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DISTRIBUTION OF CARBOHYDRATES IN THE DIFFERENT PARTS OF SMALL INTESTINE AND COLON OF BUCK BLACK GOATS

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ABSTRACT

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The aim of this study was to explore the distribution of different types of carbohydrates histochemically in the wall of different parts of small intestine and colon of native buck black goats. Eight samples of each part of small intestine (duodenum, jejunum, and ileum) and colon were used for this study. Different histochemical methods have been used to explore types and locations of carbohydrates in the wall of studied areas. It was observed that the amount of mucous secretion from the wall of colon was comparatively greater than that from the wall of small intestine; this is due to increase in the number of the goblet cells that lined the colon wall of black goats. There was noticeable variation in the concentration of carbohydrate present in small intestine and colon. Neutral glycoprotein, Carboxylated glycoproteins were found in the luminal surface of absorptive columnar cells, goblet cells and paneth cells of the intestinal glands in all parts of small intestine, while neutral glycoprotein was present in submucosal glands of the duodenal wall in black goats. Carboxylated and sulfated glycoproteins present in the surface epithelium and goblet cells of colon, while numerous goblet cells contain little amount of neutral glycoprotein. Glycogen, sulfated and Carboxylated glycosaminoglycans was not demonstrated in the wall of small intestine and colon, The enterocyte revealed no reaction with all histochemical methods used in the present study.

Key words: *Histochemistry, Glycoprotein, Small intestine, Colon.*

INTRODUCTIN

The small intestine of laboratory and domestic animals other than goat were studied morphologically and histochemically by many authors (Raul *et al.,* 1988; Zambonino *et al.,* 1989; Hall and Byrne, 1989). Some others researchers studied different aspects of small intestine in ruminants (Trahair and Robinson, 1989; Abdul Raheem, 1994; Abdul Raheem, 1995). Few data are available for the histochemistry of the small intestines of sheep and goats (Ahmed, 2006). The small intestine is highly modified for absorption and secretion (Banks, 1977). The bulk of carbohydrate digestion take place in intestine by enzyme elaborated by the animal itself (Chandrasena, 1979). The last years showed decrease in raining which cause dryness, so the food of animal became rough. These factors may affect the nature of the mucous membrane of small and large intestine to cope with these conditions. The aim of the present work is therefore to perform histochemical study of the different parts of small intestine and colon also to compare the results with the same region of other ruminant.

MATERIALS and METHODS

The three parts of the small intestine and colon of the buck black goat were collected from eight animals slaughtered at Mosul abattoir. Specimens of the duodenum were cut cranial to sigmoid loop; those of jejunum were cut at about one to one and a half meter caudal to the duodeno-jejunal flexure. Specimens of the ileum were cut (10-15 cm) from ileo –cecal folds; those of colon were cut (15-20 cm) from cecal – colon folds. The Specimens were washed in normal saline solution and fixed in neutral buffered formalin, Rossmans fluid. Paraffin section were prepared and stain by PAS stain for general identification of mucin (Luna, 1968; Culling *et al.,* 1985). Best carmine for detection Glycogen, Alcian blue (pH 1 and 2.5) for detection Sulphated, carboxylated glycoprotein and glycosaminoglycans respectively. PAS –alcian blue pH (2.5) with diastase, for identify the neutral glycoprotein (red colour) from other types of glycoprotein (blue colour) Toludine blue for detection the types of glycosaminoglycans (Kiernan, 2010).

RESULTS

The results of the histochemical staining reactions are summarized in table (1).

