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Original article

Temporospatial study of hexose transporters and mucin in the epithelial cells of chicken (*Gallus gallus domesticus*) small intestine

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Abstract

The temporospatial patterns in the localization of hexose transporters as well as in the quantitative and qualitative differences of glycoprotein mucin produced by the goblet cells of broiler chicken (*Gallus gallus domesticus*) small intestine during their first postnatal month were studied. The integral membrane proteins glucose transporter-2 and -5 (GLUT-2 and GLUT-5) that facilitate the transport of hexoses across epithelial cell layers that separate distinct compartments in organism were detected in the chicken intestinal epithelial cells using immunohistochemical labeling with polyclonal primary antibodies Rabbit anti-GLUT-2 and Rabbit anti-GLUT-5 (IHC kit, Abcam, UK). The chemical composition of mucin (neutral, acid) was carried out by applying the histochemical reactions by Alcian-Blue and periodic acid-Schiff methods.

The results revealed presence of the hexose transporters GLUT-2 and -5, immunolocalized in the enterocytes of broiler's small intestine and the temporospatial pattern of the density of goblet cells of intestinal mucosa as well as the chemical composition of mucin produced by the goblet cells in chicken immediately after hatching and in 30-days-old chicken's. Simultaneously, when goblet cells remained unstained with both antibodies in intestinal epithelium in chicken of both ages or some moderate staining was noticed in 30-days-old chickens' ileal epithelium, the increase of neutral and acid mucin- containing cells per area unit in both segments of the small intestine was detected from the first day after hatching to 30 day of life and the density of goblet cells was found to be higher in ileal than in duodenal region.

Key words: hexose transporters, GLUT-2, GLUT-5, mucin, small intestine

Introduction

Small intestine plays an important role in the digestion and absorption of ingested food. The small intestine absorptive cells (enterocytes) have apical domain with brush border, which contains microvilli. The microvilli contain intramembranous enzymes (lactase, etc.) and oligosaccharides. Hexoses that are involved in the whole-body glucose homeostasis are transported into the enterocyte by carrier proteins (Uldry and Thorens 2004). The monosaccharides, glucose, galactose, and fructose are transported from the intestinal mucosal cell into the portal circulation by glucose transporter-2 (GLUT) and -5 (GLUT-5). GLUT-2 is a transmembrane carrier protein that enables passive movement of hexoses across cell membranes. GLUT-5 is a facilitated fructose transporter expressed on the apical border of enterocytes in the small intestine. Although the small intestine plays a central role in the digestion and absorption of nutrients and the immunolocalization of hexose transporters have been well characterized in mammals, relatively little is known about the temporospatial patterns of the expression of the transporters in birds small intestine.

The intestinal tract epithelium is covered by a mucus layer composed predominantly of mucin glycoproteins, synthesized and secreted by goblet cells distributed along the villi (Uni et al. 2003). The mucus layer acts both as a medium for protection of the brush border against damage by chemicals or microorganisms and influences transport between luminal contents and the brush border (Forstner and Forstner 1994). According to their chemical composition, mucins can be histochemically identified as acid and neutral subtypes (Neutra and Forstner 1987). Recently, a mixed subtype has also been described (Duritis et al. 2013). Acid mucins can be detected by Alcian Blue (AB) pH 2.5 staining, neutral mucins by periodic acid-Schiff (PAS) staining (McManus 1948). Acid mucins can contain sulphated groups which can be detected by Alcian Blue (AB) pH 1,0 staining (Carson 1997).

As the hexose transporters, mucin subtypes and goblet cell distribution may vary spatially throughout the gastrointestinal tract and temporally during development in many mammalian species (Sheahan and Jervis 1976, Hill et al. 1990, Dunsford et al. 1991, Enss et al. 1992, Sharma and Schumacher 1995, Kandori et al. 1996); however as there is relatively few information about their distribution in bird species (Uni et al. 2003, Duritis et al. 2013, Hussar et al. 2016), the objective of the current study was to get more detailed information about the immunolocalization of hexose transporters GLUT-2 and GLUT-5 as well as on mu-

cin subtypes and the density of goblet cells in the duodenal and ileal epithelium of broiler's chicken comparatively in different postnatal ages – immediately after hatching and in the 30 days old broiler's.

