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RESEARCH ARTICLE

HISTOCHEMICAL STUDY OF CARBOHYDRATES FOUNDATION IN THE GUT OF INDIGENOUS RABBITS AT DIFFERENT POSTNATAL AGES

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ABSTRACT

This project aimed to evaluate the carbohydrate histochemical profile in the small intestine and colon of indigenous rabbits at different postnatal ages. To conduct such investigation, forty eight offspring at different postnatal ages (1, 10, 15 and 40 days of age) were collected and set equally into four groups. Specimens from the wall of duodenum, jejunum, ileum and colon were prepared for different histochemical staining techniques (PAS, Alcian blue, Aldehyde fuchsine and Toluidine blue). Microscopic findings revealed the presence of neutral and acidic mucin in the mucosal lining of the small intestine in all studied ages. The sulfated acidic mucin was abundant in kits and suckling puppies while in older rabbits, the non sulphated mucin was the dominant type. The reaction of Toluidine blue moderate in older studied ages indicating the presence of glycosaminoglycans especially in the ileum. In colon, the different reactions indicated dominant neutral mucin in newly born kits and suckling puppies, but in older studied ages, the mixed neutral and sulphated acidic mucin and glycosaminoglycans were present in the surface epithelium whereas the non sulphated acidic mucin was dominant in the crypts of Lieberkühn. It can be concluded that the quantities of sulphated acidic mucin as well as glycosaminoglycans were increased dominantly at last parts of intestine in 15 days aged preweaned rabbits. Because such developmental changes are required to parallel the fermentation process which start at this age in which the feed of animals included greenish type of food.

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INTRODUCTION

Mucin is classified into neutral and acidic groups. The latter are further subdivided into sulphated and nonsulphated groups. Neutral mucin is the predominant type expressed in gastric mucosa, whereas acidic type is expressed throughout the intestinal epithelium, dominantly in the large intestine. Classically carbohydrate histochemical staining procedures showed positive reaction of the goblet cells toward PAS which indicates the presence of neutral type of carbohydrate. Whereas, their positive reactions with Alcian blue stain (AB) (pH 2.5) indicates the presence of acidic sulphated and acidic carboxylated types. In another aspect, staining with combined Aldehyde fuchsine (AF) -Alcian blue (pH1.0) will express sulphated groups only by the Alcian blue part, whereas the Aldehyde fuchsine will react positively with acidic mucin (Krause, 2000). Numerous investigations were applied the PAS stain to demonstrate the mucous secretion of the goblet cells and the duodenal glands of the intestine. In fact, the reaction of PAS is based on the oxidation of the glycol linkages in polysaccharides by the periodic acid constituent of the stain, thus producing Aldehyde. The librated Aldehyde can react with the leucofuchsin of the Schiff's reagent subsequently produce compound of magenta color (Selim and El Nahas, 2015). The effect of feeding on the carbohydrate profile of rabbit intestine was investigated by Desantis *et al.* (2011) who found that brush border of duodenal epithelium expressed neutral/sulphated glycoconjugate and glycans sulphate mucin reactivity during fine and coarse fed, respectively. While the brush border of distal colon epithelium showed few sulfoglycans reactivity. Submucosal glands of rabbit displayed high presence of acidic/sulphated mucin in fine fed rabbits, neutral glycoconjugate and sialomucine in all rabbit species. Goblet cells exhibited in duodenum, neutral and sulfomucins in fine and coarse fed, respectively, but in distal colon acidic/sulfomucins in fine fed specimens. Crypt's cells exhibited neutral glycoconjugate in the cecum and in the distal colon also acidic/sulphated glycans.

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MATERIALS AND METHODS

Animal collection and study design

Twenty pregnant indigenous does were kept and maintained under laboratory conditions of temperature 25 ºC and 12 hrs day; 12 hrs night cycle and allowed free access of food (standard basal diet and greenish food) and tap water *ad libitum*. Animals were purchased from animal house of the college of veterinary medicine / Baghdad University. From these does, forty eight offspring at different postnatal ages (regardless to sex) were collected and set equally into four groups. The newly born kits of one day age were set in the first group (P1). The second group includes ten days aged animals which was set as suckling puppies (P10). The third group of fifteen days of age of suckling and feeding on food was set as pre-weaned rabbits (P15). Whereas, the fourth group of forty days of age was set as post-weaned rabbits fed on solid basal diet and greenish (P40). Weaning was set on day thirty of age.

