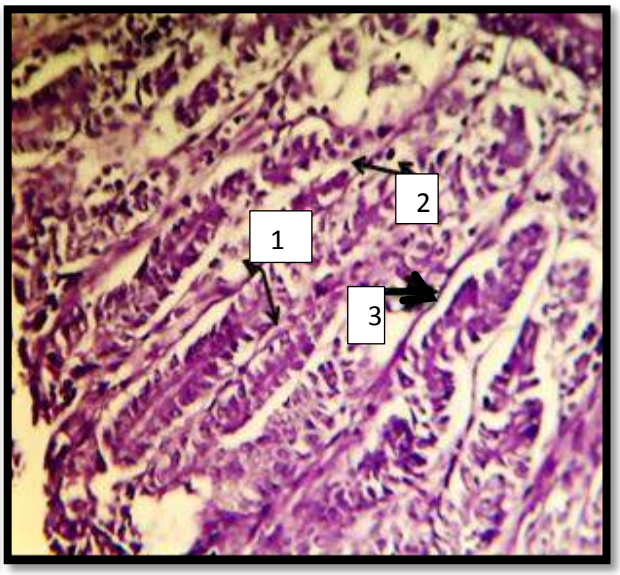
Fig(4-25) The cardiac gland of the sheep stomach(1) consist of (2) paretial cell. (3) mucus secret cell.H&E.400X



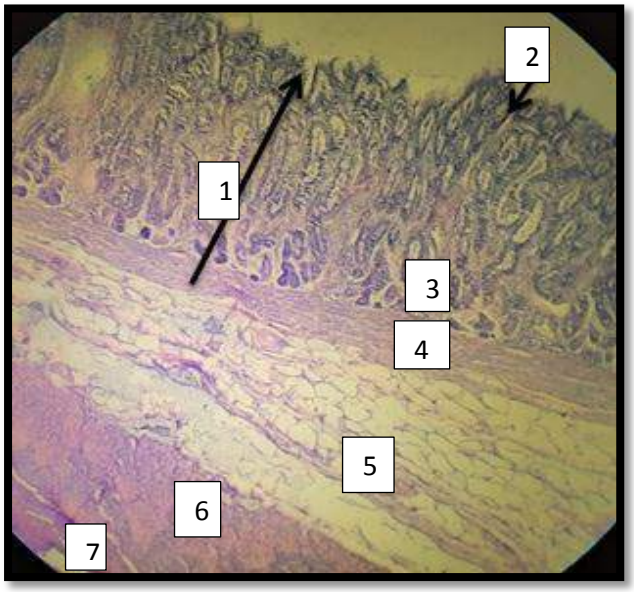
Fig(4-26) The cardiac gland of the rabbit stomach(1) consist from , (2) mucus secret cells, (3) paretial cell .H&E.400X



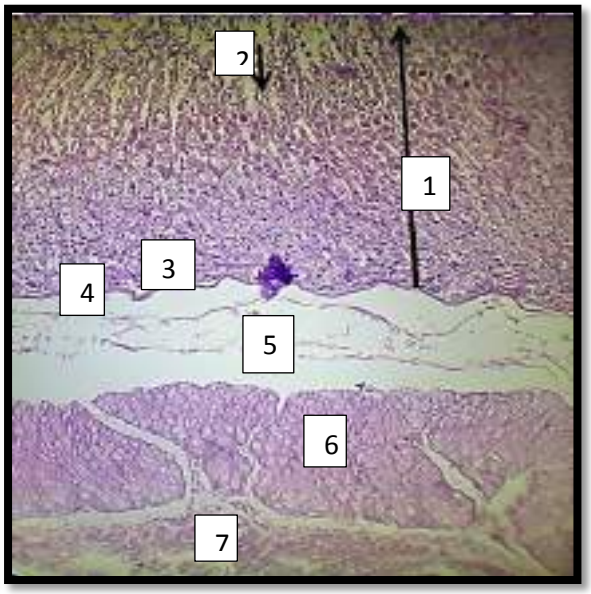
Fig(4-27) The fundus gland of the sheep stomach consist of (1) paratial cell, and (2) chief cells, (3) gland .H&E.100X



Fig(4-28) The fundus gland of the rabbit stomach consist of (1) paretial cell, and (2) chief cells, (3) gland .H&E.100X



Fig(4-29) The sheep stomach -pylorus region showing four layers (1) mucosa (2) gastric pit ,(3) lamina properia, (4) muscularis mucosa,(5) sub mucosa, (6) muscularis , (7) serosa .H&E.100X



Fig(4-30) The rabbit stomach -pylorus region showing four layers. (1) mucosa, (2) gastric pit , (3) lamina properia,(4) muscularis mucosa,(5) sub mucosa, (6) muscularis , (7) serosa .H&E.100X

4.3.2.Histomorphometeric study:

The thickness of rabbits mucosa in cardiac (695.23±90.55 μm), and fundus (648.57±78.38 μm) sections were significantly ($p<0.05$) large in comparison to the cardiac and fundus sections of the sheep stomach where the values found were (480.56±88.82 μ m) and (447.93±32.62 μ m), respectively (Table 4-2). There were non-significant ($p>0.05$) differences between the thickness of the mucosa in sheep and rabbits in the pylorus region (697.56±23.20 μ m) and (695.20±82.16 μ m), respectively (Table 4-2).

There were non-significant ($p>0.05$) differences between the thickness of the submucosa in sheep and rabbits in the cardiac region (438.56±131.40 μ m) and (317.28±112.80 μ m) (Table 4-2) respectively. The thickness of sheep submucosa in fundus (375.61±103.43 μm)and pylorus (415.27±53.65μm) sections were significantly ($p<0.05$) large in comparison to the fundus and pylorus sections of the rabbits stomach where the values found were (205.30±28.67 μ m) and (212.28±20.43 μ m), (Table 4-2) respectively.

There were non-significant ($p>0.05$) differences between the thickness of the muscularis in sheep and rabbits in the cardiac region (681.23±222.90 μ m) and (587.86±121.89 μ m) (Table4-2) respectively. The thickness of rabbits muscularis in fundus (989.16±158.29 μ m) and pylorus (349.90±77.76 μ m) sections were significantly ($p<0.05$) large in comparison to the fundus and pylorus sections of the sheep stomach where the values found were (401.27±71.94 μ m) and (1427.79±60.03), respectively (Table 4-2).

There were non-significant ($p>0.05$) differences between the thickness of the serosa in sheep and rabbits in the cardiac region (93.27±19.06 μ

m) and (83.98±16.31 µ m), respectively (Table 4-2). The thickness of rabbits serosa in fundus (209.94±19.04 µ m) and pylorus (158.62±36.13 µ m) sections were significantly ($p<0.05$) large in comparison to the fundus and pylorus sections of the sheep stomach where the values found were (139.98±24.59 µ m) and (58.32±12.29 µ m), (Table 4-2) respectively .

Table(4-2) : Mean thickness of mucosa, submucosa, muscularis, and serosa in Cardiac, Fundus and Pylorus regions of the stomach of the sheep and rabbit. (n= 10)

Thickness	Mucosa		Sub mucosa		Muscularis		Serosa	
Stomach	Sheep	Rabbit	Sheep	Rabbit	Sheep	Rabbit	Sheep	Rabbit
Cardiac	480.56 ^{a} ± 88.82	695.23 ^{b} ± 90.55b	438.56 ^{a} ± 131.40	317.28 ^{a} ± 112.80	587.86 ^{a} ± 121.89	681.23 ^{a} ± 222.90	93.27 ^{a} ± 19.06	83.98 **^{a}** ± 16.31
Fundus	447.93 ^{a} ± 32.62	648.57 ^{b} ± 78.38b	375.61 ^{a} ± 103.43	205.30 ^{b} ± 28.67b	401.27 ^{a} ± 71.94	989.16 ^{b} ± 158.29b	139.98 ^{a} ± 24.59	209.94 **^{b}** ± 19.04b
Pylorus	697.56 ^{a} ± 23.20	695.20 ^{a} ± 82.16	415.27 ^{a} ± 53.65	212.28 ^{b} ± 20.43	1427.79 ^{a} ± 60.03	349.90 ^{b} ± 77.76	58.32 ^{a} ± 12.29	158.62 **^{b}** ± 36.13

*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.

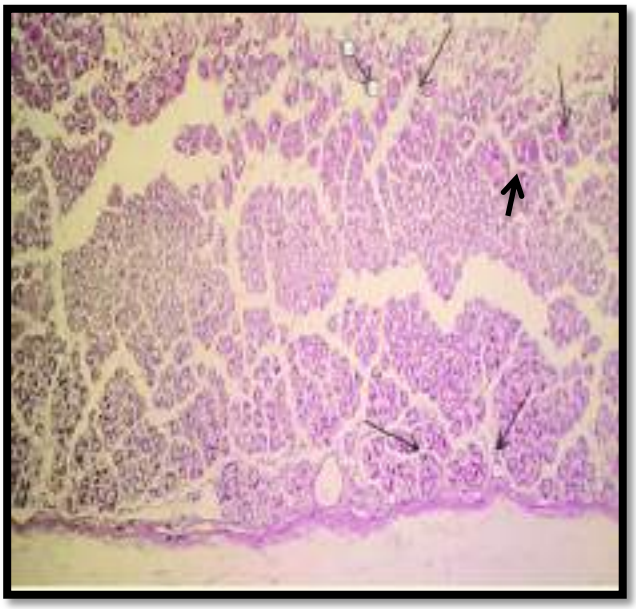
4.3.3.Histochemical study :

The mucosa layer of the cardiac region of the sheep and rabbit showed a strong reaction with PAS, distribution polysaccharides was concentrated in surface cells and body glands, showed color magenta red purple color but the stain dark in rabbit than mucosa sheep (Figure 4-31and 4-32).

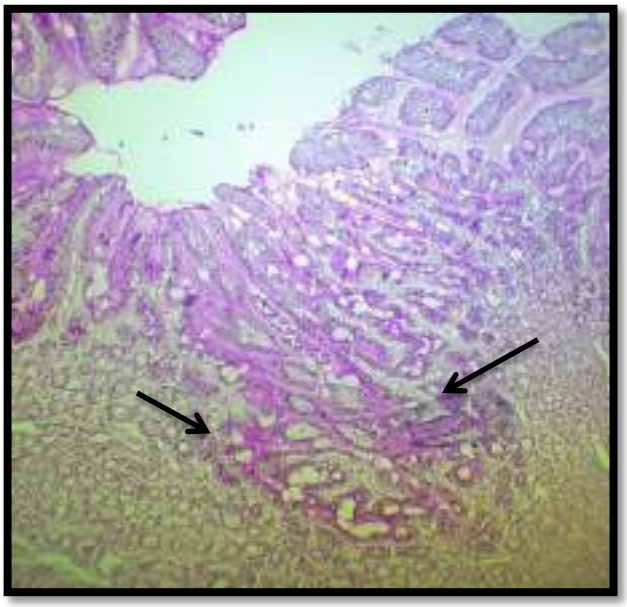
In the fundus region, the mucosa layer of the sheep and the rabbits the PAS stain showing a strong reaction with surface cells in the upper area of the mucosa layer, while reaction of PAS stain showing a weakly reaction with parietal cells and chief cells (Figure 4-33 and 4-34).

In the pylorus region, the mucosa layer of the sheep and rabbit showing a strong reaction with PAS, distribution neutral polysaccharides almost equal in pylorus glands (Figure 4-35 and 4-36).

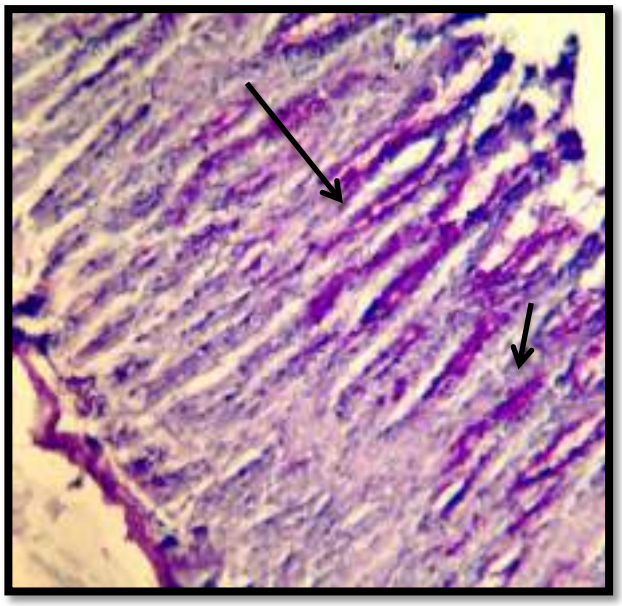
However, in both animals other layers sub mucosa and serosa showing weak reaction with PAS, but muscularis moderate reaction with PAS in cardiac (Figure 4-37 and 4-38) fundus and pylorus regions.



