Detection of cholecystokinin and glucagon like peptide in small intestine of Awassi sheep

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Abstract

The enter endocrine cells in small intestine of sheep secreting some hormones that play key roles in regulation of certain important organs. The endocrine cells of GIT are generally divided into two types, the open and close type.The aim of this study was unveil the relative frequency and regional distribution of enteroendocrine cells in some portions of small intestine of the Awassi sheep, detecting by using immunohistochemistry techniques. Specimens of small intestine from ten of both sexes with different ages of sheep *Ovis aries* were used. The Immunohistochemistry technique formed using two types of hormones cholecystokinin (CCK-8) and glucagon like peptide (GLP-1). Result of immune detection findings demonstrated that in part of small intestine (duodenum, jejunum and ileum) there is clearly expression of the CCK-8 and GLP-1 subset of cells along the villus and crypts. The cells are contained gut hormones appeared to be either triangular or flask-like in shape. I-cell which contain CCK-8 increase proximally of small intestine and decrease caudally, while L-cell which contain GLP-1 decrease proximally but increase caudally of small intestine.
Key words: Endocrine cells, intestinal tract, immunohistochemistry, hormones, sheep.

Introduction:

Iraq has a large number of livestock ruminants especially sheep, presently there are in Iraq an estimated 7-8 million. Sheep (*Ovis aries*) are compound stomach animals belonging to Bovidae family. Sheep involves the local breeds Awasi, Hamdany and Karadi which play the most important role in food industry and other associated industries (1).The small ruminant like (sheep) are an important source to produce meat and milk even in hostile environments and resistance to harsh conditions and disease, and their capacity to generate additional income in poor rural areas are increasingly appreciated. (2) The small intestine is a specialized tubular structure within the` abdomen. In ruminants lie exactly entirely to the right of the midline, packed mainly into the dorsal part of the abdomen (3). In ox and sheep existent in the right half of the abdominal cavity with a few coils caudal and ventral to the rumen, in horse are mostly in the dorsal part of the left half of abdominal cavity (4), while in camel as the *Llama* and the *Alpaca* occupy most of the caudal space and caudodorsal to the abomasum, particularly in the right para lumbar fossa (5). The wall of small intestine in ruminant is form four layers: tunica serosa the outermost layer, tunica muscularis, tunica submucosa and mucosa (6,7). The mucosa of small intestine have a simple columnar epithelium containing the columnar cell, absorptive cells, goblet cells, Panath cell and enteroendocrine cells (8), which derived from common precursor cells (Stem cells) which located in the intestinal crypts and differentiate into all four cell types present in the intestinal epithelium (9).The endocrine system mediates long-range peptide hormone signalling to broadcast changes in metabolic status to distant target tissues via the circulatory system, and have a critical role in regulation of appetite and energy balance.(10). The intestinal tract is contain 15 different endocrine cells types that release more than 100 biologically active peptides and hormones (11,12,13).The diffuse endocrine system differs region of gastrointestinal tract, the glandular endocrine system in that it must be continuously renewed, in many animals, endocrine system of the gut is the largest endocrine tissue (14). Unlike endocrine cells in the pancreas, which cluster together to form islets, the enteroendocrine cells are scattered as

individual cells throughout the gastrointestinal mucosa (15). I-cells are a subtype of enteroendocrine cells localized in duodenum that release cholecystokinin in response to ingested fat and amino-acids (16), the apical membrane of these cells connections the luminal contents whereas the basal membrane is commonly thought to be a major site of regulatory peptide release into the bloodstream (17), and it provided the establishment for digestive physiology, cholecystokinin CCK peptides are primarily synthesized in endocrine I-cells, and it's a well-established gut hormone that regulates gallbladder emptying, pancreatic enzyme secretion and pancreatic growth. It's also contributes to control the intestinal motility, inhibition of gastric acid secretion, and gastric motility. In addition, cholecytokinin (CCK) is a transmitter in central and peripheral nerves (18). The highest density of CCK-8 in the proximal part of small intestine (proximal duodenum),while its lower in density in distal part of small intestine (ileum) in mammals (17), Glucagon-like peptide-1(GLP-1) is (30) amino acid peptide hormone produced in the intestinal epithelial, form endocrine L-cells by differential processing of proglucagon, in response to glucose. L cells of the gut glucose through the same mechanisms used by taste cells of the tongue, regulates appetite, insulin secretion, and gut motility (19), and they have been identified at low density in the duodenum and higher in density in jejunum (20).