Preparation of specimens

Animals were euthanized by intra-cardiac injection of over dose of sodium pentobarbital (100 mg/kg) (AVMA, 2013). Abdominal cavity was opened using a surgical scissor, viscera were viewed and the small intestine and colon was dissected away from the abdomen. The organs were washed with phosphate buffer saline. The representative specimens of one cm were cut from each segment of small intestine (mid-duodenum, mid-jejunum, mid-ileum, proximal colon and distal colon). Most of specimens were opened longitudinally by using fine surgical scissor to evacuate the intestinal content by washing with cold normal saline and then directly immersed in 10% neutral buffered formalin for 48 hrs (More and Dubach, 1976). Specimens were dehydrated through ascending series of ethyl alcohol (70%, 80%, 90% and 100%) each for 2 hrs, then cleared with xylene for ½ hr. Samples were infiltrated with paraffin wax (58 – 60 ºC) then embedded with paraffin wax to obtain blocks of paraffin. Five micrometers paraffin sections were obtained by using rotary microtome (Culling *et al.*, 1985).

Histochemical techniques

1. Periodic acid Schiff stain (PAS) (Culling *et al.*, 1985). For demonstration of goblet cells and for accounting their number in all intestinal segments during the selected periods of the postnatal development as well as staining of Brunner's glands.
2. Combined PAS-AB (pH 2.5) technique. To differentiate neutral mucin from acidic mucin (Culling *et al.*, 1985). To evaluate the type of mucins of the goblet cells (in villi and crypts) and Brunner's glands.
3. Combined AF-AB (pH 2.5) technique. To differentiate sulfated from non-sulfated acidic mucin (Sozmen *et al.*, 1999). To evaluate the type of mucin of the goblet cells (in villi and crypts) and Brunner's glands.
4. Toluidine blue (TB) stain (Culling *et al.*, 1985). For demonstration of glycosaminoglycans (GAGs) in the goblet cells (in villi and crypts) and Brunner's glands.

RESULTS

Duodenum

Microscopic findings about to the wall structure of the duodenum of the newly born kits, revealed the presence of goblet cells in the duodenal villi which stained with the combined PAS-AB (pH 2.5) gave strong positive reaction (magenta color) toward PAS and moderate reaction (blue color) with the AB (pH2.5). In another aspect, the reaction was strong (purple color) with the combined AF-AB (pH2.5) stain. Such reactions were indicated the presence of neutral and acidic mucin and sulfated acidic mucin, respectively (Fig. 1). Post staining with the TB, it gave negative reaction indicating absence of glycosaminoglycans (GAGs) substance. Brush border of the villi were showed mixed type of neutral and acidic mucin when stained with the combined PAS-AB (pH2.5). The latter stains were negatively reacted with cellular lining of the Brunner's glands and the goblet cells present in the crypts of Lieberkühn.

Post ten days of age, goblet cells of the duodenal villi which were stained strongly with PAS were declined in their reactivity to moderate reaction, whereas remain moderately reacted with AB indicated the presence of equal amounts of neutral and sulfated acidic mucin in their structures. Similarly, results of combined PAS-AB (pH2.5) also showed mixed neutral and acidic mucin in the brush border of the duodenal villi. Toluidine blue stain gave weak reaction indicated the presence of small amount of GAGs in the villi. Most of the goblet cells of the duodenal crypts and cellular lining of Brunner's glands were moderately and strongly reacted with PAS and AB (pH2.5), respectively. Whereas, negative reactions were recorded with stains such as combined AF-AB and TB, indicated the presence of neutral and abundant non sulphated acidic mucin. Post fifteen days of age, goblet cells of the villi and crypts and the cellular lining of Brunner's gland were reacted weakly or moderately with PAS and strongly with AB (pH2.5). The reaction was weak with the combined AF-AB (purple color) and all of these reactions indicated the presence of more non sulphated acidic mucin than the neutral mucin. Toluidine blue stain also showed weak reaction in the villi indicated the presence of small amounts of GAGs in their structure. In forty days aged rabbits, the goblet cells of the duodenal villi were moderately reacted with PAS, AB and TB stains. Whereas, weakly reacted with AF (purple color) indicated the presence of non sulphated, sulfated acidic mucin, neutral mucin and GAGs, respectively. In the crypts, the goblet cells were strongly reacted with AB and moderately with PAS, but negatively reacted with AF indicated dominance of non sulphated acidic mucin and absence of the sulfated mucin type (Fig.). On the same time, the cellular lining of the Brunner's glands were strongly reacted with the AB but weakly with PAS indicated the presence of only carboxylated type of mucin (Table 12)