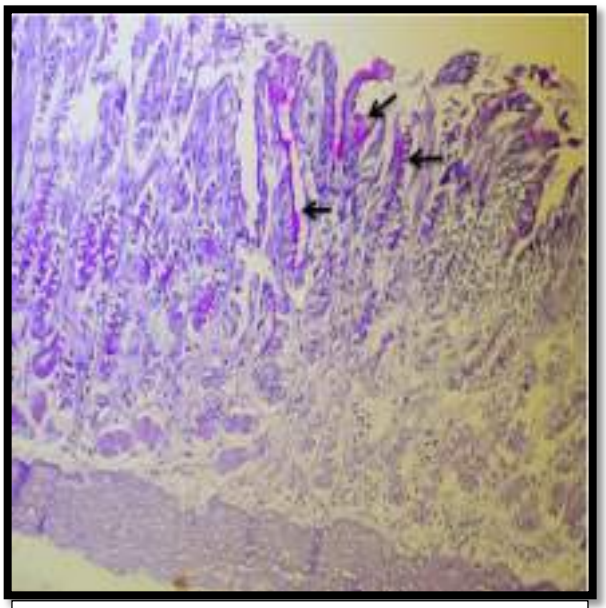
Fig(4-31) The sheep stomach -Cardiac region showing mucosa layer a strong reaction with PAS. 100X



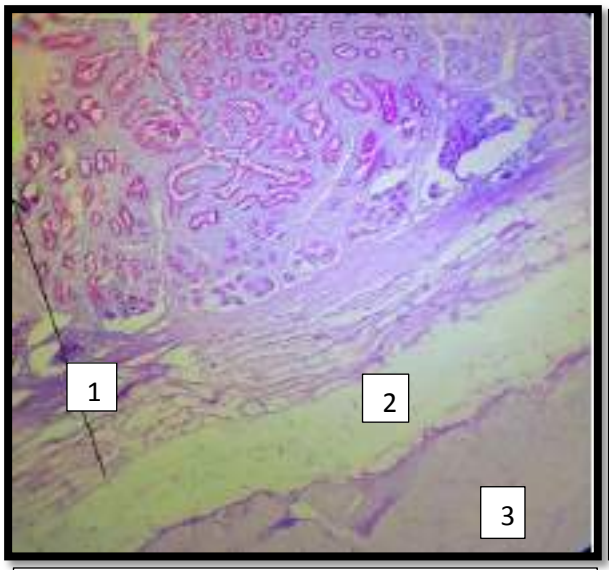
Fig(4-32) The rabbit stomach- Cardiac region showing mucosa layer a strong reaction with PAS. 100X



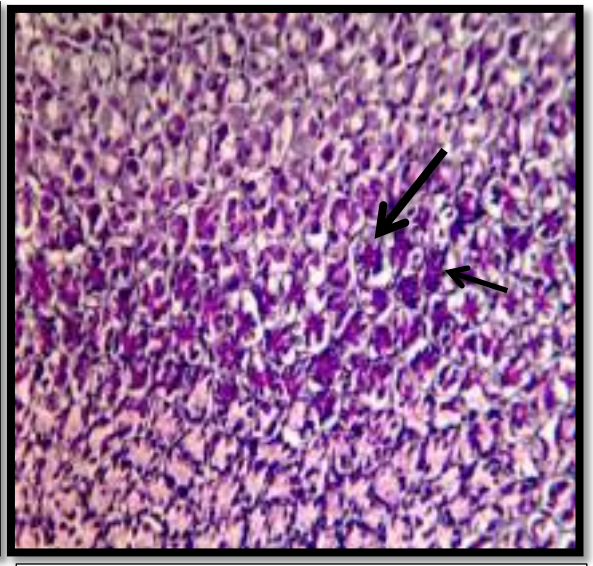
Fig(4-33)The sheep stomach- fundus region showing mucosa layer, surface cells of mucosa layer showing strong reaction with PAS. 100X



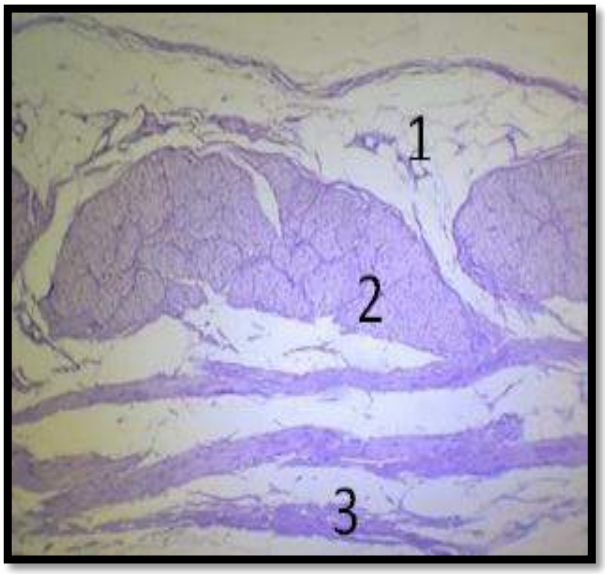
Fig(4-34) The rabbit stomach- fundus region showing mucosa layer, surface cells of mucosa layer showing strong reaction with PAS. 100X



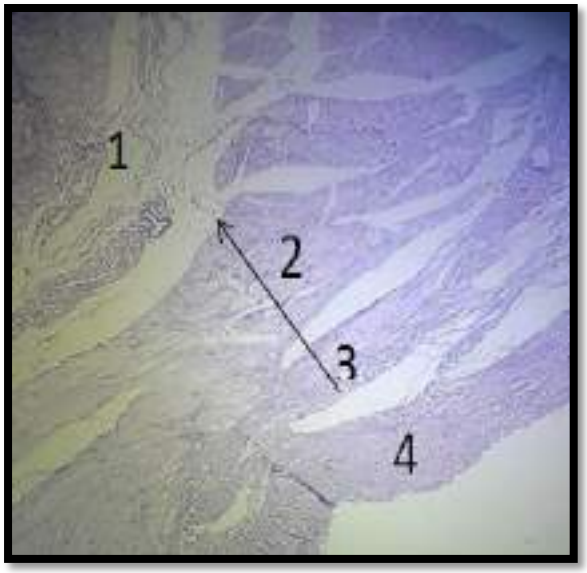
Fig(4-35) The sheep stomach- pylorus region showing (1) mucosa layer strong reaction with PAS.(2) sub mucosa showing weakly reaction with PAS, (3) muscularis layer showing moderate reaction with PAS.100X



Fig(4-36) The rabbit stomach- pylorus region showing (black arrow) mucosa layer strong reaction with PAS.100X



Fig(4-37) The sheep stomach- cardiac region showing layers (1) sub mucosa was weakly reaction with PAS,(2) muscularis was moderate reaction with PAS ,(3) serosa was weakly reaction with PAS.100X



Fig(4-38) The rabbit stomach- cardiac region showing layers (1) sub mucosa showing weakly reaction with PAS,(arrow black) muscularis (2) inner circular (3) outer longitudinal was moderate reaction with PAS, (4) serosa was weakly reaction with PAS. 100X

4.4. Small Intestine

The small intestine is a part of digestive tract, divided, as in other mammalian species, to duodenum, jejunum and ileum.

4.4.1.Histological study:

Sections of this organ appear that its wall in both animals made of four layers (tunics): mucosa, submucosa, muscularis and serosa, as other mammalian species, mucosa layer consist of from epithelium, lamina propria and muscularis mucosa. In both animals, the epithelium layer is a simple columnar epithelium (Figure 4-39 and 4-40), there are the circular folds called Plica circular consist of mucosa and sub mucosa layer, Plica circular appear in the duodenum and the jejunum (Figure 4-45) of sheep but in rabbit in the jejunum only (Figure 4-46), and it absent in ileum of both animals.

Histological examination results show that the mucosa of duodenum, jejunum and ileum have number of villi compose of absorptive (enterocytes) and goblet cells only (Figure 4-41 and 4-42).

The columnar cells have nucleus oval found in near the base, and cytoplasm was eosinophilic, while goblet cells are unicellular in its apical part becomes puffy due to mucigen droplets accumulation, and have nucleus is irregularly oval or triangular at the base and in both species have same characteristic.

The villi are projections difference in shape and size, in sheep duodenum long and thin which finger shape (Figure 4-39), but in rabbit duodenum were broad and leafy shape (Figure 4-40), the center (lacteal) of the villi consist of loose connective tissue (Fingers 4-41 and 4-42), the villi in sheep jejunum were broad and short as tongue shaped (Figure

45) while in the rabbit jejunum were thinner and taller (Figure 4-46) than those of the duodenum, in sheep, the villi of the ileum were less amount (Figure 4-47) while in rabbit more amount and more long compared to the sheep (Figure 4-48).

Lamina propria :in both animals consist from loose connective tissue containing blood vessel, nerves, lymphatic assembles, and the intestinal glands or called crypts of lieberkuhn extended to below muscularis mucosa and consists of the columnar cells, the goblet cells and the Paneth's cells (Figure 4-43 and 4-44), these glands well developed appeared simple tubular.

Intestinal glands or crypts of lieberkuhn contain Paneth's cells in each small intestine segments, these cells have pyramidal shaped, ovoid nuclei, and cytoplasm is basophilic in both animals, also they contains numerous secretory granules.

Mascolaris mucosa composed of smooth muscle fiber, it located at the base of the crypt be thin in rabbit but it more thick and very clear in sheep(Figure4-45and4-46).

Submucosa layer seen as a thin layer of loose connective tissue abundantly supplied by blood vessels, lymphocytes, collagen and elastic fiber, it locate below mucosa layer, in the duodenum contains glands called (Brunner's glands), Brunner's glands increased in density in the sub mucosa of the duodenum sheep than in rabbit, these are simple tubular (Figures 4-39 and 4- 40). Brunner's glands of rabbit characterized by their relatively wide lumen, while in sheep narrower lumen (Figure 4-39 and 4-40).

Sub mucosa layer in the jejunum and the ileum have same histological structure to those observed in duodenum, but Brunner's glands were

totally absent at all of sheep and rabbit (Figures 4-45, 4-46, 4-47 and 4-48), while in the ileum sub mucosa layer in both animals have clusters large of lymphoid nodule known as Peyer's patches located in sub mucosa of sheep and rabbit and they were large size and few number and have shape oval to elongated oval with peaked end in sheep (Figure 4-47), while the ileum of the rabbit have peyer's patches more number than sheep and their shape dome-shaped (Figure 4-48).

In both animals muscularis layer consists of two layers of smooth muscle fibers an enternal circular layer and external longtudinal layer between these layers connective tissue (small blood capillaries ,blood vessels, nerve bundles and fatty tissue).

In sheep and rabbit the serosa layer formed by the loose connective tissue with a single layer of mesothelium contain several blood vessels and nerves.

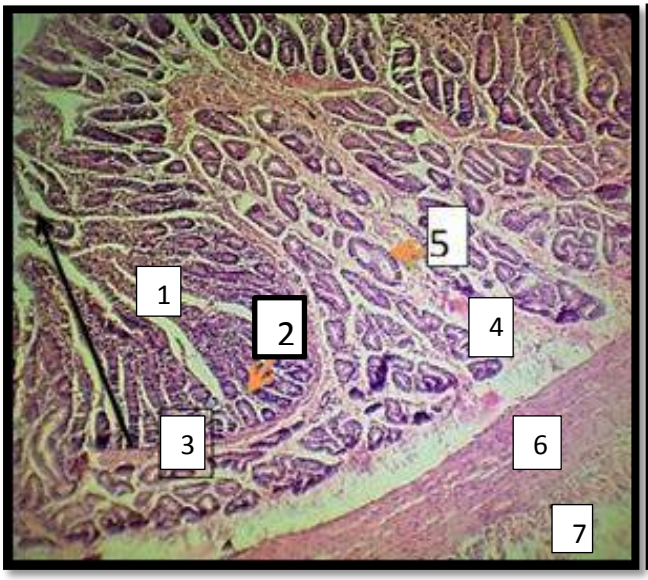


Fig (4-39) The duodenum of the sheep showing mucosa, contain villi (1), gland (crypt) of Liebrkuhn (2) . muscolaris mucosa (3). sub mucosa (4), Brunner's glands(5) . muscularis (6), serosa(7).H&E.100X