Materials and methods :

Specimen were obtained from small intestine of ten sheep (collected the specimens from AL-Qadisiyah abattoir during November (2016), approximately 50cm in length were removed from the duodenum (proximal, middle, and distal parts), jejunum and ileum (proximal, and distal parts), then washing each part by normal saline, then they are fixed in10% formalin solution for 48 hrs. at room temperature. Samples were washed with running tap water for two hrs., and then treated by routine histological processing and using the routine stain Harries Hematoxylin and Eosin (21). For immunohistochemical study, use the paraffin protocol as the instruction of kits manufacture (Table 1).

Counting of enteroendocrine cells

Counting of enter endocrine cells from each section (duodenum, jejunum and ileum) were made by Image J analysis software; data were expressed as (X±SE) (22).

Table (1): Indicate the kite used in the immunohistochemical study.

(Kite name)	Dilution	Origin
Anti-Cholicytokinine-8 Rabbit polyclonal to cholicytokinin8-azid free react with human 0.2mg/ml -20c	1:200	England Abcam
Glucagon like peptide-1 Rabbit polyclonal toGLP-1 react with: mouse, Rat, human in 0.2mg/ml in20c	1:2000	England Abcam
Immunohistochemical detection kit		USA US Biological
-Normal goat serum -Biotinylated anti-IgG -Streptavidin -biotinated -Liquid DAB -DAB -DAB buffer -Detoxification buffer	1:500	

Results:

The histological structure of wall small intestine was composed of four layers, from outside to inside: the serosa, muscularis, submucosa, and mucosa. The villi of small intestine were seen intact, and the epithelial cells attached to the basement membrane indicate the integrity of the tissue of small intestine (Fig.1). The immunohistochemistry was carried on the three parts of sheep small intestine (duodenum, jejunum and ileum) using 2 types of hormone which are (GLP-1) and (CCK-8). Depend on (Table 2) the immunohistochemistry presented clearly that the gut hormones were expressed solely in a subpopulation of cells along the villus of small intestine of the sheep.

Expression of CCK-8

(CCK IR) cells were observed in high repeatedly in the villi and the intestinal crypts in different parts of small intestine of sheep. The mean of immune reactive cell in three parts of duodenum (proximal (30±0), middle (29.6±0.24) and distal (27.8±0.8), while in two parts of jejunum (proximal (20.2±1.01), and distal (17.6±1.63) , while in the two parts of ileum CCK IR- cells few detected (proximal (17.8±1.35), and distal (16.6±1.72) (table 2). In the small intestine most of the (CCK-8) cells (I-cells) were detected flask-shaped with apices pointing towards the lumen of the gut. The relative frequency of these cells was caudally decreased along the small intestine. The I-cells expressed the CCK hormone in duodenum and subsequent-tly was decreased in jejunum and ileum. The frequency and distribution of IR endocrine cells in the duodenum on the intestinal villi, the crypts (intestinal glands) and in Brunner's glands (duodenal glands) commonly were observed in high frequency in the villi of duodenum, less frequently on the intestinal crypts and rarely in Brunner's glands (Fig. 2). In the jejunum CCK-8IR-cells were observed in moderate frequency on the intestinal villi (Fig. 3), while in the ileum CCK-8IR-cells were few detected (Fig. 4).