Jejunum

In one day-aged kits, histochemical results of stain's reactions indicated the presence of neutral and sulfated mucin in the goblet cells of villi only.

Table 1. Responses of different structures of the duodenum toward different histochemical stains at 1, 10, 15 and 40 days aged rabbits

Ages	Stains					
	Duodenal structures	**PAS**	**AB**	**AF-AB (pH 2.5)**	**PAS-AB (pH 2.5)**	**TB**
One day	Goblet cells of villi	+++	++	+++ (P) - (B)	++ (M) + (B)	-
	Goblet cells of crypts*	absent	absent	absent	absent	absent
	Cells of Brunner's glands*	absent	absent	absent	absent	absent
Ten days	Goblet cells of villi	++	++	+++ (P) - (B)	+ (M) + (B)	+
	Goblet cells of crypts	++	+++	+ (P) ++ (B)	+ (M) ++ (B)	-
	Cells of Brunner's glands	+	+++	- (P) ++ (B)	+ (M) ++ (B)	-
Fifteen days	Goblet cells of villi	++	+++	++ (P) + (B)	+ (M) ++ (B)	+
	Goblet cells of crypts	++	+++	- (P) ++ (B)	+ (M) ++ (B)	-
	Cells of Brunner's glands	+	+++	- (P) ++ (B)	+ (M) ++ (B)	-
Forty days	Goblet cells of villi	++	++	+ (P) + (B)	+ (M) + (B)	++
	Goblet cells of crypts	++	+++	- (P) ++ (B)	+ (M) ++ (B)	+
	Cells of Brunner's glands	+	+++	- (P) ++ (B)	+ (M) ++ (B)	-

*****Absent these structures at this age
PAS: Periodic acid Schiff, (+)ve for neutral mucin (glycoprotein) (magenta color)
AB (pH 2.5): Alcian blue (pH 2.5), (+)ve for acidic mucin (glycoprotein) (blue color)
AF-AB (pH2.5): Aldehyde fuchsine -Alcian blue (pH2.5), (+)ve for sulfate group (purple color) and (+)ve for carboxylated group (blue color)
TB: Toluidine blue (metachromacia), (+)ve for glycosaminoglycans (GAGs) (red- magenta color)
PAS – AB (pH2.5): (+)ve for neutral mucin (magenta color) and (+)ve for acidic mucin (blue color) and
(+)ve for mixed mucin (deep blue color)
M: magenta color
B: blue color
p: purple color

Table 2. Responses of different structures of the jejunum toward different Histochemical stains at 1, 10, 15 and 40 days-aged rabbits

Ages	Stains					
	Jejunal structures	**PAS**	**AB**	**AF-AB (pH 2.5)**	**PAS-AB (pH 2.5)**	**TB**
One day	Goblet cells of villi	++	++	+++ (P) - (B)	+ (M) + (B)	-
	Goblet cells of crypts*	absent	absent	absent	absent	absent
Ten days	Goblet cells of villi	++	++	+++(P) - (B)	+ (M) +(B)	+
	Goblet cells of crypts	++	+++	+ (P) ++ (B)	+ (M) ++ (B)	-
Fifteen days	Goblet cells of villi	++	++	++ (P) + (B)	+ (M) + (B)	++
	Goblet cells of crypts	++	+++	+ (P) ++ (B)	+ (M) ++ (B)	+
Forty days	Goblet cells of villi	+++	+++	++ (P) + (B)	+ (M) + (B)	++
	Goblet cells of crypts	++	+++	+ (P) ++ (B)	+ (M) ++ (B)	+

