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Documentation distribution of endocrine cells and expression of GIP GLP-1 and GLP-2 hormones in the small intestine of the Adult Male Bovine

A Graduation Project Submitted to the College of Veterinary Medicine/ University of Al-Qadisiyah in a partial fulfillment of the requirements for the Degree of Bachelor of Science in Veterinary Medicine and Surgery.

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

فَتَعَٰلَى ٱللَّهُ ٱلْمَلِكُ ٱلْحَقُّ ۗ وَلَا تَعْجَلْ بِٱلْقُرْءَانِ مِن قَبْلِ أَن يُقْضَىٰٓ إِلَيْكَ وَحْيُهُۥ ۖ وَقُل رَّبِّ زِدْنِى عِلْمًا ﴿١١٤﴾

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Certificate of Supervisor

I certify that the project entitled (Documentation distribution of endocrine cells and expression of GIP GLP-1 and GLP-2 hormones in the small intestine of the Adult Male Bovine" **was prepared by fatma hatif abdalla under my supervision at the College of Veterinary Medicine / University of Al-Qadissiya.**

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Dedication

This research is dedicated to my family who encouraged me to pursue my dreams and finish my study.

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Abstract

enteroendocrine cells are located along the mucosa of the small intestine in mammals. These cells are related to express a variety of incretins hormones that controlled on metabolism of digested food and absorption. Furthermore, these hormones are in charged of secretions other hormones of other accessory glands that related into food digestion and level of carbohydrates in the blood, as well as protect, and induce proliferations of injured endothelial cells of small intestine. The function of these hormones has controlled on growth of human and animals and stimulate the obesity or deficiency of basic elements of the body.

Current study had conducted on five adult male bovines, bulls were slaughtered, and small intestine identified and check from any lesions of diseases. Routine histological preparation was used for tissue of small intestine. Sections of small intestine was stained with H and E stains and identify the four tunicae that constitute duodenum, jejunum and ileum. These tunicae are involved with from internal to external "mucosa, submucosa, muscularis, serosa".

Immunofluorescence method was applied to detect expressions of three specific hormones used anti-human GIP, GLP-1 and GLP-2 antibodies at different locations of small intestine. Our findings of immunofluorescence significantly revealed that enteroendocrine cells are spread in lamina propria close to glands and also around the villi. These endocrines were huge expressed the GIP in duodenum, and jejunum but it absents in the ileum. However, GLP-1 and GLP-2 were significantly expressed in the jejunum and ileum, but not detected in the duodenum.

Inconclusions, these results strongly confirmed that antihuman GIP, GLP-1 and GLP-2 antibodies have large homology with epitope of antigens of bulls. Also, different level expressions of hormones in different locations of small intestine play vast role in regulate digestion, absorption, and intake food of bovines.

Key words: Enteroendocrine cells, Bovine, bulls, Small intestine, Duodenum, Jejunum, Ileum, Endocrine cells, GIP, GLP-1 and GLP-2.

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CHAPTER ONE :

Introduction:

Introduction

The gastrointestinal tract of mammals is filled the abdominal cavity of animal and has an important function in the body such as absorption, metabolism of food and act mechanical barrier to against microorganism invasions and their toxins. The small intestine of bulls is measured about 20 times longer than the length of the body and it is consist of three segments "duodenum, jejunum, and ileum". Additionally, the excretions of the pancreas, liver and gall bladder are drained into lumen of the small intestine, which are responsible for digestion of lipids, proteins and carbohydrates(Sherlock and Dooley 2008, Singh 2008). "Most of digestive process is completed in the small intestine and many nutrients" are absorbed via the small finger-like projections that are called villi into the blood and lymphatic systems (Ham 2002).

The villi are distributes in the epithelium of small intestine which play a huge role to increase surface area of absorption and facilitate" rapid absorption of water and nutritive materials", moreover in the sheep and cow the length of intestinal villi increase at the proximal part of small intestine are longer than in the distal part of small intestine (LUCINI, DE GIROLAMO et al. 1999, Ham 2002, Parveen, Pawan et al. 2013). The epithelium and intestinal glands of small intestine contain several types of cells involved with Paneth cells, columnar cells, absorptive cells, goblet cells, and enteroendocrine cells, subsequently" the distribution of these cell are different between the species" for example the Paneth cells the epithelium of (sheep ,water buffalo ,and camel) comprise huge number of cells and located at the crypts(LUCINI, DE GIROLAMO et al. 1999, Ali, Nyberg et al. 2007, Daly, Al-Rammahi et al. 2012, Althnaian, Alkhodair et al. 2013, Parveen, Pawan et al. 2013).