Table (2): Regional distributions and relative frequencies of the endocrine cells in the small intestine of sheep

Part of small intestine	Expression GLP-1	Expression CCK-8
Duodenum prox. part	15.5±0.6	30±0
Duodenum middle part	13.2±0.66	29.6±0.24
Duodenum distal part	12.2±0.86	27.8±0.8
Jejunum proximal part	19.8±0.86	20.2±1.01
Jejunum distal part	20.8±0.48	17.6±1.63
Ileum proximal part	25.8±0.48	17.8±1.35
Ileum distal part	28.2±0.96	16.6±1.72

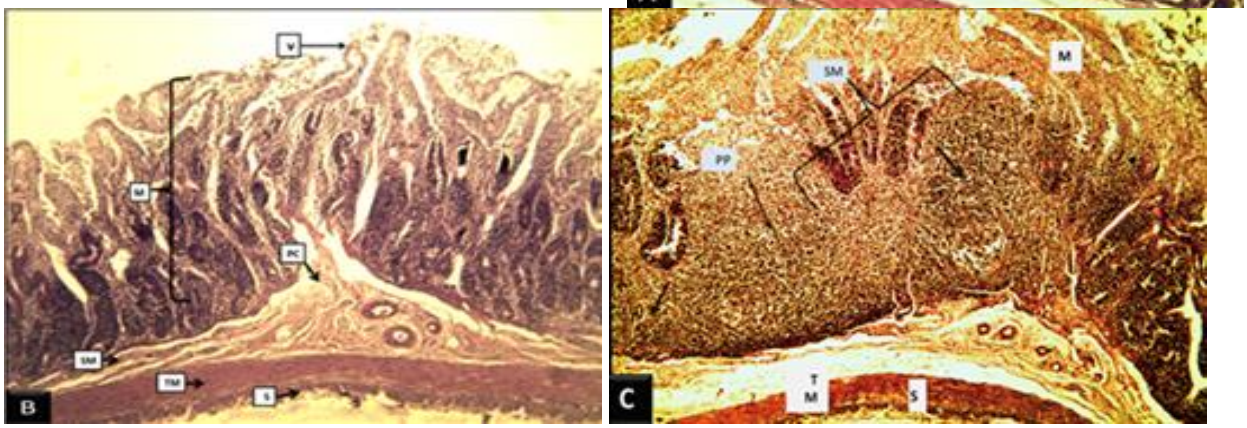

Fig. (1): Intestinal sections of control (A-duodenum, B-jejunum, C-Ileum) showing; M: mucosa, SM: submucosa, TM: tunica muscularis, S:serosa, V:villi, PC: plica circularis, PP: Payers patch (H&E X10)

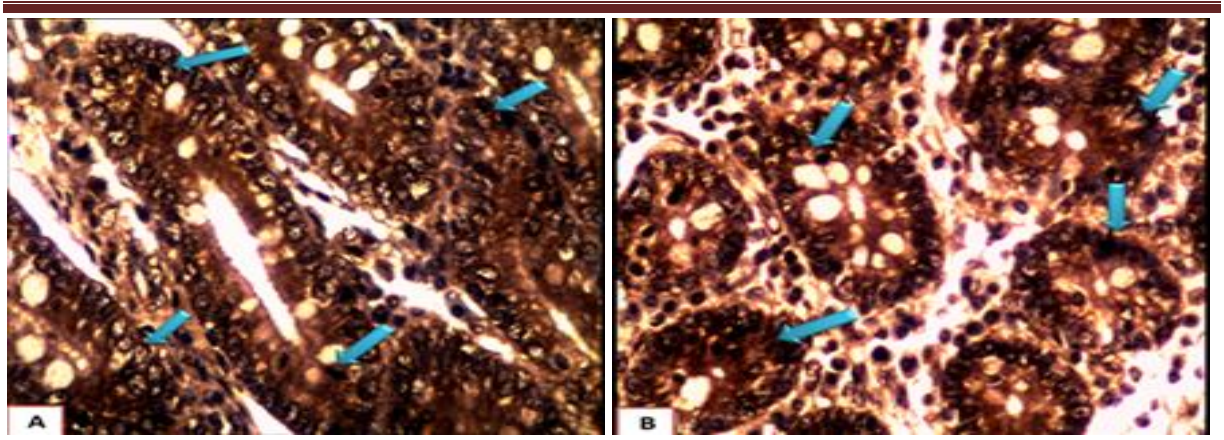

Fig. (2): Immunohistochemical sections in doudeneum experssion CCK-8 (blue arrows) higher in density in intestinal glands (A) and Brunners glands (B) (X40)