SMALL INTESTINE:

Goblet cells were very few at the surface epithelium of the villi but they increased relatively in number in the crypts of Lieberkunn, also these cells increased progressively from duodenum to ileum. The bases of the crypts showed considerable number of goblet cell

especially in the ileum. The mucous membrane, goblet cells lining villi, intestinal glands and Paneth showed moderate reaction after PAS staining. The previous structures showed weak reaction with alcian blue (pH 1), moderate to strong reaction with alcian blue (pH 2.5) after Methylation and Saponification (MS) and PAS –Alcian blue (pH 2.5) with Diastase respectively (Fig 1,2,3) and (Fig 5, 6), whereas the goblet cells that lining the superficial parts of intestinal glands showed more intense staining than those present in the deeper parts especially in alcian blue (pH2.5 \ MS technique) (Fig 7A). The previous histochemical methods assist the present of little amount of sulphated glycoprotein, moderate to considerable amount of Carboxylated glycoprotein, neutral glycoprotein respectively in all parameters of studied areas of small intestine. The amount of the carbohydrate depending on the intense of reaction. All parameters showed no reaction with Best Carmine and Toludine Blue this assist absent of glycogen, carboxylated and sulphated glycosaminoglycans. The enterocytes showed no reactions with all histochemical methods were used in the different parts of small intestine. (Fig 5, 7A) (Table 1). Duodenal gland cells exhibited positive reaction when stained with PAS –D Alcian blue (pH 2.5 techniques), this assist present considerable amount of neutral glycoprotein. (Fig 1)

COLON:

The goblet cells present in the mucosa and intestinal glands of colon were positively stained with PAS staining. The mucous membrane and goblet cells present in the mucosa of colon showed strong to moderate reaction with alcian blue (pH1) and alcian blue (pH 2.5) after Methylation and Saponification (MS) respectively (Fig 7B). The goblet cells lining the superficial parts of intestinal glands showed more intense staining than those present in the deeper parts with alcian blue (pH 2.5) after MS, while the intense of reactions with alcian blue (pH 1) were the same in all parts of intestinal glands (Fig8). The previous histochemical methods assist the present of Carboxylated glycoprotein, sulphated glycoprotein respectively in goblet cells lining the mucosa of colon and intestinal glands. No reaction in intestinal gland of colon with PAS –Alcian blue pH 2.5 with Diastase except some goblet cells in the mucosa showed weak reaction with the previous technique (Fig 4). This assured that the intestinal glands of colon of native buck black goat absent of neutral glycoprotein and the goblet cells of mucosa of colon contain little amount of this type of carbohydrate. All parameters of colon showed no reaction with Best Carmine and Toludine Blue such finding assist absent of Glycogen and Glycosaminoglycans. The enterocytes showed no reactions with all histochemical methods were used in the colon (Fig 4, 7 B, 8) (Table 1).