As the small intestine is considered endocrine organ because it has many different types of endocrine cells which product variety of" numerous peptide hormones such as, gastrin, serotonin, GIP (glucose-dependent insulinotropic" is produced by K cells(Meier and Nauck 2004, McIntosh, Widenmaier et al. 2009), and GLP1(glucagon-like peptide 1) ,GLP-2 " (glucagon-like peptide 2 and "PYY"(peptide YY), produced by L cells, CCK"(cholecystokinin) is made by I cells (Morozov 2002, Wang, Chi et al. 2003, Martin, Beck et al. 2006, Mellitzer and Gradwohl 2011).

The epithelium of small intestine is released gastric inhibitory polypeptide (GIP) which consists of 153-amino acid proprotein also known as gastroinhibitory peptide and the glucose-dependent insulinotropic peptide. This hormone considers inhibiting hormone in stomach and induce hormones secretions(Moens, Heimberg et al. 1996) it stimulate the pancreas to secret the insulin hormone which would lead to increase absorption of glucose

via epithelium of small intestine and reduce bone resorption by inhibited the osteoclast activity in bone tissue because it might be mediating nutrient-related effects on bone function (Meier and Nauck 2005, Zhong, Itokawa et al. 2007).

Also, the intestinal epithelium is secreted a 42 amino acid hormone(glucagon-like peptide 1) which are suppressed glucagon secretion, inhibits gastric emptying, and decreases appetite and food intake(Turton, O'shea et al. 1996, Flint, Raben et al. 1998). This hormone is mediated the insulin secretion may effect on the hyperinsulinaemia and reactive hypoglycaemia of this disorder(Kreymann, Ghatei et al. 1987).

The glucagon-like peptide 2 (GLP-2) is secreted in stomach, small intestine and colon and the sequence protein of it is consisted of 33 amino acid peptide encoded carboxyterminal to the sequence of GLP-1 in the proglucagon gene(Drucker 2001). This hormone is responsible on repair, growth and proliferations of epithelium and villi of small intestine after injury or complicated infections(Drucker, Erlich et al. 1996) also it is improved the nutrition absorption of digested food but impaired the GPL 2 secretions after postprandial in ileum and colon and has no effect on insulin secretion (Schmidt, Siegel et al. 1985, Jeppesen, Hartmann et al. 2001).

The enteroendocrine cells are highly specialized mucosal cell spread in the gut epithelium and scattered along the stomach into the colon that are different pattern of the distributions depending on the species. There are two kinds of these cells, first type is called open and present at the top of the epithelium and is called microvilli that project and lining the lumen of small intestine, and the second type called close" which is presented at the cellular apex (Ham 2002, Junqueira, Carneiro et al. 2005).

Many researches are investigated on the"relative frequency and spreading"of enteroendocrine cells in the intestinal tract of sheep, water buffalo, camel, equine, human, rat, mice, opossum (Krause, Yamada et al. 1985, Muta, Itsuno et al. 1994, LUCINI, DE GIROLAMO et al. 1999, xxHam 2002, Hosoyamada and Sakai 2005, Ali, Nyberg et al. 2007, Daly, Al-Rammahi et al. 2012, Althnaian, Alkhodair et al. 2013, Parveen, Pawan et al. 2013).

These three hormones (GIP, GLP-1 and GLP-2) are very important hormones because they are played role in metabolism, digestion and absorption of nutrient and intake food, and the understand and control on function of these hormones could be helped to deal with obesity in human or exploit it to increase the weight and fatty of calves. Therefore, the aims of our study were to identify and document three important hormones (GIP, GLP-1 and GLP-2) in Iraqi bovine species which might be expressed in the mucosa or submucosa of small intestine and detect the level of their expression according to the location of the intestine. In addition,

the anti-human (GIP, GLP-1 and GLP-2) bodies are labelled and probed with these hormones of different species bovine intestinal hormones which might be confirmed that there is a homology of sequence of proteins between different species, this would be interesting for researchers to develop other assays.