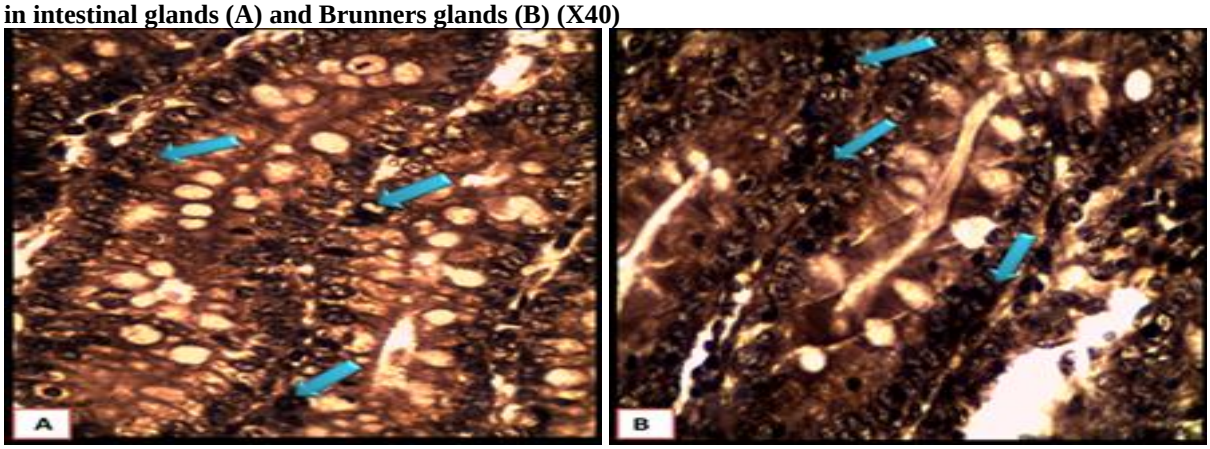

Fig. (3): Immunohistochemical sections in jejunum expression CCK-8 (blue arrows) moderate in density, A: Proximal part, B: distal part (X40)

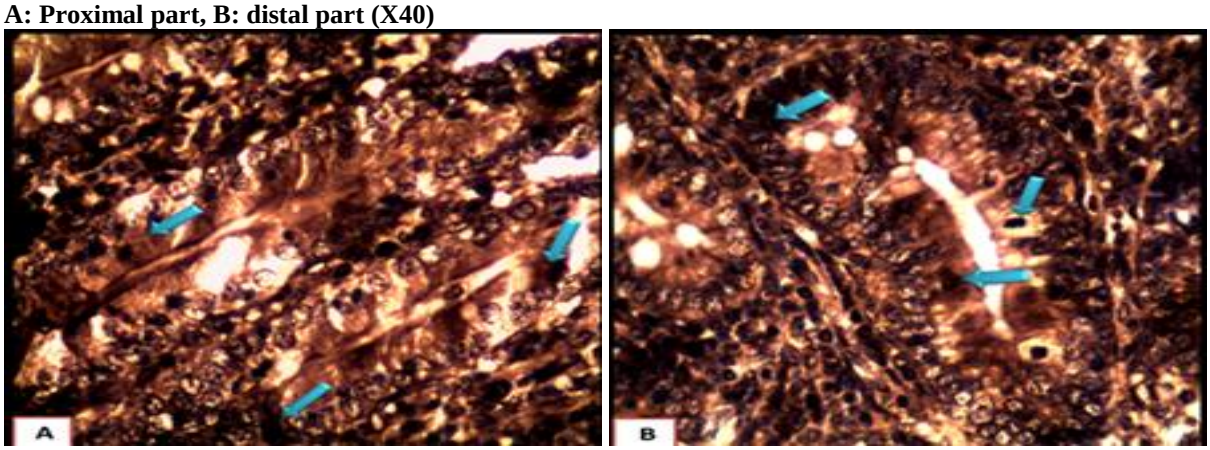

Fig. (4): Immunohistochemical sections in ileum expression CCK-8 (blue arrows) less in density A: Proximal part, B: distal part (X40)