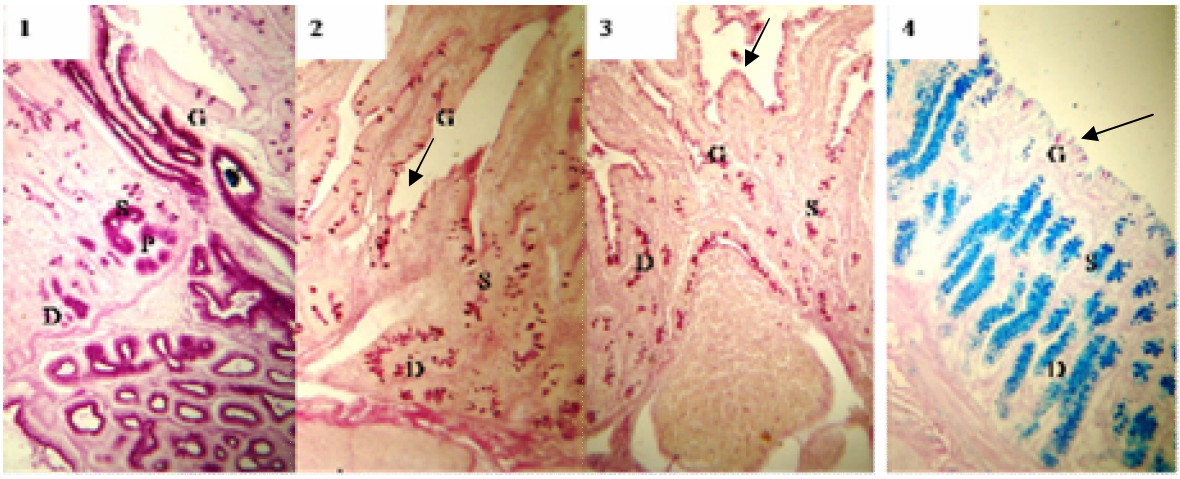

FIG (1,2,3,4): Explain the variable reaction with PAS –D alcian blue pH 2.5: Carboxylated glycoprotien with Neutral glycoprotien present in mucus membraneof duodenum (1), jejunum (2), ileum (3) (↙), while the mucus membrane of colon (4) contain Carboxylated glycoprotien only (←), while goblet cell lining villi (G) goblet cells lining superfecial parts of intestinal glands (S), goblet cells lining deep parts of intestinal glands (D), Paneth cell (p) in all parts of small intestine, red colour (neutral glycoprotien), blue colour (Carboxylated glycoprotien), megenta contain maixture of Carboxylated glycoprotien and Neutral glycoprotien. some goblet cells of colon contain little amount of neutral glycoprotien. Enterocytes showed no reaction(E) 90X

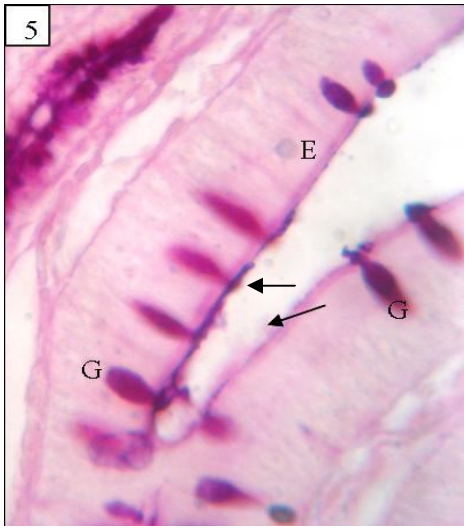

FIG (5): Explain the variable reaction with PAS –D alcian blue (pH 2.5), mixture of carboxylated glycoprotien with neutral glycoprotien (megenta) present in mucus membraneof duodenum, (←), goblet cell lining villi and intestinal glands (G) red colour (neutral glycoprotien, blue colour Carboxylated glycoprotien. Enterocytes showed no reaction(E): 300X