PAS: Periodic acid Schiff, (+)ve for neutral mucin (glycoprotein) (magenta color)
AB (pH 2.5): Alcian blue (pH 2.5), (+)ve for acidic mucin (glycoprotein) (blue color)
AF-AB (pH2.5): Aldehyde fuchsine -Alcian blue (pH2.5), (+)ve for sulfate group (purple color) and (+)ve for carboxylated group (blue color)
TB: Toluidine blue (metachromacia), (+)ve for glycosaminoglycans (GAGs) (red- magenta color)
PAS – AB (pH2.5): (+) ve for neutral mucin (magenta color) and (+) ve for acidic mucin (blue color) and (+)ve for mixed mucin (deep blue color)
M: magenta color
B: blue color
p: purple color

Table 3. Responses of different structures of the ileum toward different histochemical stains at1, 10, 15 and 40 days aged rabbits

Ages	Stains					
	Ileal structures	**PAS**	**AB**	**AF-AB (pH 2.5)**	**PAS-AB (pH 2.5)**	**TB**
One day	Goblet cells of villi	++++	++++	++ (P) + (B)	+ (M) + (B)	+
	Goblet cells of crypts*	absent	absent	absent	absent	absent
Ten days	Goblet cells of villi	+++	+	++ (P) + (B)	++ (M) + (B)	++
	Goblet cells of crypts	++	++	- (P) ++ (B)	+ (M) + (B)	-
Fifteen days	Goblet cells of villi	++	++	+ (P) ++ (B)	+ (M) + (B)	++
	Goblet cells of crypts	++	+++	- (P) ++ (B)	+ (M) ++ (B)	+
Forty days	Goblet cells of villi	++	++	+ (P) ++ (B)	+ (M) + (B)	++
	Goblet cells of crypts	++	+++	+ (P) ++ (B)	+ (M) ++ (B)	++

PAS: Periodic acid Schiff, (+)ve for neutral mucin (glycoprotein) (magenta color)
AB (pH 2.5): Alcian blue (pH 2.5), (+)ve for acidic mucin (glycoprotein) (blue color)
AF-AB (pH2.5): Aldehyde fuchsine -Alcian blue (pH2.5), (+)ve for sulfate group (purple color) and (+)ve for carboxylated group (blue color)
TB: Toluidine blue (metachromacia), (+)ve for glycosaminoglycan (GAGs) (red- magenta color
PAS – AB (pH2.5): (+)ve for neutral mucin (magenta color) and (+)ve for acidic mucin (blue color) and (+)ve for mixed mucin (deep blue color)
M: magenta color
B: blue color
p: purple color

Table 4. Responses of different structures of the colon toward different histochemical stains at 1, 10, 15 and 40 days aged rabbits

Ages	Stains					
	Colon structures		**PAS**	**AB**	**AF-AB (pH 2.5)**	**PAS-AB (pH 2.5)**	**TB**
One day and Ten days *	Proximal colon	Goblet cells of surface epithelium	+++	++	= (p) + (B)	++ (M) + (B)	-
		Goblet cells of crypts	+++	+++	= (p) + (B)	++ (M) ++ (B)	-
	Distal colon	Goblet cells of surface epithelium	++	+++	+ (P) = (B)	+ (M) ++ (B)	+
		Goblet cells of crypts	+++	+++	+ (P) + (B)	++ (M) ++(B)	-
Fifteen days	Proximal colon	Goblet cells of surface epithelium	++	++	+++ (P) = (B)	+ (M) + (B)	++
		Goblet cells of crypts	+++	+++	+ (P) + (B)	++ (M) ++ (B)	+
	Distal colon	Goblet cells of surface epithelium	++	+++	+++ (P) = (B)	+ (M) ++ (B)	+++
		Goblet cells of crypts	+++	+++	++ (P) + (B)	++ (M) ++ (B)	++
Forty days	Proximal colon	Goblet cells of surface epithelium	++	++	+++(P) -(B)	+ (M) +(B)	++
		Goblet cells of crypts	+++	+++	+(P) +(B)	++ (M) ++(B)	+
	Distal colon	Goblet cells of surface epithelium	++	+++	+++(P) -(B)	+ (M) ++(B)	+++
		Goblet cells of crypts	+++	+++	++(P) +(B)	++ (M) ++(B)	++