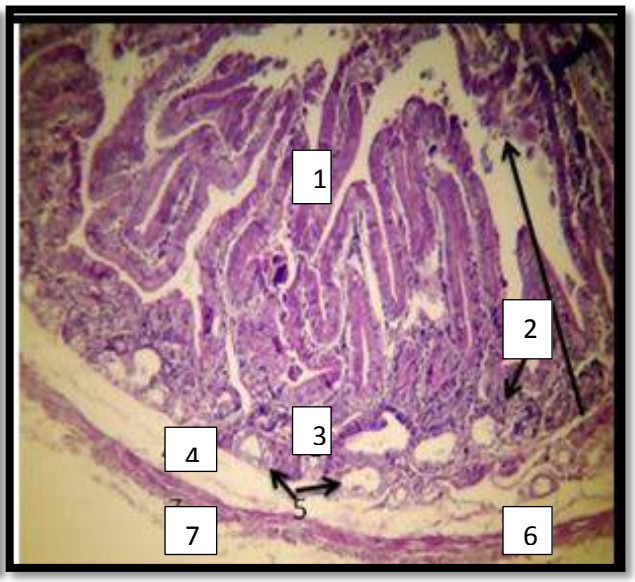


Fig (4-40)The duodenum of the rabbit showing mucosa, contain villi (1), gland (crypt) of Liebrkuhn (2) . muscolaris mucosa (3) ,sub mucosa (4), Brunner's gland(5) . muscularis (6), serosa(7). H&E.100X

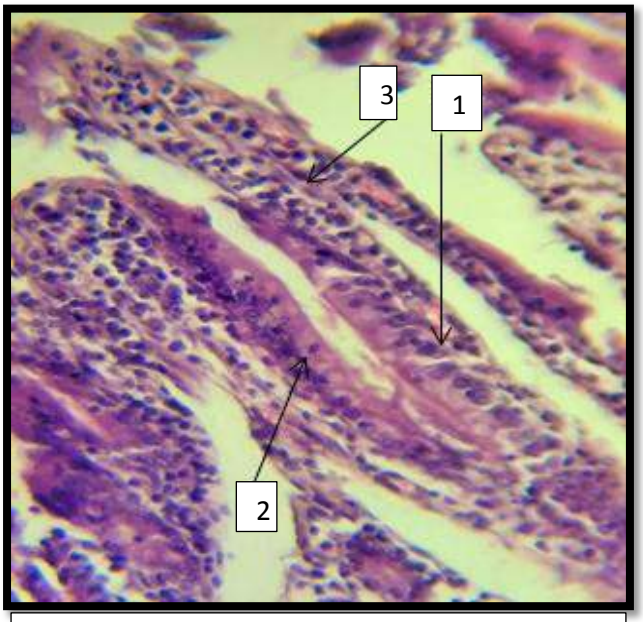


Fig (4-41) The villi of the sheep in doudenum consist of enterocytes (1) , goblet cells (2), (lacteal) (3) of villi consist of loose connective tissue H&E.100X

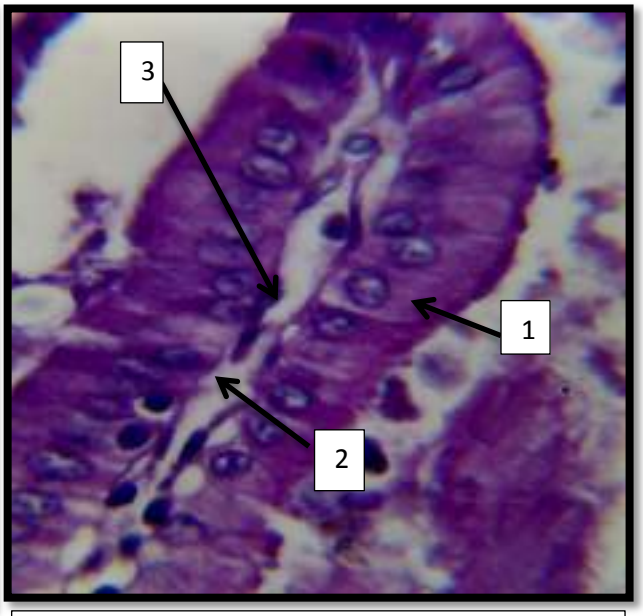


Fig (4-42) The villi of the rabbit in doudenum consist of enterocytes (1) , goblet cells (2), (lacteal) (3) of villi consist of loose connective tissue H&E.100X

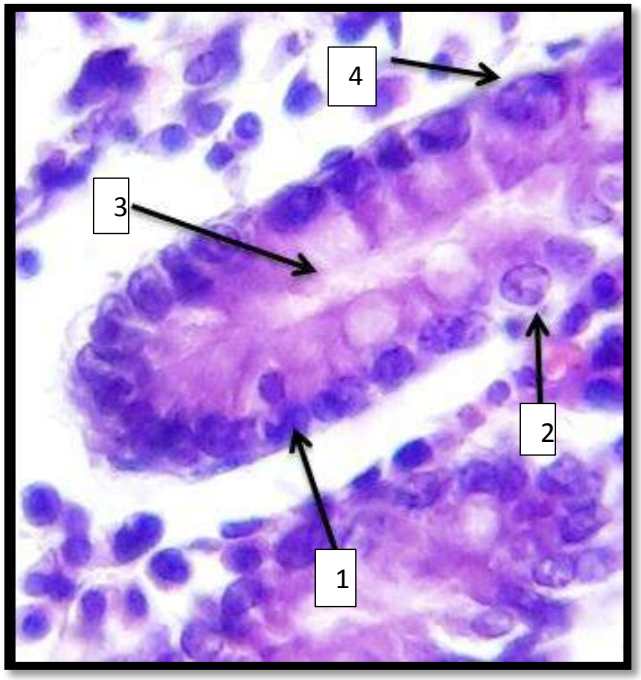


Fig (4-43) The glands (crypt) of Liebrkuhn in the sheep showing (1) goblet cell, (2) columner cells, (3) lumen, (4) Paneth cells H&E400x

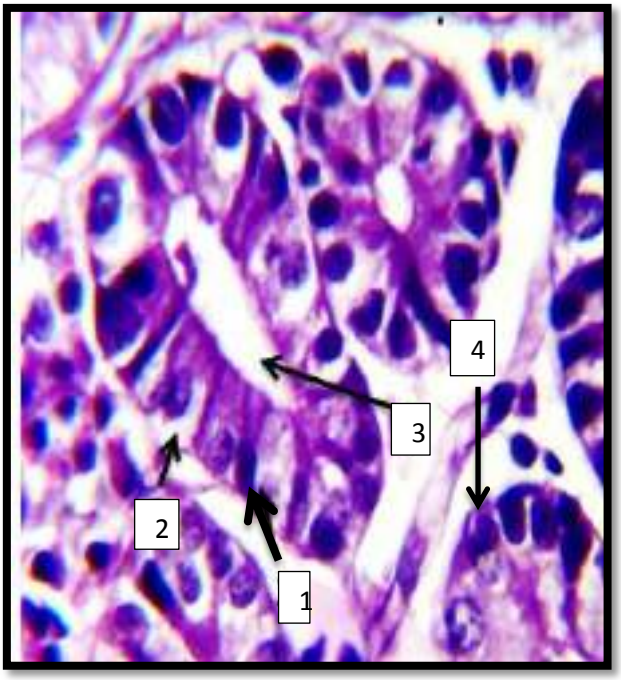


Fig (4-44) The glands (crypt) of Liebrkuhn in the rabbit showing (1) goblet cell, (2) columner cell, (3) lumen, (4) Paneth cells H&E400x

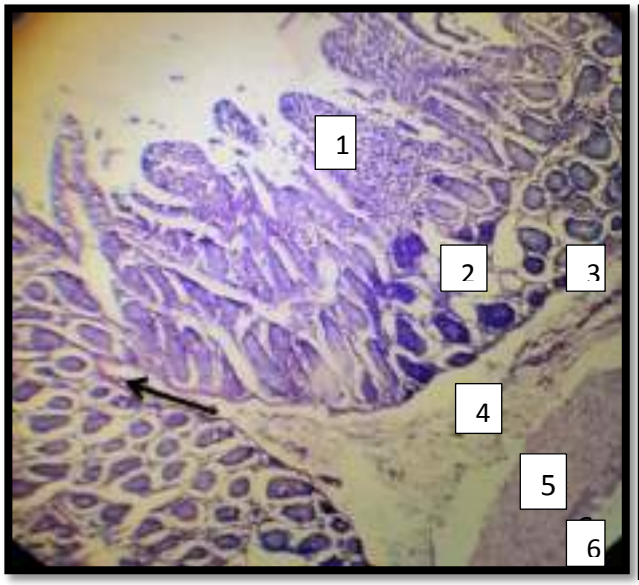


Fig (4-45) The jejunum of the sheep showing , mucosa layer consist villi (1). gland (crypt) of Liebrkuhn(2) , muscolaris mucosa (3) , sub mucosa (4), (arrow black) Plica , muscularis (5), serosa (6). H&E.100X

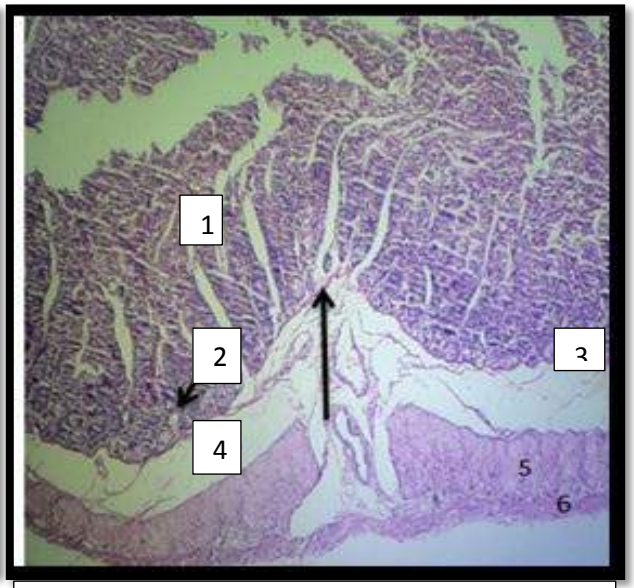


Fig (4-46) The jejunum of the rabbit showing, mucosa layer consist villi (1), gland (crypt) of Liebrkuhn(2) , muscolarismucosa (3) , sub mucosa (4), (arrow black) Plica , muscularis (5) .serosa (6). H&E.100X

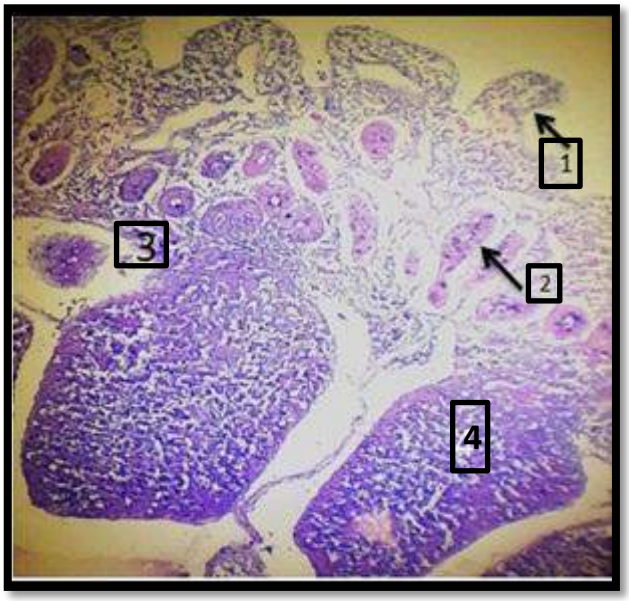


Fig (4-47) The ileum of the sheep showing mucosa consist of villi(1) , gland (crypt) of Liebrkuhn (2),muscularis mucosa (3), (4)payers patches in Sub mucosa .H &E 100X

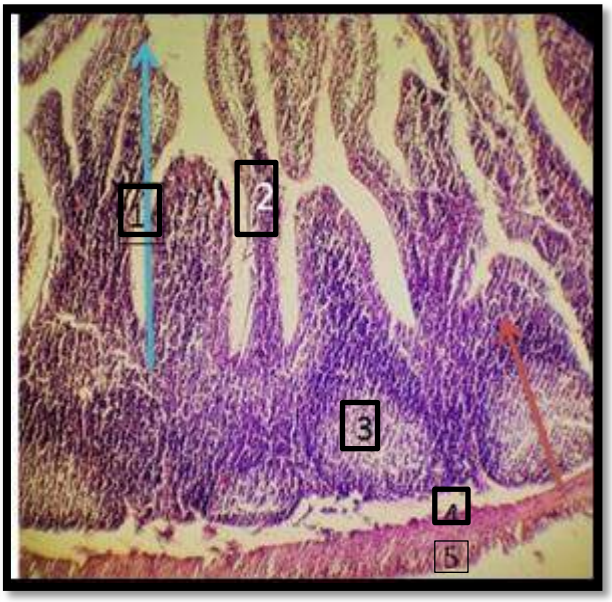


Fig (4-48) The ileum of the rabbit showing mucosa (1)(arrow blue), villi(2) , payers patches(3) , sub mucosa. (arrow red) muscularis(4) , serosa (5).H &E 100X

4.4.2.Histomorphometeric study:

The thickness of sheep mucosa layer in the duodenum (2146.31±158.24), jejunum (1814.98±210.90), and ileum, (1397.44±225.78) were sections significantly ($p<0.05$) large in comparison to the duodenum, jejunum and ileum sections of the rabbits small intestine where the values found were (846.84±55.03), (811.85±193.57) and (667.21±89.49), (Table 4-3) respectively.