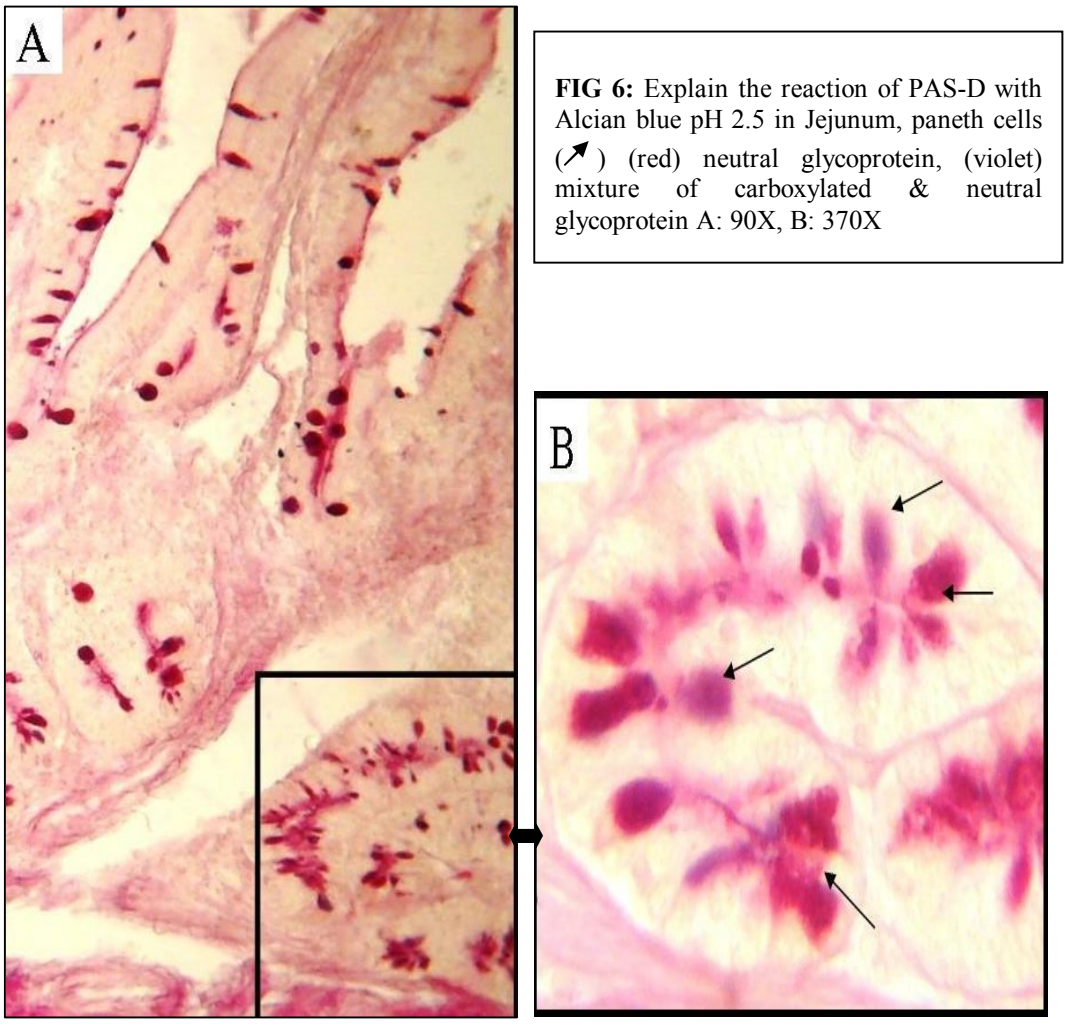

FIG 6: Explain the reaction of PAS-D with Alcian blue pH 2.5 in Jejunum, paneth cells (↗) (red) neutral glycoprotein, (violet) mixture of carboxylated & neutral glycoprotein A: 90X, B: 370X

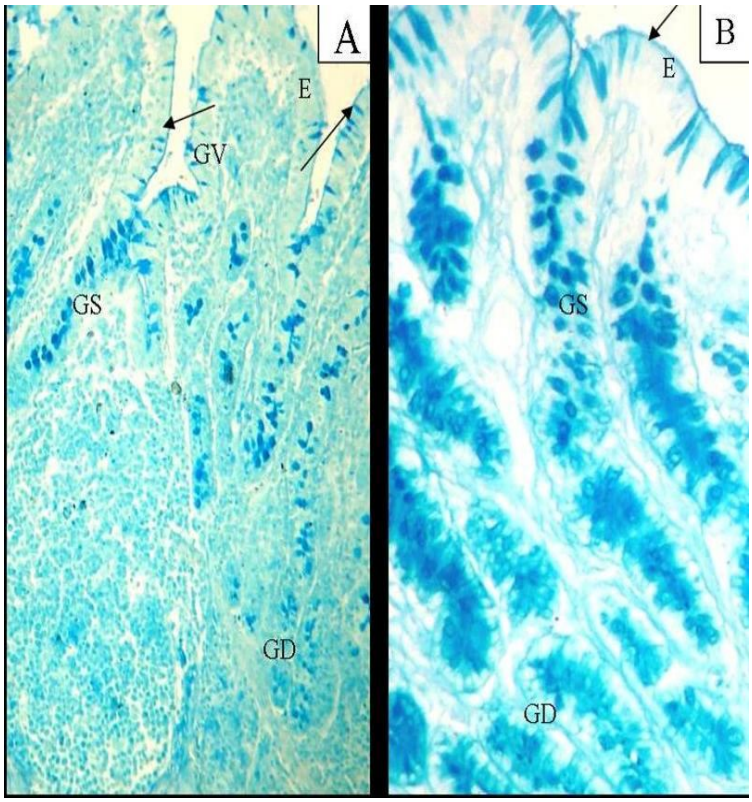

FIG 7: Explain the reaction with alcian blue PH 2.5 / MS in Ileum (A) Colon (B); There are variable mount of Carboxylated glycoprotein (↗) mucus membrane, (GV) Goblet cells lining villi, (GS) superficial parts of intestinal glands, (G D) goblet cells lining deep parts of intestinal glands. Enterocyte (E) absent of Carboxylated glycoprotein A: 70X, B: 90 X