PAS: Periodic acid Schiff, (+)ve for neutral mucin (glycoprotein) (magenta color)
AB (pH 2.5): Alcian blue (pH 2.5), (+)ve for acidic mucin (glycoprotein) (blue color)
AF-AB (pH2.5): Aldehyde fuchsine -Alcian blue (pH2.5), (+)ve for sulfate group (purple color) and (+)ve for carboxylated group (blue color)
TB: Toluidine blue (metachromacia), (+)ve for glycosaminoglycans (GAGs) (red- magenta color
PAS – AB (pH2.5): (+)ve for neutral mucin (magenta color) and (+)ve for acidic mucin (blue color) and (+)ve for mixed mucin (deep blue color)
M: magenta color
B: blue color
P: purple color

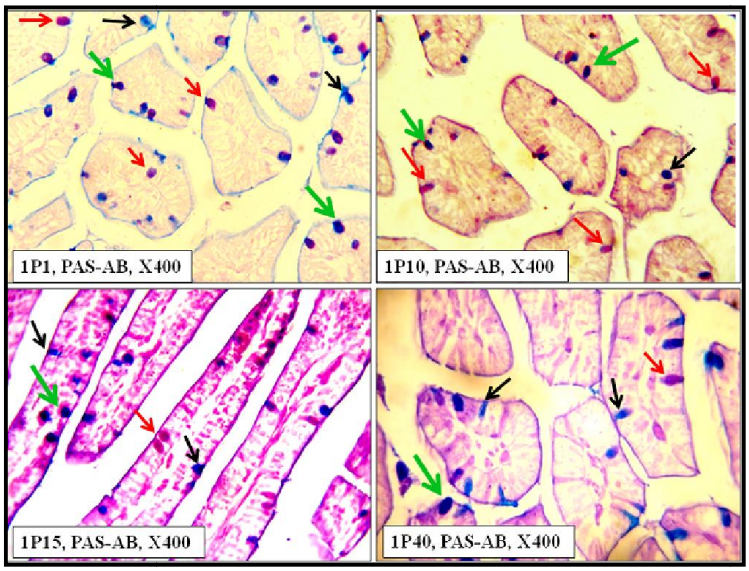

Fig. 1. Cross sections through villi of duodenum at one day kits (1P1), ten days suckling puppies (1P10), fifteen days pre-weaned rabbits (1P15) and forty days post-weaned rabbits (1P40). It showed positive reactions of goblet cells toward combined PAS-AB (pH 2.5) stain by the presence of neutral purple color (red arrows), acidic blue color (black arrows) and mixed mucin (green arrows) of goblet cells

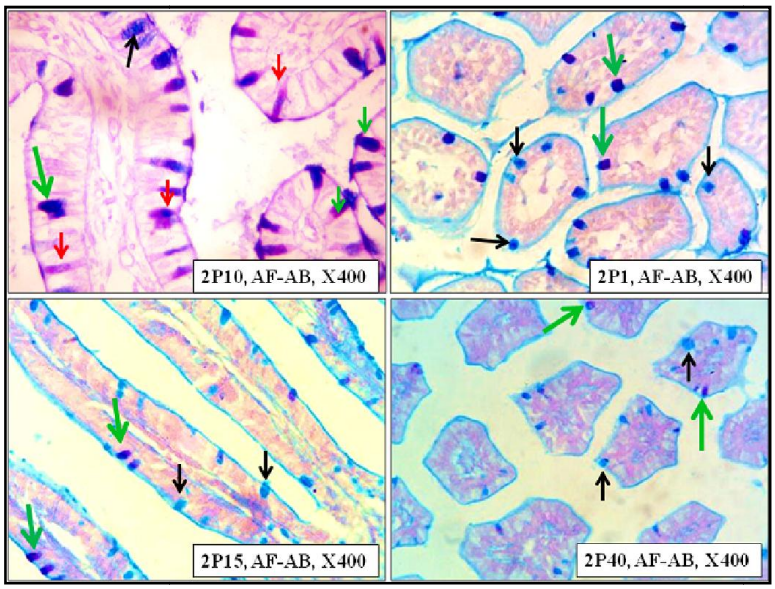

Fig. 2. Cross sections through villi of jejunum at one day kits (2P1), ten days suckling puppies (2P10), fifteen days pre-weaned rabbits (2P15) and forty days post-weaned rabbits (2P40). It showed positive reactions of goblet cells toward combined PAS-AB (pH 2.5) stain by the presence of neutral purple color (red arrows), acidic blue color (black arrows) and mixed mucin (green arrows) of goblet cells