Expression of GLP-1

Immunoreactivity for (GLP-1) was observed in duodenum, jejunum and Ileum of sheep. The density of (GLP-1 IR) cells in each intestinal region were mainly noticed in crypts and villi in duodenum (Fig. 5), and jejunum (Fig. 6), and in middle and distal ileum, were noticed in the lower part of villi and crypts (Fig. 7). (GLP-1 IR) cells appeared as pyramidal or spindle-like shape in the villus epithelium, and comma-like shape in crypts. The density of (GLP-1) labeled cells noticed decrease intensity in the duodenum but higher in distal part of small intestine (distal jejunum and the two parts of ileum). The mean of immune reactive in duodenum (proximal part (15.5±0.6), middle (13.2±0.66), distal (12.2±0.86)). In jejunum (proximal part19.8±0.86, distal 20.8±0.48), while as in ileum (proximal part 25.8±0.48, distal part 28.2±0.96) (Table 2). The density of (GLP-1 IR) cells in the ileum and jejunum were higher than that in the duodenum, while in the distal duodenum it was lower than that in the proximal duodenum.

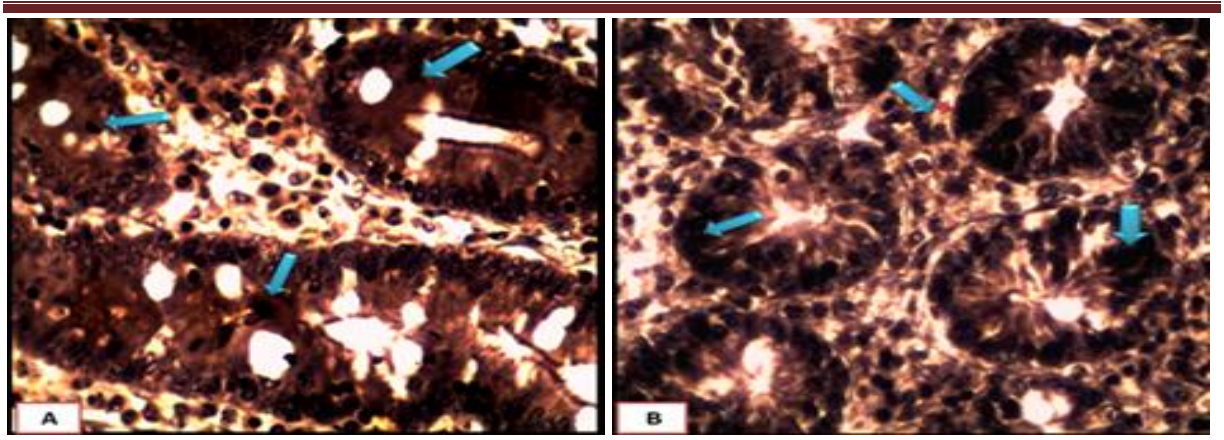

Fig.(5): Immunohistochemical sections in duodenum expression GLP-1 (blue arrows) less in density A:intestinal glands, B:Brunners glands (X40).