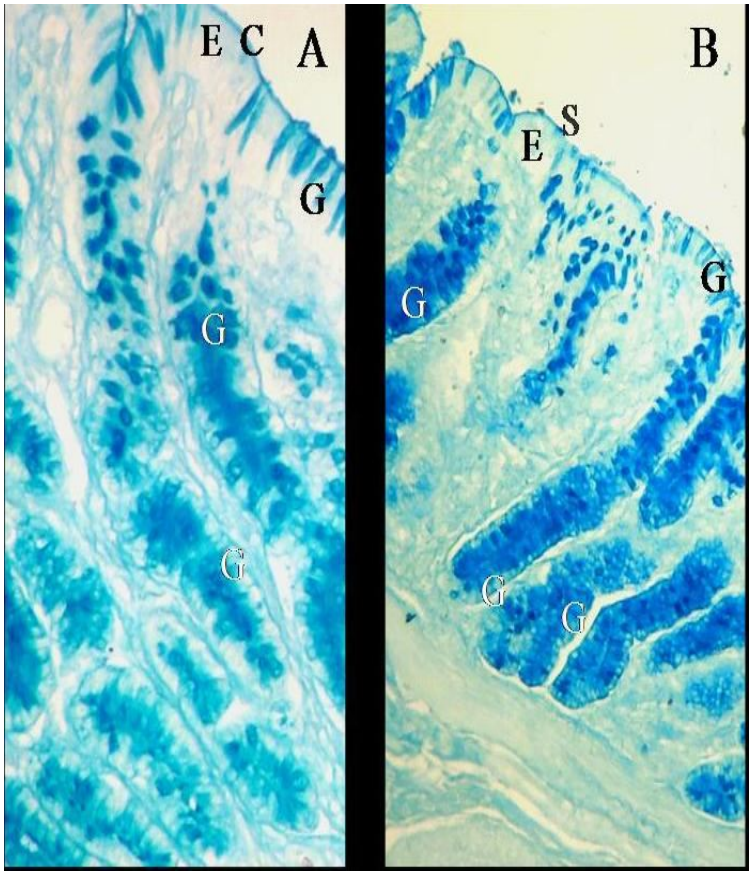

FIG 8: Explain the variable reaction with (A) alcian blue pH 2.5\MS and (B) alcian blue pH 1 in Colon ; mucus membrane of colon contain Carboxylated glycoprotien (C) and Sulphated glycoprotien (S) . Goblet cells contain carboxylated and sulphated glycoprotien,negative reaction in enterocyte (E) A:100X ; B : 90X

Table 1: Resultsof the histochemical stainig of the small intestine and colon

Studied areas	Histochemical Methods / Parameters	PAS	Best carmine	Toludine blue	Alcian blue H 1	Alcian blue pH2.5 after Methylation and Sponification	PAS-Diastase with Alcian blue pH2.5
Duodenum	Mucous membrane	+	–	–	±	+	+
	Enterocytes	–	–	–	–	–	–
	Goblet cells of surface epithelium	+	–	–	±	+	+
	Superficial parts of intestinal glands	+	–	–	±	+,++	+
	Deep parts of intestinal glands	+	–	–	±	+	+
	Paneth cells	+	–	–	±	+	+
	Sub mucosal glands	+	–	–	–	–	++
JEJINUM	Mucous membrane	+	–	–	±	+	+
	Enterocytes	–	–	–	–	–	–
	Goblet cells of surface epithelium	+	–	–	±	+	+
	Superficial parts of intestinal glands	+	–	–	±	+,++	+
	Deep parts of intestinal glands	+	–	–	±	+	+
	Paneth cells	+	–	–	±	+	+
ILEUM	Mucous membrane	+	–	–	±	+	+
	Enterocytes	–	–	–	–	–	–
	Goblet cells of surface epithelium	+	–	–	+	+	+
	Superficial parts of intestinal glands	+	–	–	+	+,++	+
	Deep parts of intestinal glands	+	–	–	+	+	+
	Paneth cells	+	–	–	±	+	+
COLON	Mucous membrane	+	–	–	+	+	–
	Enterocytes	–	–	–	–	–	–
	Goblet cells of surface epithelium	+	–	–	+	+	±,+
	Superficial parts of intestinal glands	+	–	–	++	++	–
	Deep parts of intestinal glands	+	–	–	++	+	–