Material and methods

Five small intestines of adult bulls were collected from Al-Qadisiyah's abattoir. The age of bulls were 2-3 years old and the weight was 150-175kg). Tissue were collected from different parts of small intestine after inspecting them from any pathological lesions to avoid abnormal tissue and diseases. "Tissue were directly stored in the ice until use them in the Laboratory. Briefly, tissue was prepared for histological section by following the routine of histology" "firstly tissue had fixed with 10% formaldehyde, then, dehydrated through series solutions of an ethanol-xylene, after that embedded in paraffin to make blocks and sections of tissue to stain them with antibodies and routine stain". for histological examinations, the protocol of (Luna 1968, Ramos-Vara 2005) had been followed. The samples was passaged double in "100% ethanol for 2 x 10 minutes", then leave them air dry for 10 minutes. After that sections of tissue" were circled with ImmEdge Hydrophobic Pen" and leave them to dry for 10 minutes. Subsequently, sections of slides were immersed 2 x 5 minutes in 70% ethanol. Next, slides were rehydrated 2x in dH2O for 5 minutes. "Slides were incubated "with 10% (v/v) normal donkey serum in a humidified chamber at room temperature". In the next step, sections were incubated overnight at 4○C with" primary antibodies" (Table 1). Then slides were washed in the PBS for 5 x 5 minutes. FITC-conjugated IgG/IgY (Table 1) (Stratech, Scientific Limited, Suffolk, UK) were applied at a dilution of 1:500 for 1-hour", then washed with PBS for 5 x 5 minutes. Finally, slides were "visualized using an epifluorescence microscope" (MEIJI TECHNO, Model MT4300, Japan) and images were captured with a digital camera and images were analysed.

Table (1). This is a table showed primary and secondary antibodies that have applied to identify GIP, GLP-1 and GLP-2.

Primary antibody	**Host**	**Dilutions**
"Anti-human GIP'	Goat	1:100
"Anti-human GLP-1"	Goat	1:100
"Anti-human GLP-2"	Goat	1:100
Secondary antibody	**Label**	**Dilution**
Donkey anti-goat IgG	FITC	1:500

Results

The histological segments of the small intestine were stained with routine stains "H&E" to distinguish and detect normal histological structures and the integrity of these parts, in particular, it clarified the epithelial cells of epithelium and their attached microvilli. The results showed that villi and microvilli are attached clearly into epithelium of small intestine (Fig. 1.A.B.C).

The general histology of the small intestine wall of bulls "duodenum, jejunum and ileum" were comprised with four layers, from internal to external "mucosa, submucosa, muscularis, serosa". In details, there are large similarity between parts of intestine. Small intestine was lined by simple columnar epithelium based on basement membrane, many transvers folds are projected from epithelium which are continued a long small intestine and conformed the plicae circularis, however, most these folds are more common in the jejunum (Figure .1). furthermore, many villi were revealed in epithelium, that are responsible for absorption the digested food, ions and water. These villi are very developed in duodenum. As, lamina propria displayed duodenal or Brunner glands in duodenum, and intestinal gland which are opened at the base of villi.

Similarly, Lamina propria was consisted of loose connective tissue, blood supply, lymph networks and adipose tissue. This layer was also extended into folds of intestine.

The muscularis mucosa was made up smooth muscle which are arranged longitudinally and transversely. Nevertheless, this layer in the ileum was thicker than the duodenum and

jejunum. Externally small intestine was surrounded by serosa which is comprised from connective tissue and mesothelium cells (Fig. 1 A, B &C).