The villi height of sheep in the duodenum (951.85±263.88), and ileum(1271±275.87) sections were significantly ($p<0.05$)large in comparison to the duodenum and ileum sections of rabbit small intestine where the values found were (566.90±127.8) and (510.90±78.87) (Table4- 3) respectively.

There were non- significant($p>0.05$) differences between the villi height of the jejunum in sheep and rabbit where recorded values (655.57±150.57) and (473.57±116.41), (Table 4-3).

The thickness of sheep submucosa layer in the duodenum(228.61±54.77) and jejunum (167.97±36.14) sections were significantly ($p<0.05$) large comparison to sections the duodenum and jejunum of the rabbits small intestine where the values found were (58.32±16.49) and (130.60±22.54) (Table 4-3)respectively.

There were non- significant($p>0.05$) differences between the thickness of the sub mucosa in ileum sheep and rabbit where recorded values (123.62±±33.07) and (107.28±22.52), (Table 4-3)respectively.

The thickness of sheep muscularis layer in the duodenum (264.22±101.37), jejunum (172.64±39.95), and ileum(298.62±36.14) sections significantly ($p<0.05$) large comparison to the same sections of the rabbits small intestine where the values found were (69.99±29.99), (102.63±12.02) and (104.98±12.29) (Table 4-3) respectively.

There were non- significant($p>0.05$) differences between the thickness of the serosa in sheep and rabbit in the duodenum , the jejunum and ileum, where recorded values in sheep (2013.37±5850), (62.99±15.74), (76.98±27.05) while in rabbit (46.66±21.99), (44.32±7.37) and (65.32±21.43) (Table 4-3) respectively.

Table (4-3) Mean thickness of mucosa, submucosa, muscularis, and serosa in Duodenum , Jejunum and Ileum regions of the small intestine of the sheep and rabbit (n=10).

Organ	Mucosa		Sub mucosa		Muscularis		Serosa		Height villi	
Small intestine	Sheep	Rabbit	Sheep	Rabbit	Sheep	Rabbit	Sheep	Rabbit	Sheep	Rabbit
Duodenum	2146.31 ^{a} ± 158.24	846.84 ^{b} ± 55.03	228.61 ^{a} ± 54.77	58.32 ^{b} ± 16.49	264.22 ^{a} ± 101.37	69.99 ^{b} ± 29.09 b	2013.37 ^{a} ± 5850.70	46.66 ^{a} ± 21.99	951.85 ^{a} ± 268.88	566.90 $^{\mathbf{b}}$ ± 127.81
Jejunum	1814.98 ^{a} ± 210.90	811.85 ^{b} ± 193.57	167.97 ^{a} ± 36.14	130.60 ^{b} ± 22.54	172.64 ^{a} ± 39.95	102.63 ^{b} ± 12.02	62.99 ^{a} ± 15.74	44.32 ^{a} ± 7.37	655.57 a ± 150.57	473.57 $^{\mathbf{a}}$ ± 116.41
Ileum	1397.44 ^{a} ± 225.78	667.21 ^{b} ± 89.49	123.62 ^{a} ± 33.07	107.28 ^{a} ± 22.52	298.62 ^{a} ± 36.14	104.98 ^{b} ± 12.29	76.98 ^{a} ± 27.05	65.32 ^{a} ± 21.43	1271.46 a ± 275.87	510.90 $^{\mathbf{b}}$ ± 78.87

*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.

4.4.3.Histochemical study:

The mucosa layer of the duodenum in two animals showing a strong reaction with PAS which goblet cells of villi and the Lieberkuhn crypts, but enterocytes of villi showing a weak reaction with PAS and the Lieberkuhn crypts (Figure 4-50).

However, the mucosa of the jejunum and the ileum in two animal showing reaction a strong with PAS which the villi and the crypts of Lieberkühn have color magnate (Figures 4-52,4-53,4-54 and 4-55).

In addition, Brunner's glands of the sub mucosa layer in gave a strong reaction with PAS in sheep duodenum (Figure 4-49),while in the rabbits were Brunner's glands mixed gland (serous cells a weak reaction with PAS and mucous cells moderate reaction with PAS (Figure 4-51).

While the sub mucosa layer of the jejunum gave reaction a weakly with PAS in sheep and rabbit (Figure 4-52 and 4-53), but Peyer's patches in sub mucosa of the ileum showed moderated reaction with PAS in both animals (Figure 4-54 and 4-55).

Whereas, the muscularis and the serosa showing a weakly reaction with PAS in sheep and rabbit in the duodenum, the jejunum and the ileum (Figures 4-52, 4-53 and4-55).

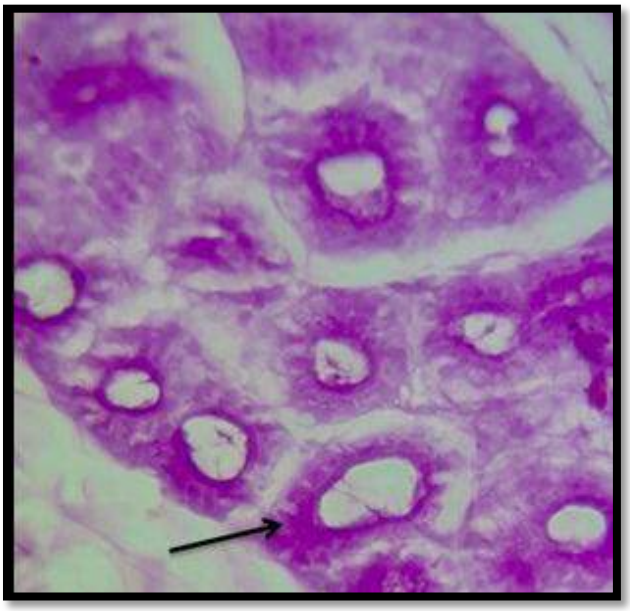


Fig (4-49) The Brunner's gland of the sheep duodenum showing (mucous acini) gave strong reaction with PAS.400X

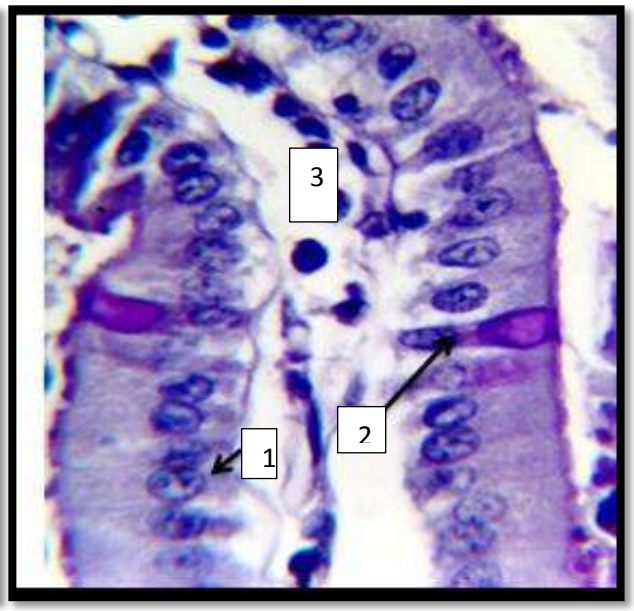


Fig (4-50) The villi of the rabbit doudenum consist of enterocytes (1) and goblet cells (2) showing strong reaction with PAS , (lacteal) (3) of villi consist of loose connective tissue.PAS.400x

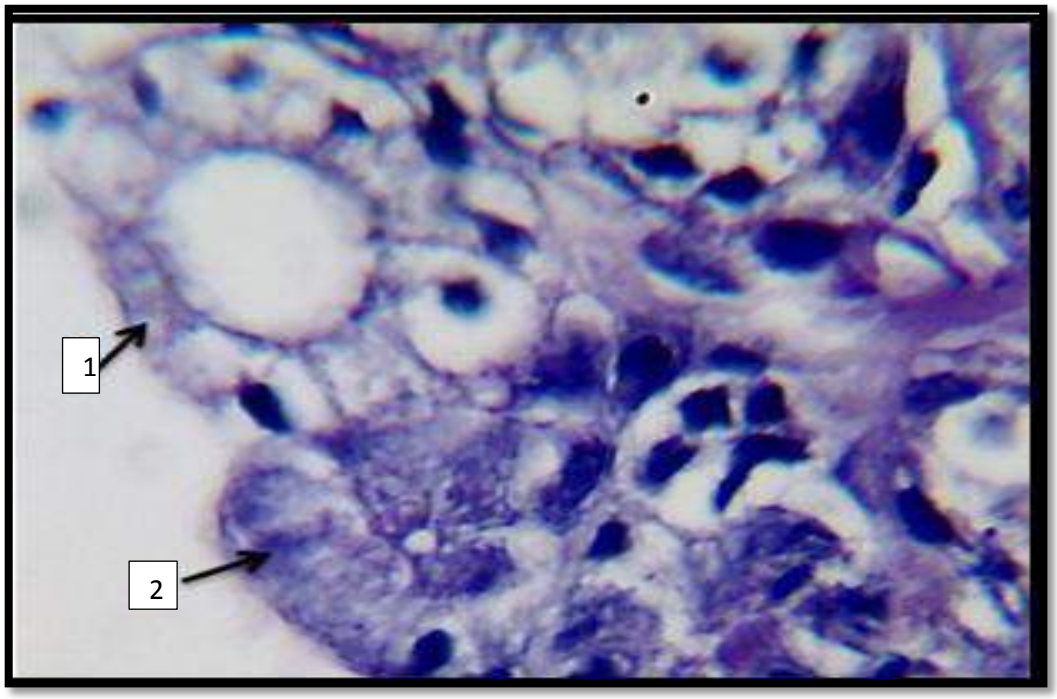


Fig (4-51) The Brunner's gland of the rabbit duodenum showing (1) serous cells gave weakly reaction with PAS, (2) mucous cells moderate reaction with PAS.400X

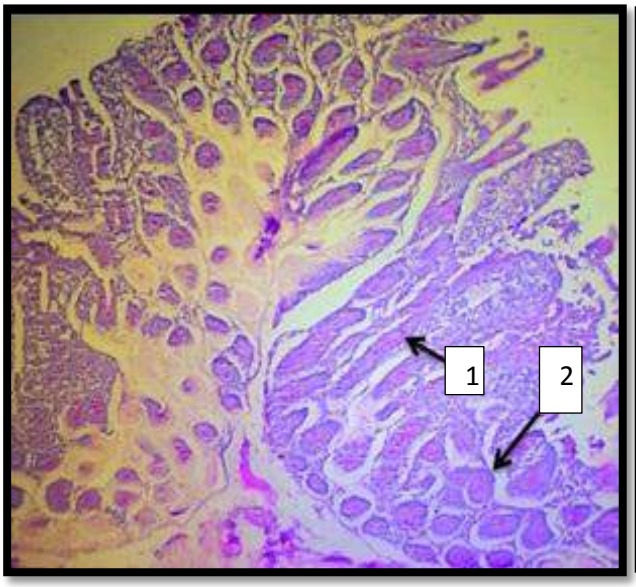


Fig (4-52) Jejunum in sheep showing (1) villi, (2)gland (crypt) of Liebrkuhn in mucosa stronge reaction with PAS.100X

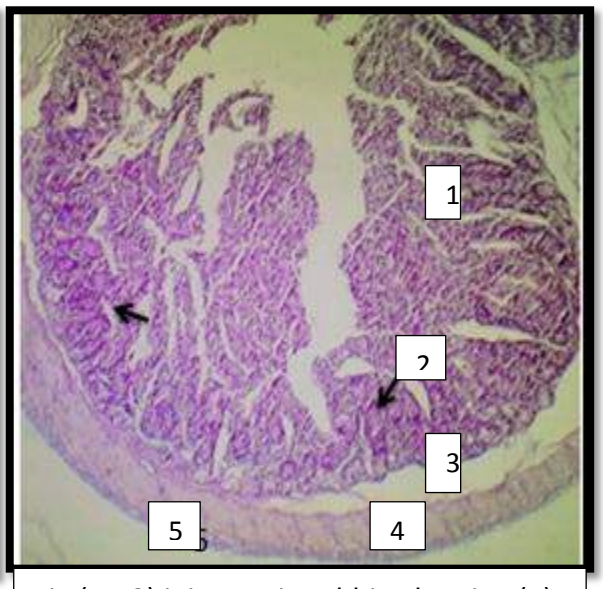


Fig (4-53) jejunum in rabbit showing (1) villi and (2) gland (crypt) of Liebrkuhn in mucosa stronge reaction with PAS, (3) sub mucosa, (4)muscularis and (5)serosa weakly reaction with PAS.100X