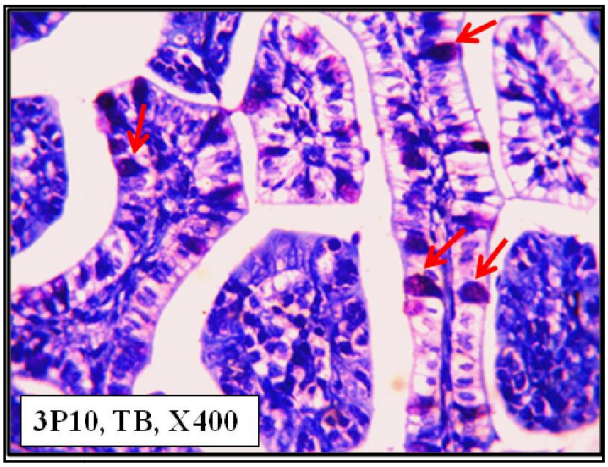

Fig. 3. Cross sections through the epithelium of ileum ten days suckling puppies (3P10) stained with Toluidine blue (TB). It showed positive reaction of goblet cells toward the stain (red arrows)

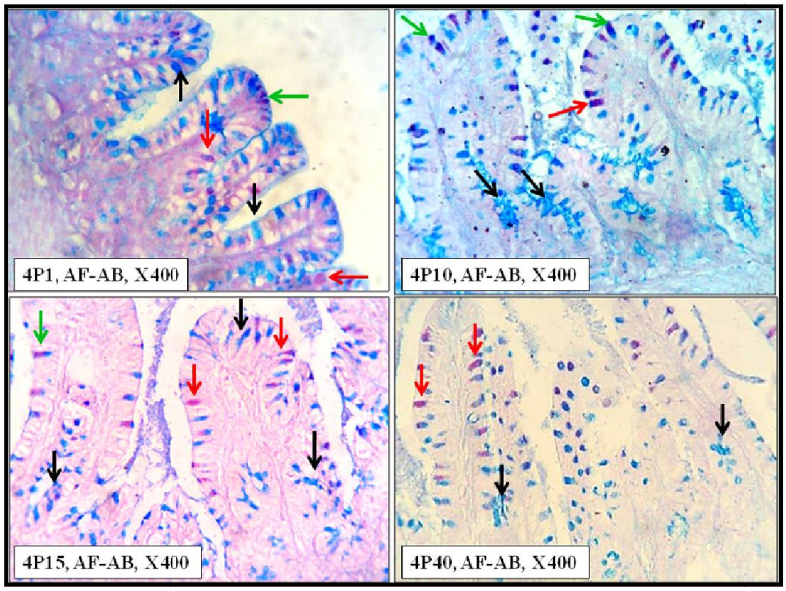

Fig. 4. Cross sections through villi of proximal colon at one day kits (4P1), ten days suckling puppies (4P10), fifteen days pre-weaned rabbits (4P15) and forty days post-weaned rabbits (4P40). It showed positive reactions of goblet cells toward combined AF-AB (pH 2.5) stain by the presence of sulphated acidic purple color (red arrows), non sulphated acidic blue color (black arrows) and mixed mucin (green arrows) of goblet cells.