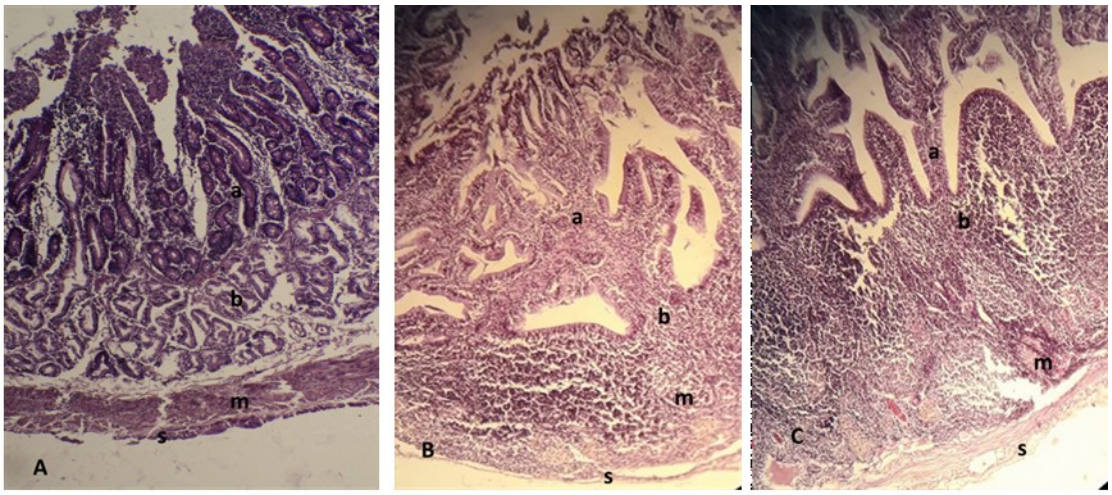

Figure .1: These images displayed the histological structure of small intestine of adult male bovine was stained with routine H&E stains. (A) This image showed the layers structures of duodenum. (B) This image showed the layers structures of jejunum. (C) This image showed the layers structures of ileum. 100 X (a, b and c) magnified.

The results of immunofluorescence were conducted and optimised by using three anti-human (GIP, GLP-1, GLP-2) antibodies of three hormones which are expressed from mucosa of small intestine and secondary antibody fluorescein isothiocyanate (FITC) to visualise them. The histological sections slides were incubated with specific different antibodies of hormones to against " GIP, GLP-1 and GLP-2" hormones and analysed them.

The assay was developed and applied on another different species to test the homology between human and bovine proteins. Result were very neat and reproducible in the different parts of small intestine. The gastric inhibitory polypeptide (GIP) was highly expressed in enteroendocrine cells of mucosa of duodenum. The expression of GIP was shiny and high signal intensity; however, it was less expression in jejunum and absent in the ileum table (2) and Figure (2). This hormone is helped to decrease the stomach acidity secretions in duodenum.

Nevertheless, the glucagon-like peptide 1 (GLP-1) was not expressed in the duodenum, but it has clear level and expression in the jejunum, and highly expressed in the ileum table (2) and Figure (3). Furthermore, the glucagon-like peptide 2 (GLP-2) was huge

intensity and significant level expression in the ileum. This evidence that this hormone has no effect on food intake in small intestine table (2) and Figure (3).

In our results were solely displayed the expression of hormones as the subpopulation of cells along with the villi of small intestine. Three hormones (GIP, GLP-1 & GLP-2) were clearly expressed in the duodenum of small intestine which are confirmed that hormones related into villi functions as well as it is related into metabolism and digestions of rest food.

Furthermore, the location of three hormones (GIP, GLP-1, and GLP-2) were different in the mucosa and distributed in the lamina propria extended into the lower part of portion of duodenal glands less than villi in the columnar epithelium.

Moreover, the enteroendocrine cells were scattered in the duodenum around the villi, crypts & Brunner's glands, but then again, the enteroendocrine cells were high commonly in villi of duodenum, less commonly on the " intestinal crypts and rarely in the Brunner's glands". While, the jejunum GIP immunoreactive cells were rarely dispersed on villi. Similarity, GIP IR-cells were not identified in histological structure of the ileum. The enteroendocrine cells was contained expression of the GLP-1 and GLP-2 that are caudally increased along of the ileum compare with duodenum and jejunum (Table 2). Additionally, GLP-2 immunoreactive cells were not observed in the histology of the duodenum and jejunum (Fig.4 C and D).

Inclusive, the expressions of " GIP, GLP-1 and GLP-2" hormones were detected using the immunostaining technique at three parts of small intestine (duodenum, jejunum, and ileum), consequently the enteroendocrine cells showed that high reaction signal for GIP hormone sited in the duodenum, even though endocrine cells are revealed high reaction for GLP-1 and GLP-2 in jejunum and ileum. Table (2).