Materials and Methods

Sample collection

Twelve Ross broilers (*Gallus gallus domesticus*) were obtained from a commercial Macedonian hatchery and were placed in temperature-controlled brooders and grown under standard conditions with free access to food and water. Broilers of different ages divided into two age groups – six chicken after hatching and six 30-days-old chicken – were killed by intracardiac overdose of sodium pentobarbital. Segments were taken from the middle parts of duodenum and ileum for histological studies.

The experiments were carried out in accordance with the Guidelines laid down by the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the Ethical Committee of Ss. Cyril & Methodius University in Skopje has approved the experiments.

Routine histology

Specimens, 0.5 – 1.0 cm in diameter, were fixed in 10% neutral buffered formalin solution, embedded into paraffin and 5 µm thick sections were cut (microtome Leica 2135) according to the standardized tissue histological procedure (Carson 1997). Slices floated on Poly-L-Lysine-coated slides (O. Kindler GmbH, Freiburg, Germany) were deparaffinized with xylene and rehydrated in a graded series of ethanol followed by the methods of routine histology, immunohistochemistry and histochemistry.

For overall histological assessment by routine histology, the tissue samples were stained with hematoxylin and eosin method (Carson 1997).

Immunohistochemistry

For the immunolocalization of hexose transporters GLUT-2 and GLUT-5 in the intestinal epithelium the sections were stained using Immunohistochemistry kit (Abcam, UK) according to the manufacture's guidelines. The sections were pre-treated using heat-mediated antigen retrieval with sodium citrate buffer (pH 6) for 20 min and incubated with primary rabbit polyclonal antibodies to glucose