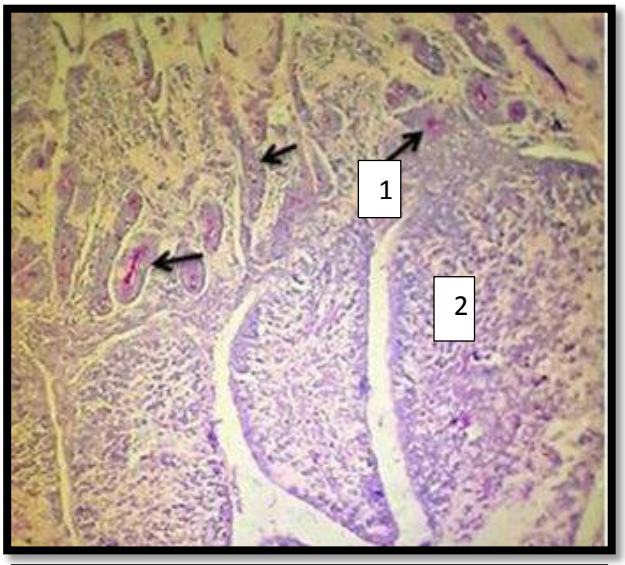


Fig (4-54) The ileum of the sheep showing (1) gland (crypt) of Liebrkuhn strong reaction with PAS, (2)payers patches showing moderate reaction with PAS 100X

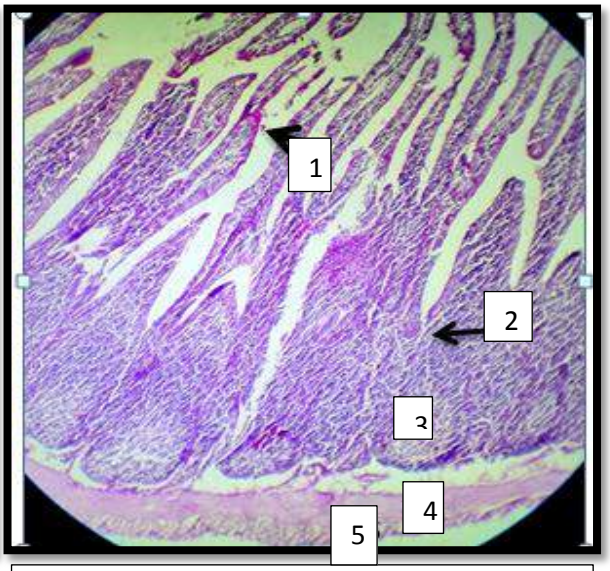


Fig (4-55) The ileum of the rabbit showing (1)villi and,(2) gland (crypt) of Liebrkuhn stronge reaction with PAS,(3) .payers patches showing moderate reaction with PAS,(4) muscularis and (5)serosa weakly reaction with PAS .100X

4.5. physiological study

4.5.1. Gastrin hormone

Results of present investigation showed that the values of gastrin did not difference significantly (p>0.05) between sheep (37.38±8.34 pg/ml) and rabbit (40.31±14.16 pg/ml) (Table 4-4).

Table (4-4): Serum gastrin level in sheep and rabbit (n=10).

Hormone	sheep	rabbit
gastrin (pg/ml)	37.38[a]±8.34	40.31[a]±14.16

*Value represent (mean± SD),

*The similar letters refer to non-significant (p>0.05) difference between values.

4.5.2. Digestive enzymes

4.5.2.1.Pepsinogen

Results of present investigation showed that the values of Pepsinogen 1 did not difference significantly (p>0.05) between sheep (1.41 ± 0.88 ng/ml) and rabbit (1.00 ± 0.00 ng/ml) .Also, the results showed the values of Pepsinogen 11 did not difference significantly (p>0.05) between sheep (1.10 ± 1.09 ng/ml) and rabbit (0.50 ± 0.00 ng/ml) as show in Table (4-5).

Table (4-5) Serum Pepsinogen 1 and Pepsinogen 11 enzymes levels in sheep and rabbit (n=10)

Enzymes	sheep	rabbit
Pepsinogen 1	1.41 [a] ±0.88 ng/ml	1.00 [a] ±0.00 ng/ml
Pepsinogen 11	1.10 [a] ±1.09 ng/ml	0.50 [a] ±0.00 ng/ml

*Value represent (mean± SD) ,

*The similar letters refer to non-significant (p>0.05) difference between values.

4.5.2.2.Pencreatic enzymes

Results revealed a significantly ($p<0.05$) height in serum α -amylase level (226.73±54.74 U/L) in rabbit in comparison to it's level in sheep(11.85±4.16 U/L). Also, the results showed there are significantly ($p<0.05$) height in serum lipase level (273.55±76.02 U/L) in rabbit in comparison to it's level in sheep (8.94±2.95 U/L) as shown in (Table 4-6)

Table (4-6) Serum α -amylase and lipase enzymes levels in sheep and rabbit (n=10)

Enzymes	sheep Mean ±SD	rabbit Mean ±SD
α -amylase U/L	11.85 [a] ±4.16	226.73 [b] ±54.74
lipase U/L	8.94 [a] ±2.95	273.55 [b] ±76.02

*Value represent (mean± SD) ,

*Different letters refer to ($p<0.05$) significant difference between values.

Chapter five

Discussion

5.Discussion

5.1.Esophagus

5.1.1.Histological study:

The presence of keratin on the surface of the epithelium supports its protection Meyer and Schnapper study (2014), who suggest that keratinization of the epithelium plays an essential role in mechanical stabilization. The presence of keratin in sheep that covers the stratified squamous epithelium may also be because these animals consume rough and dry food without good chewing. Malik and his team (2018) reported that epithelium layer 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) reported that the epithelium lining of the esophagus is the non-keratinized stratified squamous epithelium in rabbit. On the other hand, the epithelium of the esophagus of the rabbit is formed of three layes. Stratum basal are cuboidal or low columnar and are located under of the stratified epithelium; cells in the middle layers of the epithelium are polyhedral and the surface layer are; squamous cells lack keratin.

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 mucosa of rabbit esophagus consists from keratinized stratified squamous epithelium.

The muscularis mucosa consisted of smooth muscle fiber arranged longitudinally and it is more thickness in sheep than in rabbit. 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 from interwoven collagen fiber, elastic fiber, fibrocytes, lymphocytes, and blood vessels with the presence of the adipose tissue in sheep thickness 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) in sheep. According to Pawan *et al*., 2009, the presence or absence of esophageal glands was dependent on gruff feed, especially vegetable fodder.

On the other hand ,this study disagreement with results of Naghani and Andi (2012) which they reported presence of great sub mucosal glands throughout the length of the esophagus in one-humped camel and this difference in results might be due type food and species.

However, Gupta and Sharma, (1991) detected that there are seromucous tubuloalveolar glands in initial portion of the esophagus in buffalo calves. Mahmood *et al*., (2017) observed glands in esophagus of the rabbit. 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 muscular layer were 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 in ruminants (Banks,1986). Ranjan and Das (2016) wrote that muscular layer in all regions of esophagus in rabbit are formed from striated muscle, and this finding with the results of this study.

Whereas, adventia was composed of loose connective tissue gradually transformed into serosa in abdominal region which it composed of loose connective tissue and a mesothelium layer, and this findings with the study of Hussein *et al.* (2016).

5.1.2.Histomorphometeric Study

Thickness mucosa of the sheep esophagus was significantly ($p<0.05$) large in compared to thickness mucosa of rabbits esophagus. This find might be related to the fact that the rabbit has epithelium of type non-keratinized stratified squamous epithelium or because of the thickness of muscularis mucosa in sheep.

The thickness of sheep submucosa in were significantly ($p<0.05$) larger in comparison to the submucosa of the rabbits' esophagus. This find might be related to the physiological situation related to the blood supply, nervous and lymphatic system and the difference of species due to submucosa thickness of sheep once it has a great amount of adipose tissue.

In sheep, the thickness of the sub mucosa in the thoracic region was (639.32±121.06 μm) (Table 4-1). This result agrees with Malik *et al*. (2018) which observed the thickness of sub mucosa in the thoracic region was (645.5 ± 46.93 μm) in sheep. In the same context, the thickness of the sub mucosa of rabbit in the thoracic region was (319.62±85.22 μm) (Table.4-1) and this find, however, disagrees with the study of Kadhim (2019), which observed that the thick sub mucosa in this region of *(Herpestidae edwardsii)*was (131±17.7 μm). This difference might be related to the nature of the nutrition intake of the animals.

Thickness muscularis in the esophagus of sheep was significantly ($p<0.05$) larger in comparison with muscularis of the rabbit esophagus. This find might be related to a difference in the use of esophageal muscles, in sheep, function of muscularis layer help in Rumination processes.

Thickness serosa was significantly ($p<0.05$) large with the sheep in the abdominal part compared to the rabbits and this may be because presence the fat cells and blood vessels more in sheep and this conclusion according to results Hameed *et al*., (2018) in sheep.

5.1.3. Histochemical Study

The results of this study are in agreement with Malik *et al*., (2018), which mentioned that the stratum corunum 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 esophagus rabbit. Ranjan and Das (2016) observed in rabbit esophagus that the epithelium and basement membrane performed a moderately reaction with PAS.

Igbokwe *et al*., (2016) observed in rope squirrel esophagus that the mucosal layer was moderately reaction with PAS.

In both animals, the sub mucosa showed weakly reaction with PAS in each region's esophagus. This might be because of the absence of the glands. Nzalak *et al*., (2010) reported that the esophagus of the African giant rat does not have glands in the sub mucosa. 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 important factor in the protection of the esophagus from damage.

In sheep and rabbits, muscolaris layer showed reacted moderately with PAS, and this may be due to the presence of small quantities of glycogen in the muscle skeletal. 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 muscularis layer in esophagus rabbit.

5.2. Stomach

5.2.1. Histological study:

The difference between herbivores and carnivores are seen in the stomach therefore, rabbit stomach seemed like carnivores stomach while sheep stomach is classified as a multilocular (Larson and Biller, 2009). The results showed that the type of epithelium lining of the glandular (abomasum) stomach in sheep and rabbit are the same which simple columnar epithelium for its three regions cardiac, fundic and pyloric. This finding also mentioned by Mahesh *et al*., (2017) in Goat. This type of tissues epithelium secreted mucous aid make soft food and protection mucosa of effect food rough and

also prevents entry microbes to tissues that found below it (Eroschenko, 2008).

The stomach in rabbit and sheep contains folds and these folds allow to enter large amount of food and these findings were similar to results Masot *et al*., (2007), but these folds in ruminant are spiral permanent (Agungpriyon *et al*., (1992);Wang *et al*., 2015). The folds in rabbit are short because rabbit stomach always full food but this observe differs from observation of Brewer and Cruise, (1994) which observed no folds in the stomach of rabbit.

In both two animals, the lamina propria were composed of loose connective tissue contain blood vessel, lymphocytes, elastic fibers, collagen fibers and glands, and this consistent with the study Mahesh *et al*., (2017), that reinforce these results

Depending on types of glands, the stomach divided to three region cardiac, fundus, pyloric (Dyce *et al*., 2002). In rabbits the cardiac region is narrow and some animals have no cardiac region as cow or be small as horse (Reece *et al*., 2015). The gastric glands be vary according to species of animals (Banks. 1981).

The glands difference between sheep and rabbit, the cardiac glands in sheep were simple tubular. Kalita and Chandramouly, (1997) has explained that the cardiac glands were compound tubular branched and coiled in buffaloes.

The cardiac glands in rabbits were simple, branched tubular and coiled, and this finding are the same found by Cui *et al*., (2011), but this study disagree with AL-Mahmodi .(2014) which observed that cardiac glands are

tubular no coiled in rabbit . However, the cardiac region contain many of mucus secret cells and partial cells and this consistent with study Samuelson, (2007).

The fundus glands in sheep, simple, long and straight tubular. These results are similar to the study (Gallego-Huidobro and Pastor, 1996). The fundus glands in rabbit was simple, straight and branched tubular. Dyce *et al*., (2002) observed in horse that type glands in this region simple tubular glands. The parietal cells in fundus gland shown eosinophilic because contain large amount of mitochondria which have metabolic activated to secretion HCL and these cells no difference among sheep and rabbit and this conclusion according to study done by Al-Neamy (2007) which confirm the histological characteristic parietal cells are same in stomach of human, ruminant and mono-gastric animals.

There are the chief cells in fundus glands were basophilic cytoplasm because it's have more amount of rough endoplasmic reticulum in cytoplasm which have ribosome and these cells description by (Junqueira and Mescher, 2013).