-ve no reaction, ± weak reaction, + moderate reaction, ++ strong reaction.

DISCUSION

This study was performed to explore the distribution of different type of carbohydrate in the wall of small intestine and colon by using histochemical methods. (Culling *et al.,* 1985) mentioned that the animal tissue contain mucopolysaccharide that can be distinguish in normal and abnormal tissues by using histochemical methods depending on active groups that present in carbohydrate complex like aldehyde groups, carboxyl groups and sulphated salts. The amount of mucus secreted from the wall of goat's colon was comparatively greater than that in the wall of all parts of small intestine, this is due to increase in the numbers of goblet cells present in the wall of colon of buck black goat. Others authors (Ahmed, 2006) reported that the amount of mucus secreted from duodenal wall of goat greater than those in duodenal sheep, while (Krause, 1981) reported that numbers of goblet cells of duodenal European sheep, mountains goat greater than duodenal bison. The percentage of goblet cells were progressively increased from duodenum to the Ileum of camel (Abdul Raheem, 1995). This also reported in the native sheep and goat (Abdul Raheem, 1994), so the amount of mucus varies from one species to another. Many studies have shown that the dietary factors may effect goblet cell numbers and modulate the secretory activity of goblet cells in rats (Satchithanandam *et al.,* 1990; Lien *et al.,* 2001). The mucus membrane of the lining epithelium contain mixture of carboxylated and neutral glycoprotein in duodenal epithelium, increase gradually toward jejunum, ileum progressively while mixture of sulphated and carboxylated glycoprotein present in the mucus membrane of colon, the same finding was reported in the small intestine of sheep and goats (Banks, 1977) and small intestine of camel (Chandrasena *et al.,* 1979). (Geneser, 1986) established that the majority of intestinal enzymes are located in the epithelium brush border of the absorptive cells. (Ham, 1979) reported that the thick coat of absorptive cells contain enzymes convert disaccharides into monosaccharide, some research (Egberts, 1984; Damino, 1987) revealed that glycoconjugates are important constituents of the intestinal mucosal barrier which involved in digestion and absorption of nutrient, protection from possible damage caused by ingested material and interaction between cells and pathogen in the intestinal lumen. Thus it can be suggested that presence of variable amounts of glycoprotein are related to the nature of organs, while (More *et al.,* 1987; Chae, 1997) revealed that intestinal glycoconjugates vary in relationship with growth and diet, intestinal disease may also alter the nature of these complex carbohydrates.