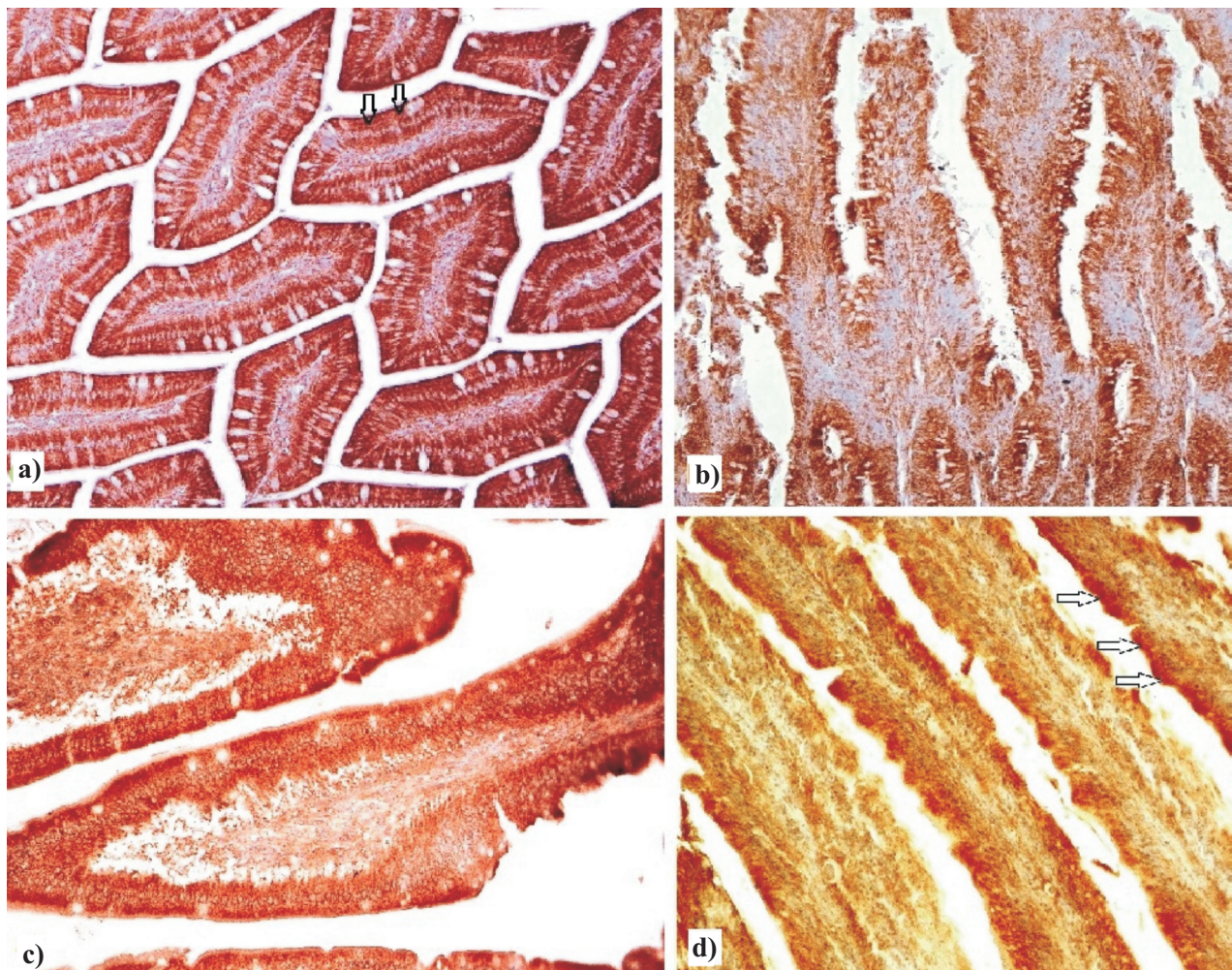


Fig. 1. Duodenum: a) Duodenal epithelium in chicken after hatching. Note the strongly stained enterocytes (arrows) for GLUT-2, x200; b) Duodenal epithelium of 30-days-old chicken, stained for GLUT-2, x200; c) Enterocytes stained for GLUT-5 in duodenal epithelium in chicken after hatching, x400; d) Apical parts of enterocytes stained for GLUT-5 (arrows) in 30-days-old chicken's duodenal epithelium, x200.

transporter 2 (GLUT-2) and – 5 (GLUT-5) primary antibodies (Abcam, UK) at 1/1000 dilution, for 30 min at 37°C. Biotinylated secondary antibody and streptavidin-conjugated peroxidase were used for detection using DAB as chromogen. Nuclei were counterstained with Harris Hematoxylin. Negative controls contained antibody diluent (Dako, S0809) instead of primary antibodies. Ostriches chicken's intestinal tissue sections for identifying GLUT-2 and GLUT-5 were used as positive controls (Hussar et al. 2016).

Histochemistry

For identification of epithelial mucosubstances, the following histochemical reactions were applied: periodic acid-Schiff test (*Bio-Optica*) (PAS) for identification of the neutral glycoproteins; Alcian Blue at pH 1.0 (AB) (*Sigma-Aldrich*) for identification

of the acid glycoproteins (sulfomucin); Alcian Blue at pH 2.5 – periodic acid-Schiff test (AB/PAS) (*Bio-Optica*) for determination of the acid glycoproteins (Carson 1997, Kiernan 2008).

By using the histochemical reactions, the differentiation of the goblet cells by the chemical composition of glycoproteins was carried out into the acid (AB+, pH 2,5), neutral (PAS+) and sulphated (AB+, pH 1,0) glycoproteins-containing cells. The density of the goblet cells was determined in 10 microscopic fields (0.083 mm²), at 400 x magnification, in duodenal and ileal mucosa of each preparation of each chicken.

Photos of all slides were taken with the microscope Zeiss Axioplan-2 Imaging (Germany) and saved to the computer for analyzing by visual control using camera (AxioCam HRC, Germany) connected to the microscope.