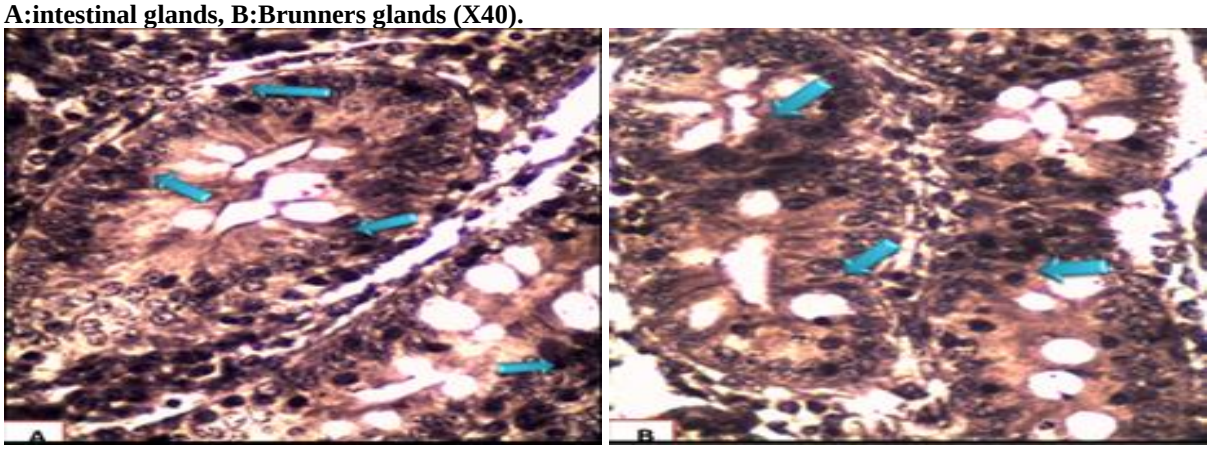

Fig.(6): Immunohistochemical sections in jejunum expression GLP-1 (blue arrows) moderate in density A:proximal part, B:distal part (X40).

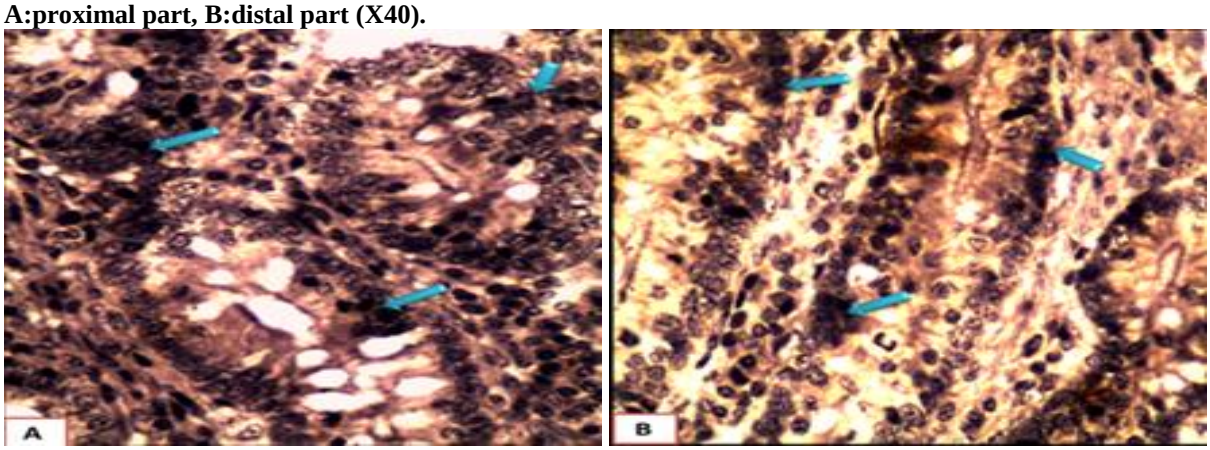

Fig.(7): Immunohistochemical sections in ileum expression GLP-1 (blue arrows) higher in density A:proximal part, B:distal part (X40).