The histochemical study of black goat showed that the concentration of carboxylated and neutral glycoprotein dominated respectively in goblet cells lining villi and intestinal glands, while sulphated glycoproteins were dominated in goblet cells of intestinal glands of colon and the concentration of the previous carbohydrates was greater in the superficial parts of the glands than deep parts especially carboxylated glycoproteins. (Pakawadee *et al.,* 2009) showed that the deep purple color in the goblet cells of descending colonic epithelium of the Swamp Buffalo stained by AB (pH 2.5) - PAS was due to the mixed presence of acid and neutral glycoconjugates, which showed as deep blue and magenta, respectively. The results of AB (pH 2.5) -PAS staining of goblet cells from the upper and the lower crypts were the same, while the descending colon of the buffalo consists of numerous goblet cells in the upper and the lower part of crypts, which contain acid and neutral glycoconjugates. These results were not agreed with the current study but similar to (Sheahan and Jervis, 1976; Chen *et al.,* 1993) which referred that the acid mucosubstances have been found predominantly in the large intestine of mammals. (Pongket *et al.,* 2001; Specian and Oliver, 1991) reported that goblet cells of the descending colon of the swamp buffalo consist of acid glycoconjugates with sulfomucin, which is considered to be an indicator of mucin maturity and is associated with increased protection of the epithelium, while (Rhodes, 1989) referred that colonic epithelia are often terminated with sialic acid or sulphate groups, which increase the charge of the mucus and therefore its intensity, which in turn increase its potential to resist attacks by bacterial enzymes. (Freeman *et al.,* 1980 and Caldero *et al.,* 1988) assisted that the sulphated mucin in the lower parts of crypts has the role of a lubricant, while upper parts of crypts contained sulfomucin plus more carboxylated glycoconjugates that play important role in resisting the invasion of potential pathogens, so the previous factors could be help in function of colon of native black goat.

Paneth cells revealed the presence of carboxylated and neutral glycoprotein in the all parts of small intestine of native black goat. (Charlootte, 2006) showed the same finding concerning the paneth cells of mammalian, but was not determined the types of carbohydrate. Submucosal glands of duodenum of native black goat contain neutral glycoprotein only, while (Schmacher *et al.,* 2004 recorded that the carbohydrate present in goblet cells differ from carbohydrate present in the submucosal glands of mammalian duodenum. In bovine (Takehena, 1991) reported that there are two types of mucous cells in bovine duodenal glands while in camel composed only one type of mucous cells containing acidic carbohydrate, the previous study have shown that the secretion of duodenal glands neutralizes gastric hydrochloric acid in cooperation with pancreatic juice, bile and intestinal juice.

There negative noticeable in enterocyte for any types of carbohydrate in all studied areas of native buck black goats could be due to absorptive activities of these cells.

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توزيع المواد الكربوهيدراتيه في الاجزاء المختلفه للامعاء الدقيقه والقولون في ذكور الماعز الاسود المحلي

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تم دراسه الكيمياء النسجيه لتوزيع المواد الكربوهيدراتيه المتواجده في جدار الامعاء الدفيفه والقولون للماعز الاسود المحلي. استخدم لهذا الغرض (٨) عينات من الاثنى عشر، الصائم ، اللفائفي والقولون من ذكور الماعز الاسود المحلي. استخدمت تقنيات الكيمياء النسجيه المختلفه للكشف عن انواع واماكن وجود المواد الكربوهيدراتيه للمناطق المدروسه. تبين ان المخاط المفرز من جدار القولون اكثر عما هو عليه في جدار الامعاء الدقيقه ، ويعزى ذلك لكثرة الخلايا الكأسية المتواجده في جدار القولون للماعز الاسود. لوحظ وجود تفاوتا واضحا في تركيز المواد الكربوهيدراتيه بين الامعاء الدقيقه والقولون للحيوان قيد الدراسه. تواجدت مادتي البروتينات السكريه الكربوكسيليه والمتعادله في الغشاء المخاطي المبطن للخلايا العماديه الماصه والخلاياالكأسيه وخلايا بانث المتواجده في الاجزاء المختلفه للامعاء الدقيقه ، بينما اقتصر تواجد البروتينات السكريه المتعادله في الغدد تحت المخاطيه لعفج الماعز الاسود المحلي. لوحظ تواجد البروتينات السكريه الكربوكسيليه والكبريتاتيه في الغشاء المخاطي والخلايا الكأسيه للقولون، كما تواجدت كميات ضئيله من البروتينات السكريه المتعادله في بعض الخلايا الكأسيه للقولون في الماعز الاسود المحلي. لم يلاحظ تواجد الجلايكوجين والسكريدات السيامينيه السكريه بنوعيها لجميع المناطق المدروسه. لم تظهر الخلايا العماديه أي تفاعلا مع جميع تقنيات الكيمياء النسجيه في الدراسه الحاليه.