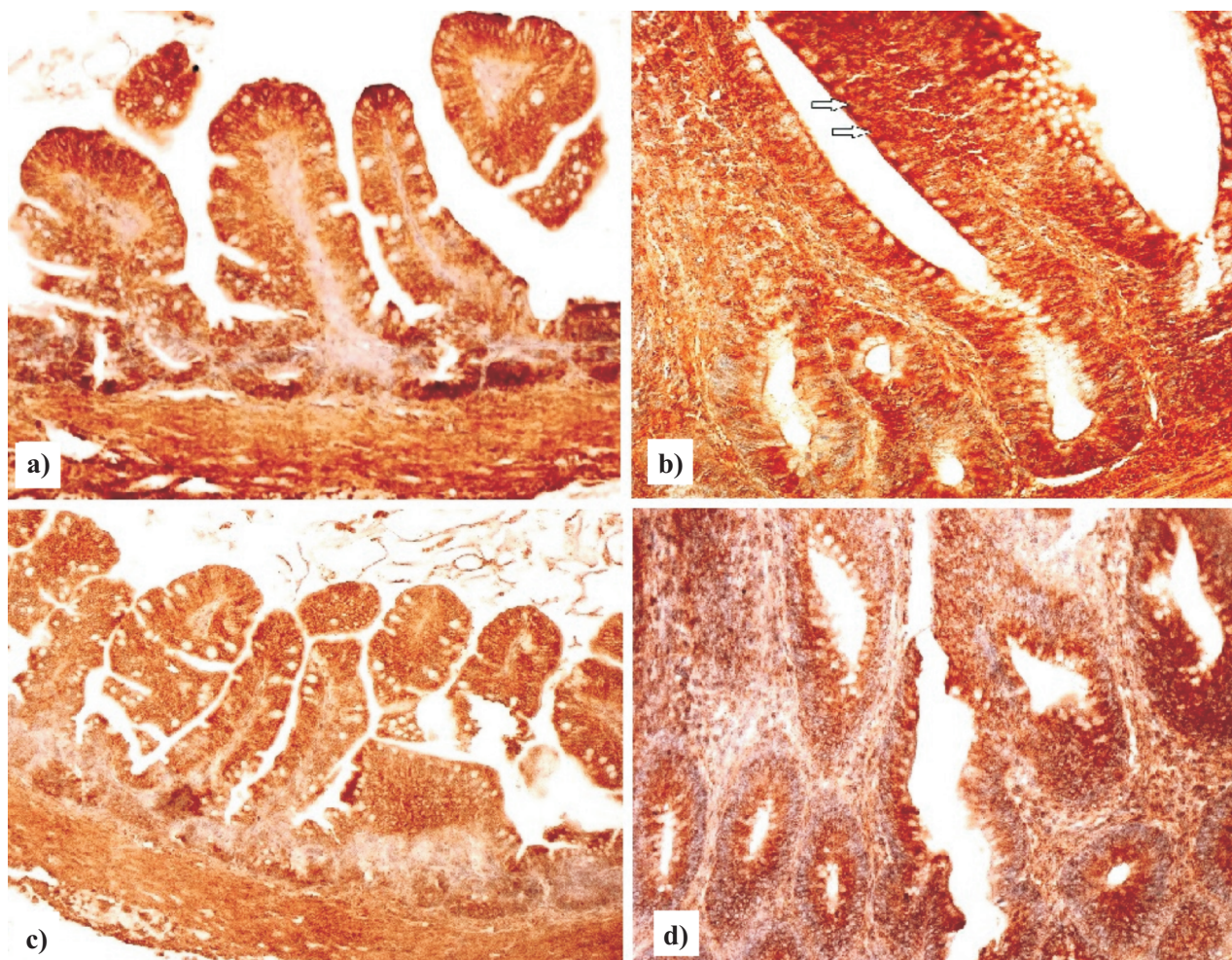


Fig. 2. Ileum: a) Ileal epithelium, after hatching, GLUT-2, x200; b) Moderate staining of goblet cells (arrows) for GLUT-2 in 30 days old broiler's ileal epithelium, x400; c) Strongly stained enterocytes and unstained goblet cells in chickens' ileal epithelium after hatching, x200; d) Ileal epithelium in 30 days old chicken stained for GLUT-5, x400.

Statistical analysis

The data obtained in the study were statistically processed by SPSS 17.5 software program. Arithmetic mean value and the standard error (SEM) were calculated for each parameter. To compare the mean parameters between age groups, the multifactor dispersion analysis ANOVA was applied for comparison of the mean values for several unrelated samples, as well as the T-test for comparison of related samples (within the same age group) (Arhipova et al. 2003).

Results

Morphology

The sections of duodenum and ileum of the chicken in the different age groups – immediately after hatching and 30-days-old-chicken – were exam-

ined by light microscopy for the general histological overview.

The duodenal and ileal villi were lined by simple columnar epithelium. In both age groups the villi were higher in duodenum compared to ileum. From the first day after hatching towards the 30th day the ileal villi became bigger and broader and the number of goblet cells in lamina epithelium grew larger.