Discussion:

In the small intestine, neuroenteroendocrine cells are highly specialized mucosal cells that produce a wide range of hormones with specific regional distribution and play a vital role in the function of the digestive system with enteric nervous system (23). The endocrine cells in each part of the gastrointestinal tract differ remarkably between animal species in term of regional distribution, relative frequency and cell type (24). Enteroendocrine cells constitute 1% of the cells lining the intestinal epithelium, and there are twenty or more subtypes of enteroendocrine cells based on the major products they secrete (25). I-enteroendocrine cells secrete CCK-8, while L-enteroendocrine cells secrete GLP-1 in response to dietary carbohydrates, amino acids and lipids (26,27). In the present study, we have demonstrated the expression and distribution of two types of neuroendocrine cells in the proximal, mid and distal parts of small intestine of the sheep using immunohistochemical techniques. This study though is the

first to clarify immunohistochemically the type and distribution of neuroentero-endocrine cells in small intestine of sheep.

Cholecystokinin-8(CCK-8)

In duodenum of sheep in the present study, the CCK-8 was localized in a subset of cells along the villus and crypts and Brunner's glands. Most of the CCK-immunoreactive (IR) cells were flask shaped with apices pointing towards the lumen of the gut. This expression of the CCK has been found in agreement with (23) in camel (28) in sheep, the CCK hormone in duodenum and subsequently was decreased in jejunum and ileum, this finding was supported by (23, 29, 30) in mammals and camel. Cholecystokinin (CCK-8) was released by lipid in the intestine to initiates satiety by acting at cholecystokinin type 1 receptors (CCK1Rs) located on vagal afferent nerve terminals located in the wall of the gastrointestinal tract (27), CCK in the upper small intestine may be associated to the role of these hormones in the stimulation of intestinal and gallbladder smooth muscle and pancreatic secretion (31,32,33).The cells are located to the crypts and the villi, they are more numerous in the crypts compared to the villi, the shape of the cells varies according to the segment of the gut, the neurotransmitters and neuropeptide-IR cells were generally spherical or spindle shaped (open type cells), while cells that were rounded in shape (closed-type cells) were occasionally seen. The pattern of distribution of these neuroendocrine cells is in line with recorded in other mammals including buffalo (34), human (35), (36, 37) in sheep (38) in guinea pig.

Glucagon Like Peptide -1(GLP-1)

The cells are located to the crypts and villi, they are more numerous in the crypts compared to the villi, the shape of the cells varies according to the segment of the gut, the neurotransmitters and neuropeptide-IR cells were generally spherical or spindle shaped (open type cells), while cells that were rounded in shape (closed-type cells) were occasionally seen. The pattern of distribution of these neuroendocrine cells is in line with what recorded in other mammals including human (35), (36,37) in sheep (38) in piglet, (34) in buffalo. On this study indicate that GLP-1 expressed of cells along the villus and crypts in the small intestine of sheep, these result showed similar findings in dromedary camel (22), (39,40) pig, calf and sheep, (26) in horse**.** The duodenum, jejunum and ileum of sheep expression of GLP-1 was increase caudally along the length of the small intestine of sheep this result in agreement with (41) in camel but disagreement with (42) in canine, who mention the enteroendocraine L cell increase in middle part of small intestine more than the proximal and distal parts of jejunum. Increase GLP-1 induce regulation of insulin release by enteric-derived incretions, distribution of the GLP-1in small intestine has been seen in other mammals (39). GLP-1 IR cells were mainly showed in the middle part of intestinal villi of the duodenum and jejunum and in the lower part of intestinal villi and crypts of ileum, in the ileum and jejunum observed the highest density similar to (43) in camel. Increasing intensity of glucagon like peptide-1 (GLP-1) from enter-oendocrine L-cells in ileum due response to carbohydrate and fat ingestion and mainly involved in stimulating gastrointestinal motility, crypt cell proliferation, and nutrient absorption in the small intestine (44). Glucagon like peptide-1 GLP-1 contain in ileum by proprotien-convertase2 (PC2), proprotien-convertase3 (PC3). PC3 is the enzyme responsible for synthesis of glucagon and glucagon like peptide-1 GLP-1(45). In the duodenum of sheep the presence of the GLP-1was beneficial for the metabolism of the carbohydrate (46) who recorded low level of expression in gastrointestinal of lesser mouse deer, and low expression of GLP-1in duodenum of the two humped camel (47).

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