The pylorus glands in sheep are simple, branched tubular and coiled and this the results same of study by Wang *et al*., (2015). Eurell and Frappier (2006) described that the pyloric glands were simple branched coiled tubular glands and relatively shorter compared to other gastric glands.

However, the pyloric glands in rabbit simple tubular. This different in type of glands indicated on function all region in stomach.

On the other hand. (Sujana. 2017) descripted that pyloric glands in pigs were devoid of parietal cells but had mucous cells. However enteroendocrine

cells were not identifiable in this study, because it required specialized silver stains to localize it (Igbokwe and Obinna, 2016).

Muscularis mucosa consist of smooth muscle fibers be longitudinal and contract these muscles help secretion the glands (Junqueira and Mescher, 2013).

Furthermore, in sheep and rabbit sub mucosa layer consists of connective tissue contains reticular fiber, blood vessels, nerve plexus few, elastic fibers and collagen fibers in (cardiac, fundus and pyloric region) (Frye and Aughey, 2001).

Nevertheless, muscularis layer in both animals compose from two smooth muscle layers thick inner circular layer and a thin outer longitudinal layer , the circular muscle layer prevents food from traveling backward, while the longitudinal layer shortens the tract, Auerbach's plexus are present between the two muscularis layer which serve of mechanosensory (Cui *et al*., 2011).

In sheep and rabbit, serosa layer formed of loose connective tissue cover with mesothelium (Cui *et al*., (2011).

5.2.2. Histomorphometeric Study

The mucosa layer of the cardiac and the fundus regions in the rabbits were significantly ($p<0.05$) larger in compared with sheep and this may be due rabbit food contain amount large of protein that are come of soft feces and this requires large number of cells that have capacity for digestive where stomach full food always consequently these cells increase thickness this layer, and conclusion according to (Mendes *et al*., 2000) stated that

Cecotrophy contains high amount of protein and water content, and is richer in vitamins and poorer in fiber than normal feces.

Furthermore, the thickness of mucosa in fundus region of the rabbit (648.57 ± 78.38 µ m) and this result agree with the study done by Khalel, (2012), which observed thickness of the mucosa in males rabbits of the fundus region was (694.5 ± 29.157 µ m). While this finding disagree with Igbokwe and Obinna, (2016) which observed in rope squirrel thickness the mucosa in same region was (472.5 ± 0.7µm) and this difference in thickness may be attributed to quality and nutrition nature.

While thickness the mucosa of the cardiac region in sheep was (480.56 ± 88.82 µ m) and this result agree with study Malik *et al*., (2018) they observed thickness this layer in sheep of the cardiac region was (474.20 ± 12.47 µ m) but this study disagree a with AL-Haaik .(2009), who observed thickness of mucosa layer in pylorus region of sheep was (714 ± 56.8 µ m) and this difference in thickness may be attributed to the age and species of the sheep uses in this study.

Thickness of sub mucosa layer of rabbits in the pylorus region was (212.28 ± 20.43) and this result disagree with the study done by Khalel, (2012) which observed that sub mucosa thickness in rabbit female of the pylorus region was (177.75 ± 8.735 µ m) and this difference in thickness may be attributed explained to differe in the sex where present study adopted on only sex males.

Thickness of muscularis layer in the sheep in the pylorus region was significantly ($p<0.05$) larger than in rabbits where was (1427.79 ± 60.03 µ m) and this result consistent with study Malik *et al*., (2018) which found

that thickness of muscularis layer of the pylorus region in sheep (1365.80 ± 48.37μ m).

The pylorus region considered sphincter according to study Johnson-Delaney.(2006) which explained that increase thickness of mascularis layer may be help prevents back food from duodenum to stomach.

Thickness of serosa layer of the rabbits in the fundus and pylorus regions were significantly ($p<0.05$) larger than in sheep and this maybe because difference in species or amount food that sheep eat which this factor has effect of connective tissue cells, where was thickness this layer of the rabbits in pylorus region (158.62 ± 36.13μm) and this result disagree with study Kadhim., (2019), which observed serosa thickness to this area in *Grey Mongoose* was (98 ±13.4 μ m).

5.2.3. Histochemical Study

The results showing that the mucosa layer in the cardiac region in sheep and rabbits showed reaction a strong with PAS where appear as pink or magenta color in surface cells and body of gland and this indicator that these cells secreted mucous to counteract the acid of the stomach wall, but there are different in distribution neutral polysaccharides, while rabbit showing mucosa layer darkly color stain than mucosa of the sheep this may be due stain remove by washing during routine tissue preparation of mucosa sheep and this study similar to study Khalel, (2012) in rabbit, and on the other hand, Al-Saffar and Eyhab, (2016) reported that presence of neutral polysaccharidesis important to protection the stomach wall against acid digestion.

In the fundus region also the mucosa layer showed a strong reaction with PAS in both sheep and rabbits and this indicator that mucosa layer cells secreted mucous, but distribution neutral polysaccharide are only in surface cells of the mucosa layer, while basal fundus gland showed negative reaction with PAS and this may be due to found partial cells and chief cells where these cells was negative reaction with PAS because the function of these cells are limited which parietal cells secret HCL and chief cells secret pepsin, and this result consistent with (Khalel, 2012) in rabbit. Ranjan and Das, (2018) observed that parietal cells in rabbit showed positive reaction with PAS .

However, pylorus region mucosa showed strong reaction with PAS of both animal which was distribution neutral polysaccharides almost equal in all glands but this region more intense than other regions because these glands mostly made of cells secrete mucus which appear as magenta color and considered as mucus producing cells and few of parietal cells and this study agree with Ahmed *et al*., (2009) ; Wang *et al*., (2015) but this study disagree with study Igbokwe and Obinna, (2016) who observed that pyloric glands were weakly reaction with PAS.

Karakoc *et al*., (2016) reported that polysaccharides were present in the mucosa and the glands of the cardiac, fundus, and pyloric of the abomasums of both bulls and rams and this result agree with present study. There are doesn't difference at the reactivity of the gastric glands of sheep and rabbit with PAS and this may by a reflection functions of these glands is secreted mucus in stomach of sheep and rabbit.

5.3. Small Intestine

5.3. 1. Histological study

The walls of small intestine in all species of mammalian composed of four layers: the mucosa, sub-mucosa, muscularis and serosa and present findings was similarity with study (Călămar *et al*., (2014).

The mucosa layer in all segments of small intestine were covering by simple columnar epithelium and this study agree with study Pérez *et al*., (2011). However, the stem cells give rise to four major epithelial cells: the absorptive (enterocytes) which make up about 80% from small intestinal epithelial cells; the goblet cells which produces a variety of mucins and trefoil peptides needed for epithelial growth and repair; the enteroendocrine cells which export peptide hormones; and the paneth cells which secretes antimicrobial cryptdins or defensins, digestive enzymes, and growth factors (Snoeck *et al*., 2005).

The columnar cells, have nucleus oval, found in the base, and cytoplasm was eosinophilic. These results are similar to the study Parveen *et al*., (2013).

The goblet cells are unicellular mucous cells, in its apical part becomes puffy due to mucigen droplets accumulation, and have nucleus is irregularly oval or triangular at the base in both animals and this similarity with study Korkmaz and Kum , (2016).

In this respect, Goblet cells dispersed between the columnar cells of villi and Lieberkuhn crypts epitheliums and their function was secretion the alkaline mucus for neutralize of the ingesta (Junqueira and Carneiro, 2005). In sheep, Ergun and his team (2003) stated that absence paneth cells in the

villi of small intestine and that these cells were differentiated as such toward the base of the crypts.

Hassan and Moussa,(2015) observed that the crypts contain paneth cells in each three segments of the small intestine and were characteristic the presence of large acidophilic granules in the cytoplasm.

In another aspect, paneth cells are play role at innate immunity in the gut by their antimicrobial activity by their secreted peptides, Because these cells are also involved in linking innate and adaptive immunity, their role in human diseases become increasingly of top interest (Sabatino *et al*., 2008). Lamina propria under villi contain the secretory units of crypts Lieberkühn in rabbit and sheep where this glands show simple tubular in all regions small intestine .

Lamina propria observed also contain on blood vessel and lymphocytes, collagen, elastic fibers and glands, and this finding similarity with (Hassan and Moussa, 2015).

The muscularis mucosa was thin layer of smooth muscle fibers at the under the crypt but seen more thickness in sheep, and these consistent with (Lesson et al.,1988).

On the other hand, the mucosa layer of small intestine distinguish by the presence of villi, but these villi varies between is small intestine regions where are in sheep duodenum long and thin which finger shape and this result consistent with Saleh *et al*., (2012). While duodenum villi in the rabbit was broad and leafy shape and these findings are similar to study khamees and kareem , (2017).

Yu and Chiou, (1997) who considered that sources of dietary fiber i.e. pectin and cellulose may cause the villi to become blunted and compact in rabbits and rats.

The mucosa of Jejunum has villi vary ones from duodenum villi where are thinner and taller in rabbit and this agree with study Al-Haaik. (2016). While villi in sheep Jejunum broad and short tongue shaped and this result agree with Kumar *et al*., (2014).

Chiou *et al*., (1996) suggest that the various fiber components affect the height of villi and the thickness of the muscle layer of the jejunum and affect the crypt depth and ileum in local rabbits.

The submucosa layer in small intestine regions composed of connective tissue, but in both animals sub mucosa only in the duodenum has Brunner's glands are simple tubular. The duodenum is the site of most of the breakdown of the food passing through it (Elnasharty *et al*., 2013).

Brunner's glands have function nutralizes chyme entering to the duodenum from the pylorus, protecting the mucous membrane, and bringing the intestinal contents to the optimum pH for pancreatic enzyme action (Ergun *et al*., 2010).

The ducts of these glands penetrate the muscularis mucosae and usually pierce the base of the crypts of lieberkuhn to deliver their secretory product into the lumen of the duodenum (Mohammadpour. 2011). These secretions are respond to parasympathetic vagal stimuli (Moore *et al*., 2000). Moreover, the sub mucosa layer of Jejunum lack Brunner's glands in sheep and rabbit and this study compatible with (Hamza and Al-Mansor, 2017).

Hassan and Moussa, (2015) reported that duodenum goat didn't exhibit any glands of Brunner in the sub mucosa layer nor did the sub mucosa of jejunum contains any glands or lymphoid nodules.

Verdiglione *et al*., (2002) reported that the jejunum in large herbivores and pigs contain Brunner's glands.

The sub mucosa layer in sheep and rabbit ileum have peyer's patches different in shape and size, in sheep they well developed and have shape oval to elongated oval elongated with peaked end and large size, and this study similarity with study Kumar *et al*., (2015).

While ileum of rabbit have peyer's patches more number than sheep and their shape dome-shaped and this similar findings were reported in rabbit (Al-haaik. 2016).

Voutilainen *et al*., (2002) stated that aggregate lymphoid nodules which lie in intestinal tract at other animals and humans was the key transferred position that the lymphoid tissue executed immune response.

The muscularis layer composed by an inner circular and an outer longitudinal layer of smooth muscles and between connective tissue, similar findings were reported in sheep Kumar *et al*., (2015).

The serosa layer formed by the loose connective tissue had collagen, elastic and also reticular fibers along with varying amounts of fatty tissue, few blood capillaries, and flat mesothelial cells layer as reported indomestic animals (Stinson and Calhoun, 1993).

5.3. 2. Histomorphometeric Study

Thickness of the sheep mucosa layer in the duodenum, jejunum and ileum significantly ($p<0.05$) large in comparison to the duodenum, jejunum and ileum sections of the rabbits small intestine and this may be back to muscularis mucosa thicker than it is in rabbit.

In this respect, Mandir *et al*., (2005) consider that increase in thickness of intestinal epithelial tissue as well as the development of the gut itself can occur during three main mechanisms, that are elevation of cell production from the intestinal crypts, raise in number of crypt (by crypt fission)or by altered apoptosis.

The thickness of the mucosa in rabbit duodenum (846.84±55.03) and this result disagree with study Tomaszewska *et al*., (2014), which observed in guinea pigs female mucosa thickness of duodenum was (488.3 ±99.81) , this difference might be related to the nature of the nutrition intake of the animals.

Alves *et al*., (2004) reported that measurements of villi height gives an indication of the likely maturity and functional capacity of enterocytes.

Villi height was in the mucosa duodenum of rabbit was (566.90±127.81) and this result accordance with study Yu and Chiou, (1997) which was their result (543± 15) in rabbit. As wall this result disagree with Mohammad pour. (2011) observed villi high of guinea pig in mucosa duodenum was (785.00 ± 87.67), this difference might be related due vary in species. While recorded villi high of the ileum in rabbit was (510±78.87) and this finding disagree with study Al-haaik. (2016) observed villi high of rabbit in age 40 day was (318.00±2.33).