Localization of glucose transporters

Strong positive staining of duodenal and ileal enterocytes' cytoplasm by the GLUT-2 and GLUT-5 antibodies was noted in the epithelium of both regions and age groups (Fig. 1 and 2). The staining was equally strong for GLUT-2 in enterocyte's both – apical and basolateral- parts (Fig. 1a). For GLUT-5 the staining was stronger in apical parts of the cells (Fig. 1d). Brush border membranes and goblet cells

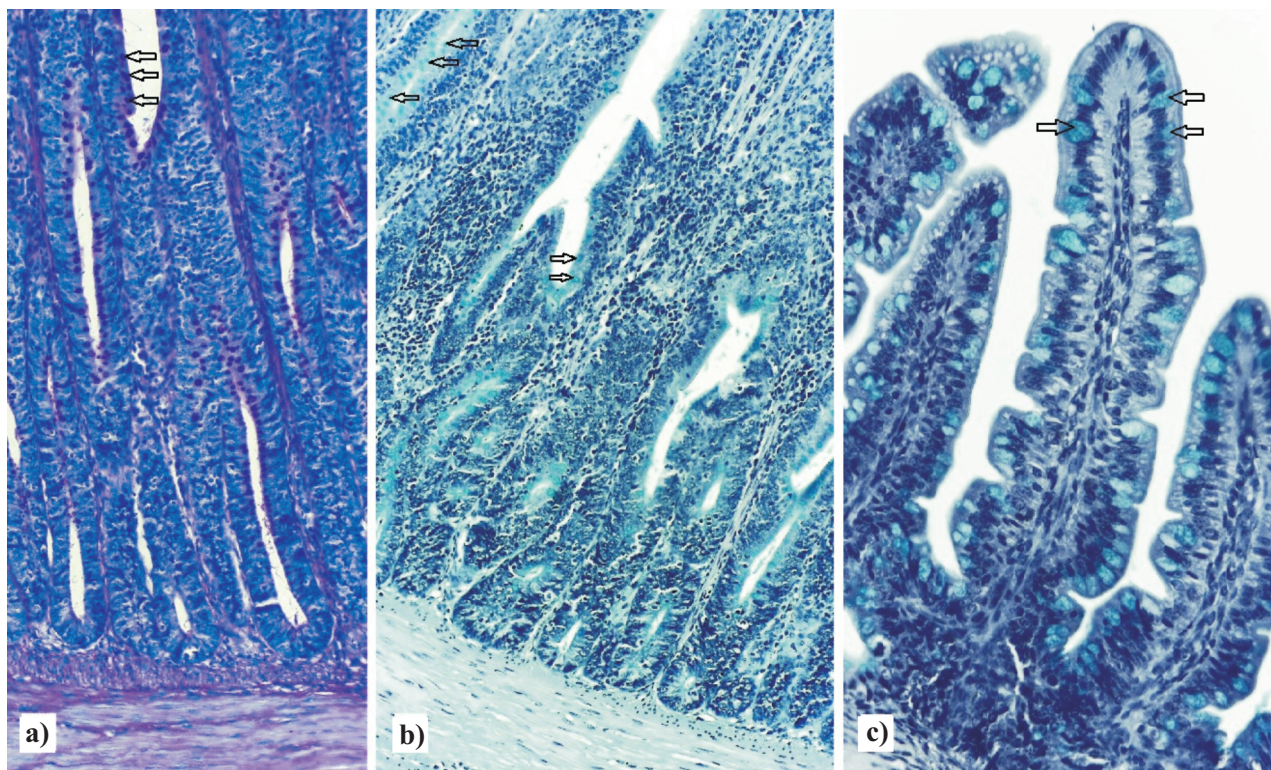


Fig. 3. a) Goblet cells (arrows) stained with PAS+ in 30 days old chicken duodenal epithelium, x100; b) Cross section of 30 days old chicken ileum. Goblet cells (arrows) stained by Alcian blue (pH 2,5), x100; c) Ileal villi of chicken after hatching. Note the goblet cells (arrows) stained by Alcian blue (pH 1), x400.

remained mostly unstained with both antibodies in intestinal (both duodenal and ileal) epithelium in chicken after hatching. Some weak or moderate staining of goblet cells was noticed only in 30-days-old chickens' ileal epithelium (Fig. 2b).