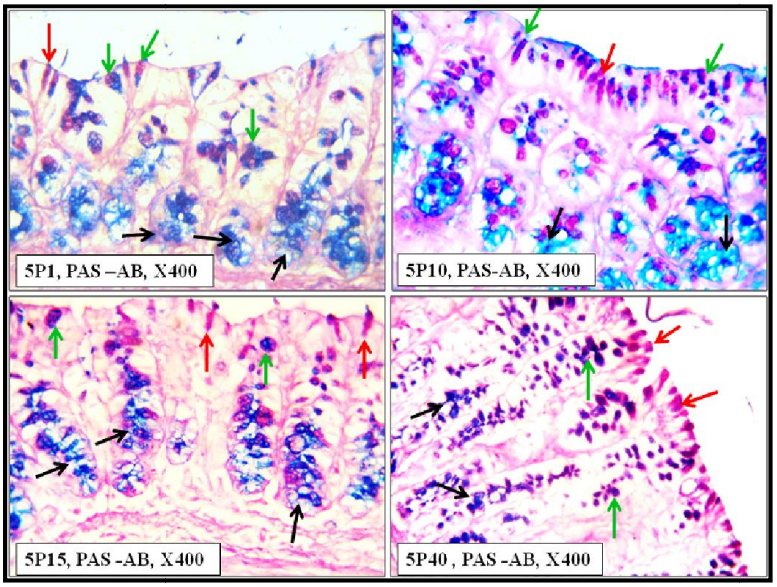

Fig. 5. Cross sections through the mucosa of distal colon at one day kits (5P1), ten days suckling puppies (5P10), fifteen days pre-weaned rabbits (5P15) and forty days post-weaned rabbits (5P40). It showed positive reactions of goblet cells and crypts cells toward combined PAS-AB (pH 2.5) stain by the presence of positive reactions of neutral purple color (red arrows), acidic (black arrows) blue color and mixed mucin (green arrows).

Post ten days of age, as in duodenum, the intensity of reaction of goblet cells of villi decreased with PAS into moderate reaction but remain moderately reacted with AB indicating the presence of equal amounts of neutral and sulfated acidic mucin. The reaction with TB stain was weak indicated the presence of small amounts of GAGs in the villi. Most of the goblet cells of crypts were moderately and strongly reacted with PAS and AB (pH2.5), respectively. Whereas, negative reactions was recorded post staining with the combined AF-AB and TB stains, indicated the presence of neutral and abundant non sulphated acidic mucin. In age of 15 days, as in duodenum the goblet cells of villi and crypts were weakly to moderately react with PAS but strongly reacted with AB (pH2.5) and the reaction was decreased with the combined AF-AB (purple). All of these reactions revealed the presence of extra non sulphated (carboxylated) acidic mucin than the neutral mucin. Staining with the TB stain also showed moderate reaction in the villi only indicated the increased amounts of GAGs in their structure.

In forty days aged weaned rabbits, the findings of histochemical staining revealed the presence of neutral mucin and sulfated mucin more evidently than the non sulphated (carboxylated type) of mucin in the goblet cells of the jejunal villi, in addition to GAGs. While in crypts, the neutral and carboxylated acidic mucin were more apparent than the sulfated mucin with the presence of lowest amounts of GAGs (Table 2) (Fig. 2).

Ileum

In one day aged kits, the results of staining with PAS, AB (pH2.5), combined PAS-AB (pH2.5), combined AF-AB (pH2.5) and TB showed the presence apparently neutral mucin more than the sulfated acidic, in addition to the presence of small amount of GAGs in the structure of the goblet cells existed in the ileal villi (Fig. 3). Post ten days of age, the ileal mucosa of the suckling rabbits showed abundant amount of neutral mucin which was found in the goblet cells of the ileal villi. Whereas, decreased amounts of acidic non sulphated mucin and increased sulfated GAGs were recorded. The cellular lining of the ileal crypts revealed mixed neutral and non sulphated acidic mucin only. Findings related to those of pre-weaned rabbits of fifteen days, were similar and not apparently differed from those recorded in ten days aged rabbits. In age of forty, goblet cells of ileal villi and crypts revealed equal amounts of neutral mucin, non sulphated acidic mucin and GAGs, with scarce amounts of sulphated acidic mucin (Table 3). Generally, histochemical findings revealed the presence of neutral mucin in the mucosal lining of the small intestine in all studied ages. The sulphated acidic mucin was abundant in kits and suckling rabbits (one and ten days of age), while in contrary the case was inverted in older ages (15 and 40 days of age), the non sulphated mucin was the dominant type. The reaction of combined PAS-AB (pH2.5) indicated the presence of mixed mucin (neutral and acidic) in all investigated ages and for the brush border in all small intestinal segments. The reaction of TB which is considered one of the metachromatic stain for GAGs was absent in one day of age, weak in the subsequent ten days of age and moderate in age of 15 days.