AL-Masri *et al*. (2015) observed that aging has the effect on structural changes in the intestinal mucosa of local rabbits.

Thickness of the sheep submucosa layer in the duodenum and jejunum were significantly ($p<0.05$) large comparison to sections the duodenum and jejunum of the rabbits small intestine, where was the thickness of sheep submucosa layer in the jejunum sheep (167.97±36.14 µm) and this result disagree with Firmansyah *et al*., (2019) observed thickness of tunica sub mucosa in Aceh cattle was (227.98±7.8 µm), this difference might be related due vary in species.

Thickness of muscularis layer in sheep significantly ($p<0.05$) large in comparison with rabbits, where muscularis thickness was in sheep duodenum (264.22±101.37) and this finding accordance with Mohammadpou. (2011) observed muscularis thikness in ganna pig duodenum was (293.18 ± 37.23).

In addition, thickness muscularis in rabbit jejunum was (102.63±12.02) and in ileum was its thickness (104.98±12.29) these result disagree with study Al-Haaik, . (2016) observed muscularis thickness in rabbit age 15 day in jejuinum was (77.28±2.05) and in ileum was (47.42±1.02) and this different in results may be due age and sex.

5.3. 3. Histochemical study

In both animals, the columnar cells gave a weakly reaction with PAS in each regions of small intestine and this may be indicate a lack of mucus secretion by these cells and this finding consistent with study Andleeb *et al*., (2009).

Goblet cells in sheep and rabbits, these cells gave strong reaction with PAS and this is evidence of neutral mucus in duodenum, jejunum and ileum.

Moreover, Kadadi. (2012) stated that duodenal goblet cells in sheep stained with magenta indicating presence of PAS positive material in their secretion.

Kumar *et al*., (2014) observed that goblet cell and crypts of Lieberkuhn were strong reaction with PAS in jejunum sheep (Ovis Aries). Jawad *et al*., (2019) reported that crypts of Lieberkuhn in the tunica mucosa of the duodenum in rabbits positive reaction with PAS.

The PAS staining properties of duodenal sub mucosal glands showed marked differences in this study, where Brunner's glands in sheep be mucous acina only because gave strong reaction with PAS which this is evidence of the presence of neutral carbohydrates for of neutralize of the acidic chyme come from the stomach, and this result agree with study Kadadi. (2012) which reported that Brunner's glands in sheep showed positive reaction with PAS.

However, Sub mucosal glands is vary with species and there are three types of acini (mucus, serous and mixed) Al-haaik. (2016). Previous study, Mohammadpou. (2011);Krause, (2000) observed that Brunner's glands in ganna pig and moose they mucos acina only.

The studies uses different techniques depend on species, duodenal sub-mucosal glands were reported to contains neutral or acidic mucin glycoproteins or the combination of both types of mucin (Takehana *et al*., 1991; Krause, 2000).

Andleeb *et al*., (2009) reported that Brunner's glands in Gaddi goat gave strong reaction with PAS and this is evidence of the presence of neutral carbohydrates.

On the other hand, Hamza and Al-Mansor (2017) observed that Brunner's glands in Gazelle mixed acina and showing weakly reaction with PAS and this result disagree with present study and this different may be due species. Although similarities between the duodenal glands of different animals have been emphasized in the past, physiological evidence of significant differences between species has been reported in recent chemical tissue studies (Krause, 2000; Morre *et al*., 2000).

Brunner's glands in rabbit mixed acina but mostly serous this may indicate the secretion of enzymes and not mucous substances and this conclusion according to study Kirkegaard *et al*., (1984) mentioned that the secretory cells of Brunner's glands secrete lysozyme continuously which a bactericidal enzyme in humans.

Elnasharty *et al*., (2013); Jawad *et al* .(2019) observed that there are groups of serous-type cells lying between the mucous acini of Brunner's glands in rabbit, these findings were similar to ours result.

Ergun *et al.*, (2003) stated that the mucous cells in the acini of Brunner's glands contained neutral, carboxylic and sulpho acidic mucin,

Makovicky *et al*., (2014) reported that rabbits very specific animal models due of the functional and morphological specificities of their digestive tracts due to the quality of their specific herbivorous feed.

The results of this study agree with study Ergun *et al*., (2010), which observed Brunner's glands mixed acini in rabbit Angora. While this study disagree with Rashmi and Prasad, (2016) found that the Brunner's glands of guinea pig were composed of only mucous acini and this difference in results may be due natural nutrition.

Ergun *et al*., (2003) stated that serous cells of Brunner's glands contain neutral mucopolysaccharides.

Schumacher *et al*., (2004) mentioned that staining properties of Brunner glands are marked differences, in bison, deer, voles and domestic rabbit they contain acidic sulphated and carboxylate mucins, whereas in humans, cats, raccoons and rats they contain neutral mucins.

There are numerous groups of serous type cells lying among the mucous acini in rabbit. It has long been believed that these cells are of a nature similar to those of the pancreatic acini (Jawad *et al*., 2019).

Andleeb *et al*., (2009) stated that the lamina propria and the lamina muscularis mucosae have not shown any PAS and reaction at the ileum as also reported at Gaddi goats.

5.4. Gastrin Hormone

As no differences between sheep and rabbits in hormone level and this is may be evidence of the similarity of the physiological characteristics between these animals, as they are herbivores or both animals are characterized by a high degree of acidity in the stomach.

In this study the results showed non-significant ($p>0.05$) differences between hormone level of serum gastrin in sheep and rabbit. This results are agree to the study done by Amure and Omole, (1971), they stated that non-significant differences in gastrin levels among goat, rabbit and cattle and addition that these animals have highest gastrin concentration comparative non-herbivorous species (cat, dog, man and pig).

They herbivorous, especially those with ruminant, stomach secrete considerable amounts of gastric juice, even more than in the non-herbivores- and since the cephalic phase of gastric secretion is either absent or unimportant in the herbivores, the voluminous secretion of juice in these species would necessarily require a large production of gastrin, in herbivores too, the concentration of hydrochloric acid in the gastric juice appears to be less than is found in the non-herbivores (Amure and Omole, 1971).

Dietary fibre because its water binding properties (distension effect), can activate the vagal and intramural nervous reflexes in the area of the stomach and secretory mechanisms, may increase GIT tract cells proliferation and gastrin released (Korczynski *et al*., 2004).

Kataria *et al*. (2008) stated that feeding did not affect the levels of gastrin in abomasum sheep ,it could be due to the fact that in ruminants the abomasum receives a continuous, though variable inflow of forestomach material.

The stomach of rabbit is very acidic with a pH of 1 to 2 (Beasley *et al*., 2015). Abdel-Maged *et al*. (2013) stated that the normal range of serum gastrin in rabbits is (87.93±0.94 pg/ml). The elevation of gastric pH is accompanied by higher gastrin and pepsinogen release (Lawton *et al*., 1996).

In addition to, Lawton. (1995) stated that the normal range, serum gastrin in sheep is (12-64 pg/ml).While, Kataria *et al*. (2008) observed Plasma levels of gastrin in Marwari sheep is (103.45± 10. 41 pg/ml).

Morisset. (2005) stated that gastrin hormone has two shapes, they were named gastrin I, the non-sulfated form, and gastrin II the sulfated form, but similar forms of gastrin were later found in cat, cow, sheep, dog, goat, rat, guinea pig and rabbit.

5.5. Digestive enzymes.

5.5. 1. Pepsinogen.

The levels of PG I and PG II, which might be confounding for comparative with studies other, and this is may be evidence of the similarity types nutrition between these animals, as they are herbivores or both animals are characterized by a high degree of acidity in the stomach.

Pepsinogens are zymogens of pepsins, aspartic proteases working as digestive enzymes in the vertebrate stomach (Yasugi. 2002). It is protein of molecular weight (mol. wt). 43,000 and activated to pepsin by acidification with the loss of a number of peptides, totalling about 5,000 mol.wt (Hirschowitz. 1984). Pepsin has been studied extensively in a vast range of animals such as; pigs, chickens and cold-water fish, displaying pepsin is present in two main isoforms - PG A and PG C (Peter *et al*., 2019).

Serum PG concentrations have been shown to reflect the morphological and functional status of the gastric mucosa. As the fundic gland mucosa

reduces, PG I levels gradually decrease, whereas PG II levels remain constant (Normura *et al*., 2005). The majority of PGs directly enter the stomach cavity, but a small amount also enters the gastric mucosal capillaries and into the bloodstream, which can then be detected in serum. Following synthesis, much of the PG is activated into pepsin. Thus, PG can be used to determine gastric mucosal status (Liu *et al*., 2019). Morgado *et al* ., (2014) mentioned that pepsinogen converted to pepsin in an acid environment. The conversion begins at a gastric pH of approximately 5.0, and its optimal activity occurs at pH values between 1.8 and 3.5. The normal serum values of the enzyme range from zero to 5.0 IU/L in cattle. Also, Hajimohammadi *et al*., (2010) reported that the reference range for the mean pepsinogen level in cattle is 2.54–3.54 IU/l. Also, reported that low or normal levels of serum pepsinogen (< 5.0 IU/l) may be useful as a predictor for low susceptibility in major changes to the mucous membrane of the abomasum (Mesaric, 2005). Kataria *et al*., (2008) found that blood levels of pepsinogen can be used in the diagnosis of abomasal parasitism or disorders. Increase plasma levels of pepsinogen are due to its leakage into the blood vessels from damaged abomasal mucosa and increased activation of pepsinogen into pepsin by enhanced acidity of gastric contents can cause ulcers in humans and animal.

In study done by Noori and Jassim (2016) found the value of pepsinogen in the healthy sheep about (3.13 IU/I). Hirschowitz (1984) stated the pepsinogen occurred in all stomach, but molecule of the same weight and structure in all species.

5.5.2. Pancreatic Enzymes.

The differences obtained in sheep and rabbit ranges can be explained by the different physiology of the two species.the reason for the low level of these enzymes in sheep is due to the fact that sheep and rabbit are different in species and likewise in the quantity and quality of food intake where the sheep need a large amount of food compared to rabbits or perhaps because of the presence of the two enzymes in the rumen fluid of the sheep as that is, the microorganisms produce the enzyme amylase and lipase there are effect on carbohydrates and fats and this conclusion according to Moharrery and Das (2001) which they study enzymatic activities were estimated for urease, cellulose, protease, amylase, and lipase in various fractions of rumen fluid in sheep.

The pattern of enzyme activity is closely associated with type of feed and intestine transit time. Intestinal muscular contractions shift chyme, water and enzymes along the GIT, so the enzyme activity is found deeper within the intestine as time passes from consumption(Snoeck *et al*.,2004).

The presence of digestive enzymes within the gut lumen prior to the arrival of the ingested feed bolus is essential in order to ensure the highest level of enzyme activity. This mirrors the natural condition in which enzymes are released after the cephalic phase of pancreatic enzyme stimulation (Konturek *et al*.,2003).

In the non-ruminant, starch digestion occurs mainly in the small intestine. The situation in the ruminant differs due to the action of microorganisms in the rumen (Cerrilla and Martínez, 2003).

On the other hand, Cerrilla and Martínez (2003) found that in the non-ruminant, starch digestion occurs mainly in the small intestine, the situation

in the ruminant differs due to the action of microorganisms in the rumen. The capacity of the ruminant small intestine to digest large amounts of starch has been questioned as a consequence of the low levels of pancreatic amylase, intestinal maltase and isomaltase (Cerrilla and Martíne, 2003).

Swanson *et al*. (2000) stated that the presence of glucose or starch hydrolysate in the small intestine decreases secretion of amylase in cattle . Al-Abedi and his colleagues (2020) observed amylase enzyme in sheep was (18.45 ± 3.02) and lipase enzyme was (23.30 ± 2.20) where was sheep food treatment were fed concentrate diet containing 14% crude protein and this result different with ours results may be due to differ in food quality. Kreikemeier and Harmon, (1991) suggest that the amount of protein in the diet could play an important role in starch digestion in the small intestine.

Furthermore, Burski *et al*. (2004) they suggest that choosing serum α-amylase as an indicator of the exocrine pancreatic function is based on the enzyme's relative stability, long breakdown period, high sensitivity and specificity, in comparison with other pancreatic enzymes being also used in daily clinical practice.

On the other hand, Das *et al*. (2017) they recorded normal level of the enzyme amylase (92.2 ±11.47) in non-descript sheep and this result disagree with ours results may be due different in strain species or food. Moreover, these enzymes high level in rabbit than in sheep and this probably due its food rich in protein which source feces and this ours conclusion according to Johnson *et al*. (1977) ; Cerrilla and Martínez (2003) they suggest the stimulatory effect of protein on pancreatic amylase secretion has been shown to occur in non-ruminants, fed a high carbohydrate diet to rats,

finding that amylase synthesis was stimulated only in the presence of high quality proteins.