Mucin subtypes in intestinal goblet cells

The goblet cells of the duodenal and ileal mucosa were examined at the first day after hatching and at the 30th day after hatching to determine the density and types of the goblet cells in the intestinal mucosa (Table 1 and 2).

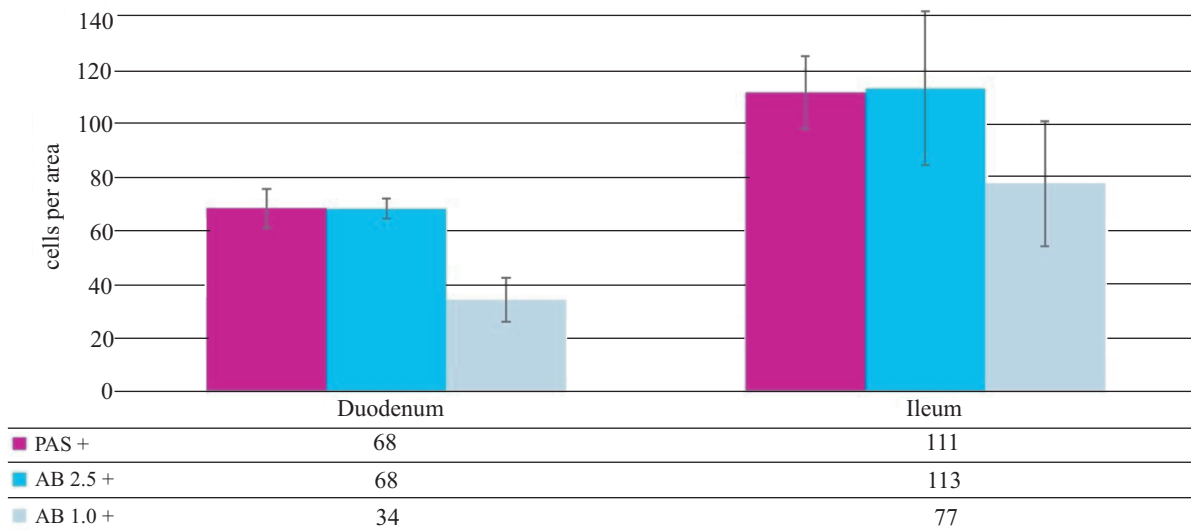
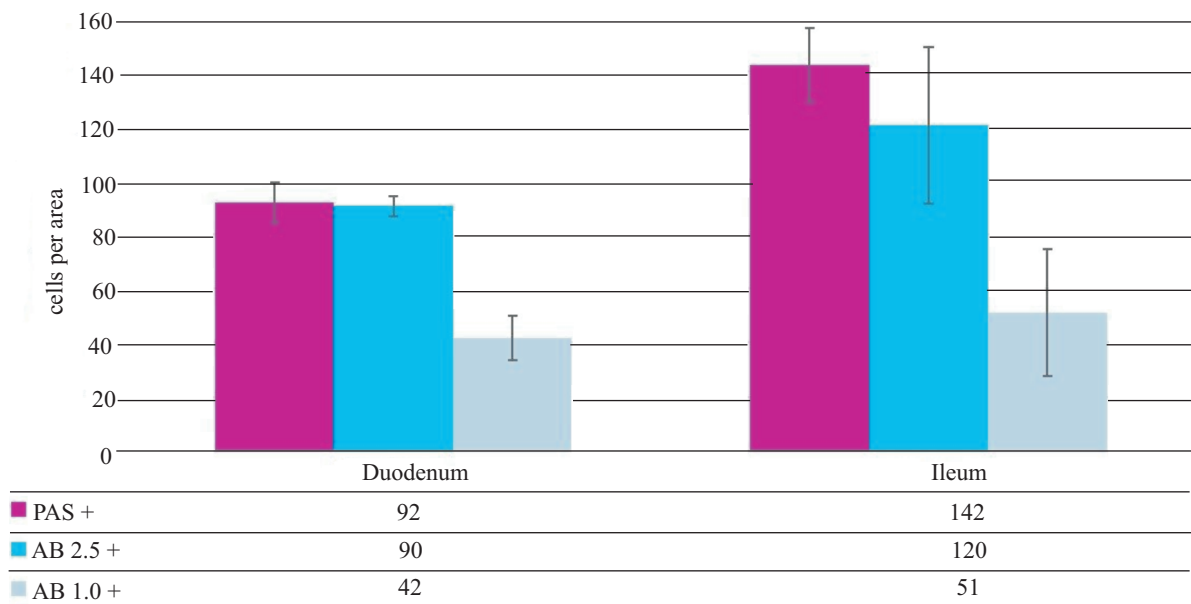
In the intestinal epithelium goblet cells containing the neutral and acid mucin were detected in both part of small intestine. About 50% of all acid mucin-containing cells, showed positive reaction to (or contain) sulphated mucin groups (staining by Alcian Blue, pH 1.0). From the first day after hatching to 30th day of life, increase of neutral and acid mucin containing cells per area unit (field of view) in both segments of the small intestine was detected, furthermore, in duodenum it was significantly ($p < 0.01$) higher in 30-day-old chicks (Table 2).