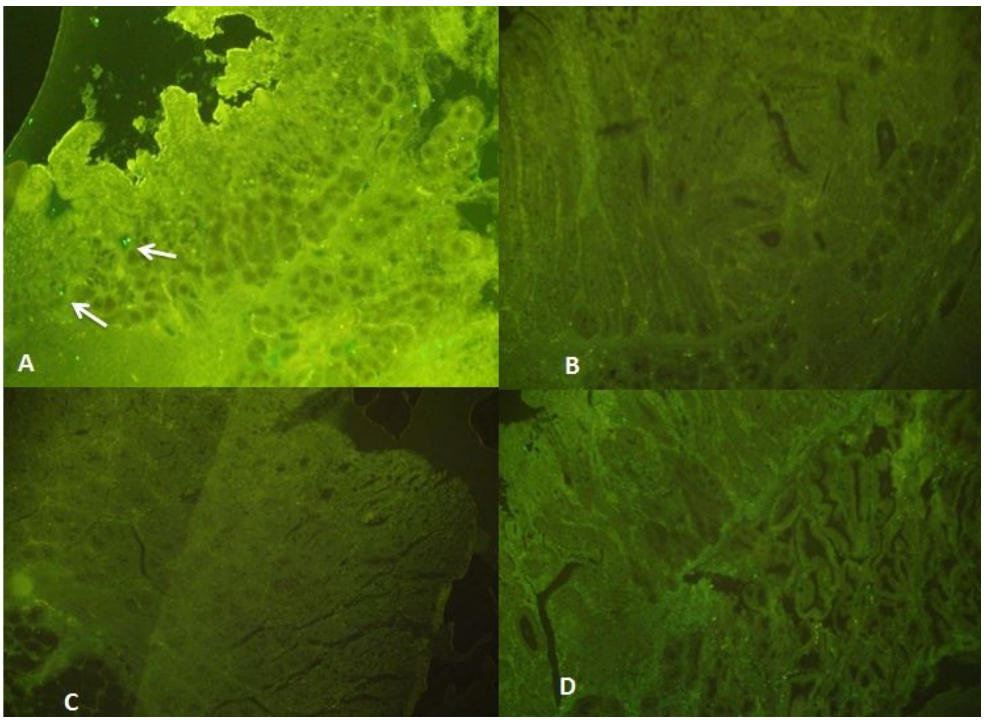

Figure (2): These images displayed the duodenal part of small intestine of adult male bovine was incubated with anti-human "GIP, GLP-1, GLP-2 antibodies". (A) typical image showed the expression of (GIP) in the duodenal section and detected close the villi, upper and lower half of the glands of duodenum. (B) and (C) these images showed the duodenal sections were incubated with anti-human (GLP-1, GLP-2) antibodies and were not detected in duodenum. (D) This image showed the duodenal sections were only incubated with secondary antibody. 200X magnified. Arrow(s). Indicate positive staining.