However, Suckow *et al*. (2012) observed the normal value activity of amylase in rabbit is (200-500 U/L) and this result agree with ours results in rabbit . Whereas, Burski and his team.(2004) observed the normal level of activity amylase is 124 U/L in rabbits and this finding different with ours results may be due different in food quality . Khan *et al*.(2019) recorded an normal level of the lipase and amylase enzymes in rabbit as (109 ± 1.00 U/L; 53 ±1.58 U/L) respectively.

Gutiérrez *et al.* (2002) who found that amylase is already present from birth but markedly increase after weaning from day 24 onwards suggesting that the feed greatly stimulates amylase biosynthesis. The role and mechanism of nonparallel pancreatic secretion of digestive enzymes, in which enzymes proportions change in rapidly regulated fashion, remain controversial (Adelson *et al*.,1995) .

In ruminants, pancreatic α -amylase is the primary enzyme responsible for the initial hydrolysis of α -linked glucose in small intestine lumen (Swanson *et al*., 2000) .

Ruminants don’t have salivary α-amylase (Al-Abedi *et al*.,2020), but secrete pancreatic α-amylase which hydrolyzes starch within the intestinal lumen (Kreikemeier . 1991).

In ruminants, Pancreatic lipase has the same nature and function of nonruminants (Al-Abedi *et al*.,2020).

Xu *et al*.(2006) observed that that pancreatic amylase activity was decreased with a lower starch diet.

Al-Abedi *et al*.(2020) reported that in non -ruminants pancreatic lipase increases in response to increased dietary triglyceride.

Murai *et al*. (2000) their found that production pancreatic amylase is regulated mainly by neural factors instead of gut hormones.

Contreras-Aguilar *et al*.(2019) they were considered lipase enzymes as a biomarker of stress in sheep.

Carriere *et al*.,(1993) they suggested that gastric lipase cannot replace the pancreatic lipase under conditions of pancreatic insufficiency. In addition , Capolino and his team (2011) reported that the rabbit stomach is known to be an abundant source of gastric lipase which is resistant and active under acidic pH conditions.

Whereas, Borel *et al*.(1991) gastric lipase shows an adaptive response to dietary fat in the rabbit. most preduodenal lipase activity is supported by gastric lipase. gastric lipase is the only lipolytic enzyme in the stomach.

Chapter six

Conclusion and Recommendation

6.1. Conclusions

1- This study presented information histochemical and microscopic features of the esophagus, stomach and small intestine of sheep and rabbits, this information can be used as a basis for further studies of these organs and consequently, determine any pathological changes in these species.

2- The results of the current study indicated similarities and differences in, the histological structures of the esophagus, stomach and small intestine between sheep and rabbits and this help in the ongoing struggle to clarify the degree of kinship between these animals.

3- The type of epithelium lining the esophagus in sheep was a keratinized epithelial layer, while in rabbit it was not keratinized epithelial layer.

4- There are differences in the types of glands in the stomach areas between the sheep and the rabbits. Also, There is a difference in the type of the Brunner's glands in rabbits, were glands is mixed, while in sheep were mucous glands.

5- The layers thickness of the esophagus and small intestine were large in sheep than rabbit while thickness some layers in stomach were large in rabbit than sheep.

6- There were no significant differences in the level of gastrin hormone between sheep and rabbits. Also, There were no significant differences in the level of pepsinogen 1and pepsinogen 11 enzymes between sheep and rabbits.

7-High level a-amylase and lipase in rabbit than sheep , may be these results as a reference can researches use in diagnosis..

6.2. Recommendations

1- Future study included large number of animals to give a clearer picture on the hormone and digestive enzyme in sheep and rabbits.

2- Interest in studying farm animals which have a financial return to the country, because of their characteristics that differ from other animals.

3- Study the effect of different feeding on the level of the gastrin hormone in sheep and rabbits.

4- Further study to elucidate the presence, distribution and functions of the duodenum endocrine cells in rabbits and sheep.

5- Study the activity of the microorganisms in the rumen, and it's relationship with deficiency of pancreatic enzymes in sheep.

6- Genetic studies about the digestive enzymes and hormones in farm animals.

7- Conducting studies on animals at different ages such as young and old or before and after weaning.

8- Study the impact of the climatic and environmental factors on digestive enzymes and hormones in farm animals

9- Study the effects of some medications on the level of pepsinogen enzyme and the gastrin hormone.

Chapter seven

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Appendix

Appendix Appendix 1: Hematoxylin stain

Hematoxylin Solution (Ehrlich's)

ethanol (100%)……..100 mL

glycerol……100 mL

glacial acetic acid……..10 mL

hematoxylin…….2g

Humotoxylin is mixed with ethanol alcohol, then added glycerol and glacial acetic acid, then added a quantity of potassium alum ,these components place in glass bottle exposed to sunlight. The vial is opened for a period, then closed and shaken. This process is repeated for several weeks until the dye matures.

Appendix 2: eosin Y stain

Eosin Y ..... 1 g
Distilled water 20ml
95% Ethanol..... 80 ml

Mix to dissolve and store in room temperature.

Appendix 3: Periodic Acid Schiff PAS technique

Solutions

Periodic Acid Solution:

Periodic acid ..... 1 g

Distilled water..... 100 ml

Schiff Reagent:

Basic fuchsin.....1.0 gm.

Sodium metabisulphite .....2 gm.

Distilled water.....100 ml

Hydrochloric acid..... 2 ml

Charcoal activated ..... 0.3 gm

Dissolve basic fuchsin in boiling water, cool at 50 ^{0}C and filter. add sodium metabisulphite and HCl.store at dark room at room temperature overnight. Add charcoal, shake for one minute and filter.

Results: Glycogen, neutral glycoprotein : magenta

الخلاصة

أجريت الدراسة الحالية لمقارنه التركيب النسيجي القياسي والكيميائي النسيجي لكل من المريء والمعدة والأمعاء الدقيقة بين الأغنام والأرانب وقياس بعض المعايير الفسيولوجية لهذه الحيوانات. ولتحقيق هذا الهدف تم جمع العينات من سوق ميسان المحلي والمسلخ المحلي على التوالي. اشتملت الدراسة على 20 حيوانًا ، عشرة ذكور من الأغنام وعشرة ذكور من الأرانب ، تراوحت أوزانهم من 35-45 كجم للأغنام و1.5-2.5 كجم للارانب. كانت فترة الدراسة من (2019/11/4 إلى 2020/7/4) ، استخدمت للدراسات النسيجية نوعين من الصبغات ، الهيماتوكسيلين والأيوسن ، والصبغه الخاصة (PAS).

أظهرت الدراسة النسيجية للمريء اختلافات في نوع ظهارة الغشاء المخاطي المبطن للمريء بين الأغنام والأرانب ، وكانت البطانة الظهارية مكونة من ظهارة حرشفية طبقية متقرنة في الأغنام بينما في الأرانب كانت مكونة من طبقة حرشفية غير متقرنة. في كلا الحيوانين لا تمتلك طبقة تحت المخاطية للمريء غددًا. تتكون الطبقة العضلية لكليهما من عضلات مخططة ،كلا الحيوانين يحتويان على طبقة خارجية من النسيج الضام الرخو تسمى الطبقه المصليه. أظهرت جميع الطبقات للمريء الأغنام سماكة أكبر من تلك الموجودة في الأرانب.

أظهرت الدراسة الكيميائية النسيجية أن التفاعل مع صبغة PAS كان متشابهًا بين الحيوانات وفي أماكن مختلفة. اذ أظهرتا الطبقة القرنية لغشاء ظهارة لمرى الأغنام والطبقه السطحيه لغشاء ظهارة لمرى الأرنب تفاعلًا قويًا مع هذه الصبغه. في المقابل ، كانت بقية الطبقات الغشاء المخاطي والطبقات العضلية تفاعلات معتدلة مع صبغة PAS في جميع مناطق مريء الأغنام والأرانب. أظهر الغشاء تحت المخاطي والمصليه تفاعل ضعيف مع صبغة PAS في كلا الحيوانين.

من ناحية أخرى ، أظهرت الدراسة النسيجية للمعدة تشابه في نوع ظهارة الغشاء المخاطي المبطن للمعدة في الأغنام والأرانب ، حيث تتكون البطانة الظهارية من طبقه عمودية بسيطة ، بينما تختلف الغدد المعدية بين الأغنام والأرانب ، حيث كانت الغدد cardiac في الأغنام أنبوبية بسيطة ولكن في الأرانب أنبوبية بسيطة ،ملفوفة ومتفرعة .لكن أظهرت غدد قاع في الأغنام أنبوبية بسيطة ومستقيمة، في الأرانب بسيطة ،مستقيمة ومتفرعة. في حين أن غدد البوابيه في الأغنام بسيطة ، متفرعة أنبوبية وملفوفة ، في الأرانب كانت أنبوبية بسيطة .

طبقة الغشاء تحت المخاطي في المعدة لا تمتلك غددًا في المعدة ، تتكون الطبقة العضلية لكليهما من ألياف عضلية ملساء في جميع مناطق المعدة ، تتكون الطبقة الخارجية من المعدة من نسيج ضام رخو تسمى المصليه. بينما سمك الطبقات في المعدة كان مختلفا بين الحيوانين.

بالإضافة إلى أن الطبقة المخاطية لمنطقة cardiac تظهر تفاعلاً قوياً مع صبغه PAS للأغنام والأرانب وتوزيع سكريات يتركز في الخلايا السطحية وجسم الغده . تظهر صبغه PAS في منطقه قاع الأغنام والأرانب تفاعلًا قويًا مع الخلايا السطحية للطبقة المخاطية ، بينما تظهر صبغه ذاتها تفاعلًا ضعيفًا في الخلايا الجدارية والخلايا الرئيسية في المنطقة البوابيه ، أعطت الطبقة المخاطية في الأغنام والأرانب تفاعلًا قويًا مع صبغه PAS ، وتوزيع السكريات المحايدة متساوية تقريبًا في جميع أجزاء لغدد البوابيه.

في الأمعاء الدقيقة الطبقة المخاطية تظهر عبارة عن ظهارة عمودية بسيطة . تظهر نتائج الفحص النسيجي أن الغشاء المخاطي للاثني عشر والصائم واللفائفي يحتوي على عدد من الزغابات المكونة من الخلايا المعوية والخلايا الكأسية فقط. تختلف نتوءات الزغابات في الشكل والحجم ، في الاثني عشر الأغنام طويلة ورفيعة أي شكلها إصبعي ، ولكن في الاثني عشر أرنب كانت عريضه وورقيه الشكل المركز الزغابات يتكون من النسيج الضام الرخو الصفيحه الاساسيه في الامعاء الدقيقه تتكون في كلا الحيوانين من للنسيج الضام يحتوي على الغدد المعوية أو تسمى خبايا ليبيركون وتتكون من خلايا عمودية وخلايا كأسيه وخلايا Paneth.

ظهرت هذه الغدد بشكل متطور جدا تظهر أنبوبيه بسيطه من جهه اخرى، أعطت غدد برونر في الطبقة تحت المخاطية تفاعلًا قويًا مع صبغه حامض الدوري اي غدد مخاطيه في الأغنام ، بينما في الأرانب كانت غدد برونر مختلطة (الخلايا المصلية والخلايا المخاطية). بينما أعطت الطبقة تحت المخاطية في الصائم تفاعلًا ضعيفًا مع صبغه حامض الدوري في الأغنام والأرانب، لكن بقع باير في الغشاء تحت المخاطي للفائفي أظهرت تفاعلًا معتدلًا مع صبغهPAS في كلا الحيوانين.

من الناحية الفسيولوجية ، أظهرت نتائج الدراسة الحالية أن قيم هرمون الجاسترين لم تكن معنوية ($p>0.05$) في الأغنام والأرنب. أظهرت نتائج الدراسة الحالية أن قيم الببسينوجين 1و البيبسينوجين 11 لم تختلف معنويا ($P> 0.05$) بين الحيوانين. من ناحية أخرى ، هناك ارتفاع معنوي ($p<0.05$) في مستوى انزيم ألفا الأميليز ومستوى انزيم الليباز في المصل الأرانب مقارنة

في الأغنام. تم تحليل جميع النتائج بواسطة (T-test). في الختام أوضحت هذه الدراسة أن الأغنام والأرانب لها أوجه تشابه واختلاف في المريء والمعدة والأمعاء الدقيقة. أي أن طبقات هذه الاعضاء لها سمك مختلفة وتستجيب بشكل مختلف لـ PAS.

وزارة التعليم العالي والبحث العلمي

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دراسة مقارنة نسيجية وفسلجية بين الارانب و الاغنام لبعض مناطق القناة الهضمية

رسالة مقدمة

الى مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

من قبل

بكالوريوس علوم /علوم الحياة (2015)

بأشراف

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