The density (number of cells per field) of goblet cells was different in both parts of small intestine

mucosa. On 30th postnatal day number of neutral mucin containing cells was significant ($p < 0.05$) higher in ileum than in duodenum. Also number of acid mucin-containing cells, containing sulfated mucin, tends to be higher in both age groups in ileum.

Discussion

Glucose transporters play a pivotal role in the transfer of glucose across epithelial cell layers that separate distinct compartments in the organism (Takata 1996). Based on sequence comparison, the GLUT isoforms can be grouped into three classes: class I comprises GLUT1-4; class II, GLUT6, 8, 10, and 12 and class III, GLUT5, 7, 9, 11 and HMIT. Tissue- and cell-specific expression of the well-characterized GLUT isoforms underlies their specific role in the control of whole-body glucose homeostasis. Numerous studies with transgenic or knockout mice confirm an important role for these transporters in the control of glucose utilization, glucose storage and glucose sensing. The uptake of hexoses into intestinal enterocytes is mediated by Na⁺/glucose co-transporter (SGLT) and the facilitated-diffusion glucose transporters (GLUT). GLUT-2 is up-regulated at the brush border membrane, enhancing the capacity

Table 1. Number of Goblet cells in chicken small intestine on the first day of life (mean \pm SD).Table 2. Number of Goblet cells in chicken small intestine on the 30th day of life (mean \pm SD).

of the transport of hexoses (Uldry and Thorens 2004). GLUT-2 is found in small intestine of the basolateral membranes, but also in apical parts of enterocytes (Kellett and Brot-Laroche 2005). GLUT-5 allows for fructose to be transported from the intestinal lumen into the enterocyte by facilitated diffusion due to fructose's high concentration in the intestinal lumen and is shown to be located in the mammals' intestinal epithelium (Ferraris 2001). Our previous study on glucose transporters in ostrich's gastrointestinal system comparatively in different age groups showed in growing ostrich's chicken the gradual increase of staining intensity for GLUT-2 and GLUT-5 in the epithelial cells which could be related to the ostrich's chicken

capability of transportation of carbohydrates as the ostrich's chicken mostly begin to eat only at the end of the first week after hatching (Hussar et al. 2016). Our present, temporospatial study of hexose transporters on broilers' chicken, showed GLUT-2 and -5 to be expressed in duodenal and ileal enterocytes with equal strength in the both studied age groups which is in accordance with our presumption, as broilers' chicken begin to eat immediately after hatching.

The mucus layer coating the gastrointestinal tract is the front line of innate host defense, largely because of the secretory products of intestinal goblet cells. Goblet cells synthesize secretory mucin glycoproteins (*MUC2*) and bioactive molecules such as epithelial

membrane-bound mucins (*MUC1*, *MUC3*, *MUC17*), trefoil factor peptides (TFF), resistin-like molecule β (RELM β), and Fc- γ binding protein (Fcgbp) (Kim and Ho 2010). Our studies on mucin showed the increase of neutral- and acid mucin-containing cells per area unit in both segments of the small intestine. On 30th postnatal day number of both types of mucin-containing cells was higher in ileum than in duodenum. The density of the goblet cells of broiler's chicken per area unit from the 1st day after hatching to the age of 30 days of life tended to increase in both segments of the small intestine. Our results characterize both the quantitative and qualitative differences of glycoproteins secretion that is possibly connected with specific roles of the small intestine segments in the processes of nutrients absorption.

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