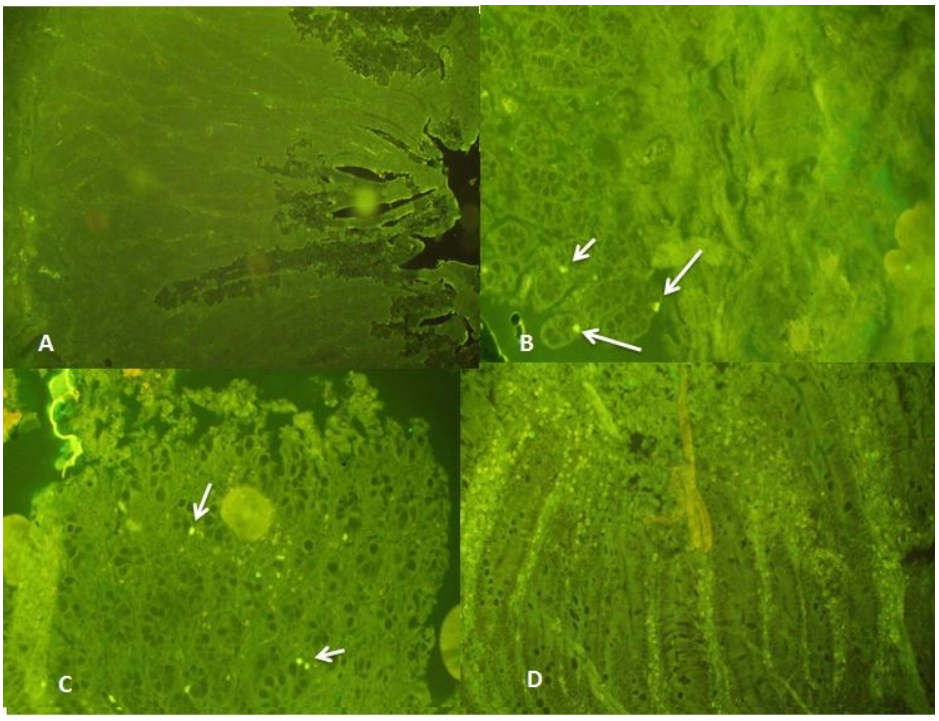

Figure (3): These images displayed the jejunal part of small intestine of adult male bovine was incubated with anti-human "GIP, GLP-1, GLP-2 antibodies". (A) GIP hormone was not detected in the jejunal sections. (B) and (C) these images showed the jejunal sections were incubated with anti-human (GLP-1, GLP-2) antibodies and were detected in jejunal part in" villi, upper and lower half of the glands of jejunum". (D) This image showed the jejunal sections were only incubated with secondary antibody. 200X magnified. Arrow(s). Indicate positive staining.

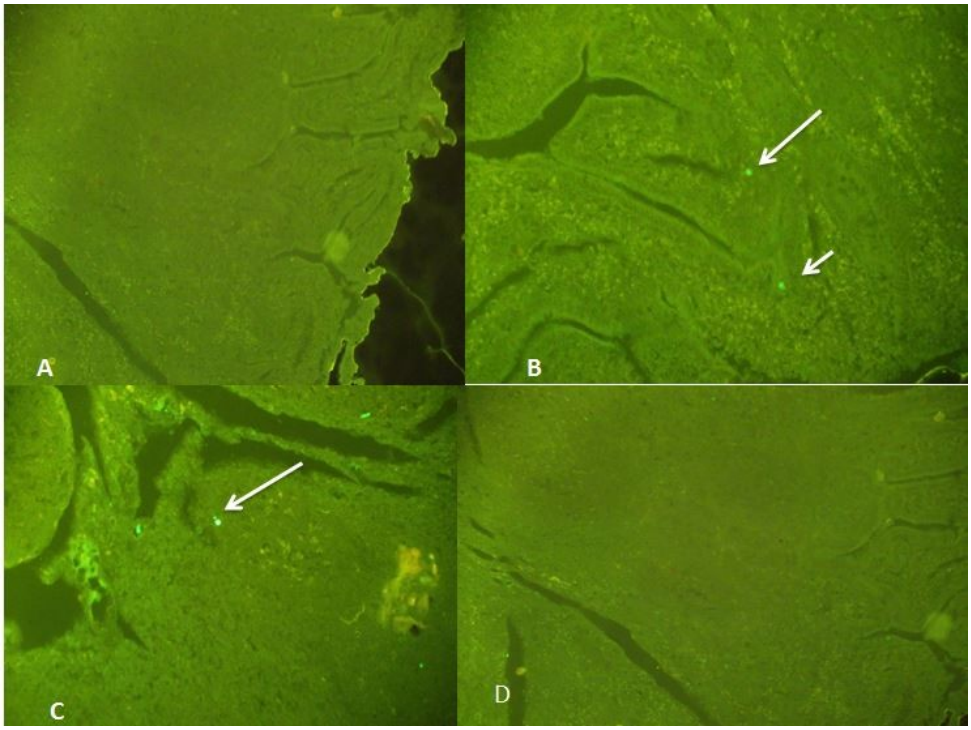

Figure (4): These images displayed the j Ileum part of small intestine of adult male bovine was incubated with anti-human "GIP, GLP-1, GLP-2" antibodies. (A) GIP hormone was not detected in the Ileum sections. (B) and (C) these images showed the Ileum sections were incubated with anti-human (GLP-1, GLP-2) antibodies and were detected in Ileum part in" villi, upper and lower half of the glands of Ileum". (D) This image showed the jejunal sections were only incubated with secondary antibody. 200X magnified. Arrow(s). Indicate positive staining

Table (2): This table revealed the expression and absent expression of GIP, GLP-1 and GLP-2 hormones in the small intestine of adult male bovine.