Proximal colon

In kits of one day of age and suckling rabbits of ten days of age, sections from proximal colon stained with histochemical stains showed that the goblet cells of the surface epithelium reacted moderately with PAS but strongly with AB (pH2.5), while it negatively reacted with AF and TB stains. These reactions indicated the presence of neutral mucin more than non sulphated type of mucin with absence of GAGs substances. Goblet cells of crypts were equally reacted with both PAS and AB (pH2.5) stains indicating the presence of equal amount of neutral and non sulphated acidic mucin in their structures. In pre-weaned rabbits of fifteen days of age, the goblet cells of surface epithelium showed moderate reaction with PAS and AB (pH2.5) but strongly reacted with AF (purple color) indicating the presence of equal amounts of neutral and sulphated acidic mucin, as well as, the GAGs substances were moderately reacted with TB. In the crypts, the reactions of PAS, AB (pH2.5) and AF were strong indicated the existence of neutral and non sulphated acidic mucin, GAGs gave weak reaction with TB. Results at forty days aged rabbits were similar to those described of fifteen days **(**Fig. 4**) (**Table 4)

Distal colon

In kits of one day of age and suckling rabbits of ten days of age, sections of distal colon showed dissimilar reactions to proximal colon in which the goblet cells of surface epithelium showed neutral and sulfated acidic mucin in addition to lowest amounts of GAGs. In the crypts, the goblet cells gave strong reaction with PAS and AB (pH2.5) and positively reacted with AF indicating the existence of equal amounts of neutral and sulfated acidic mucin only. Findings at age of fifteen showed dramatic increasing of sulfated acidic mucin and GAGs with the presence of moderate amounts of neutral mucin in the goblet cells of surface epithelium. In the crypts, the reactions were as same as in the surface epithelium with the addition of dominant of the non-sulphated groups also. Results at forty days aged rabbits were similar to those recorded to the age of fifteen days (Fig. 5) (Table 4).

DISCUSSION

The gastrointestinal secretions in vertebrates contain number of mucosubstances that can differ according to cell type, anatomical region, functional status, pathological condition, age, sex and species (Filipe, 1989, Sato and Spicer, 1980, Allen, 1981, Pedini et al., 2001, Liquori et al., 2002, Choi et al., 2003 and Schumacher et al., 2004). The goblet cells and Brunner's glands secrete large quantities of mucous into the small intestine. The distribution of mucins secreted by these cells into the lumen of this organ, as detected by the series of histochemical tests, revealed the presence of neutral mucin in its mucosal lining in all studied ages. The sulphated acidic mucin was abundant in newly born and suckling puppies (one and ten days of age), while in contrary, the case was inverted in older rabbits (15 and 40 days of age), and the non sulphated (carboxylated) mucin was the dominant type. The reaction of combined PAS-AB indicated the presence of mixed mucin (neutral and acidic) in all investigated ages and for the brush border in all small intestinal segments. Current results were in agreement with Zanuzzi *et al* (2010) and Desantis *et al* (2011) in New Zealand and white Italian rabbits. Comparative mucin histochemical study for 13 mammalian species (rats, voles, guinea pigs and rabbits) identified a similar mucin histochemical profile (Schumacher *et al*., 2004). Since the functional modulation of gastrointestinal mechanisms is closely associated with the structural alterations of the mucosa and its mucin substances, the changes in mucosal architecture and histochemical composition of mucin have received increasing attention since these are linked to the pathogenesis of various inflammatory and neoplastic gastrointestinal disorders (Forstner *et al*., 1982; Jacobs and Huber, 1985). The reaction of TB which is considered one of the metachromatic stain for GAGs was absent in one day of age, weak in the subsequent ten days of age and moderate in age of 15 days. In ileum the GAGs was more than that detected in duodenum and jejunum even in one day aged rabbits. In fact, at ileum and proximal colon which are nearest organs to the cecum, the fermentation processes is takes place. The latter activity produces GAGs because of the existence of bacteria. The GAGs is highly acidic mucins contain the carbohydrates hyaluronic acid and chondroitin sulphate. Hyaluronic acid is very hydrophilic and absorbs water to from the mucus gel. This gel in turn protects the mucus membrane by binding pathogenic bacteria, parasites (and their toxins) and arresting their movement (Law, 2000).

Conflict of Interests

The authors have not declared any conflict of interests.

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