Hormones	(Duodenum)	(Jejunum)	(Ileum)
"GIP"	+++	±	-
"GLP-1"	-	++	+++
"GLP-2"	-	+++	+++
(+++: high, ++: moderate, +: low, ±: low , -: not signal)			

Discussion

The present research is covered three specific hormones in the small intestine of the adult male bovine. The small intestine is histologically contain four tunicae "mucosa, submucosa, muscularis and serosa", mucosa is lined by simple columnar epithelium, which is based on basement membrane. lamina propria was beneath the epithelium and both extended in branched folds. Also, the tubuloalveolar duodenal gland was "scattered in the submucosa at the upper part of the duodenum" and reduced in numbers toward the end and disappeared(Hampson 1986, Cesta 2006).

The mucosa of small intestine is contained massive endocrine glands in the body which are might be produced the hormones and abundant number of enteroendocrine cells. These have many functions of the small intestine such the "absorption and digestion of the food" combine with enzymes that are secreted from the endocrine gland(Krause, Yamada et al. 1985, Muta, Itsuno et al. 1994, Ham 2002, Hosoyamada and Sakai 2005, Samuelson 2007, Bacha Jr and Bacha 2012).

Our result showed that distribution arrangement of GIP hormone of male adult bovine was identified in the duodenum, less in the jejunum, but it is not observed in the ileum, but not GLP-1 expression was detected in duodenum, however, the incretin hormones GIP and GLP-1 were detected in all parts of endocrine cells of small intestine of the porcine, rat, and human(Mortensen, Christensen et al. 2003). This suggested that expression GIP hormones in duodenum would be helped to reduce the acidity products of stomach and protect the small intestine.

Results was not detected the expression of glucagon-like peptide 2 (GLP-2) in the duodenum, and detected high level expression in the jejunum and ileum, but this hormone was identified in the gastrointestinal tract of human and rodents (Munroe, Gupta et al. 1999, Yusta, Huang et al. 2000). Our result strongly distinguished that glucagon-like peptide 2 (GLP-2) has humble effect in the duodenum due to high concentrations of GIP hormones.

Enteroendocrine cells are highly specialized mucosal cells and are differ in location and interposed in mucosa and submucosa of small intestine between species of animal in the regional distribution, relative frequency and cell type(Wang, Chi et al. 2003, Martin, Beck et al. 2006). These cells are secreted hormones which are effect on food intake, maintain blood glucose levels, reduce cholesterol levels, and various other physiological functions (Ahlman

and Nilsson 2001). However, these cells have different activity in the small intestine according to their locations and distributions.

The cells which are responsible for secreting the (GLP-1 and GIP-2) are called L-enteroendocrine cells, these cells are produced and secreted hormones as response to absorb the nutritive carbohydrates, amino acids and lipids (Longuet, Sinclair et al. 2008) and located in the crypts and the villi and had different shapes, also the shape of subunits of endocrine cells were commonly round or axle shape and open form and consider as second type of cell that is secreted" GIP called K-enteroendocrine" (Hosoyamada and Sakai 2005).

Generally, in mammals, the co-expression of the GIP with chromogranin is considered a typical evidence of enteroendocrine that approves the site an expression to be in enteroendocrine cells(Jang, Kokrashvili et al. 2007).

In same way, our study exhibited that "GIP was expressed exclusively along the villus in the duodenum of small intestine" and the cells expressing GIP are looked triangular or flask in shape at middle and distal portion of small intestine. But, the scattering of K cells is decreased along the length of the small intestine, this pattern of the GIP showed in the middle and distal portion of small intestine, same these results had been documented in other mammals(Theodorakis, Carlson et al. 2006, Moran, Al-Rammahi et al. 2010).

The tunica mucosa of duodenum plays an significant role in digestion, pancreatic secretions and gall bladder functions in mammals under affecting the gastrointestinal hormones that are released from the" proximal part of small intestine". The first part of the duodenum is protected the epithelium of duodenum from acid-pepsin which released from the stomach on adult opossum. (Krause, Yamada et al. 1985).

In conclusion, there are different expression of GIP, GLP-1, GLP-2 hormones in the small intestine of bulls, which are secreted by enteroendocrine cells. Our findings support that the expression of the incretin hormones play a role in regulate the intake of foods and induce other hormones which helped in accomplished process of metabolism of the bull.

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