UNIVERSITY OF TARTU
FACULTY OF SCIENCE AND TECHNOLOGY
INSTITUTE OF MOLECULAR AND CELL BIOLOGY
DEPARTMENT OF DEVELOPMENTAL BIOLOGY

Aleksandra Rotmistrova

Glucose transporters in ostrich gastrointestinal tract

Master Thesis

Supervisors: Piret Hussar, M.D., D.M.Sci
Martin Kärner, M.Sc
Ilmars Duritis, D.Vet.Sci (Latvia University of Agriculture)

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ABBREVIATIONS

ABC method – avidin-biotin complex method

BSA – bovine serum albumin

DAB – 3,3'-diaminobenzidine

EU – European Union

GIT – gastrointestinal tract

GLUT – glucose transporter

H&E – hematoxylin and eosin

HIER – heat-induced epitope retrieval

HMIT – proton driven myoinositol transporter

IHC – immunohistochemistry

Km – Michaelis constant

LSAB method – labeled streptavidin-biotin method

MFS – major facilitator superfamily

NBF – neutral buffered formalin

PBS – phosphate buffered saline

SGLT – sodium-glucose transport protein

TBS – tris buffered saline

TM – transmembrane

INTRODUCTION

Ostrich farming is very popular in agriculture due to wide use of their products in medicine, food, cosmetic and pharmaceutical industries. It can be said, that this is the bird of XXI century.

Although the anatomical and histological structure of avian was studied more than 100 years ago, the gastrointestinal tract (GIT) of the ostrich has been studied recently.

Due to lack information on ostrich digestive system morphology and glucose transporters localization, there are farming difficulties connected to feeding of the animal that lead to high mortality of birds in the early ages. In order to enhance commercial efficiency of the ostrich production, there is a need in more detailed scientific research in this field.

Carbohydrates, which are the main energy source in food, are degradated in small intestine into monosaccharides. Hexoses, including glucose, belong to this class of monosaccharides.

Glucose is transported across the epithelial cell layers by two groups of transporters (integral membrane proteins). The first group is represented by the classical active Na^+ dependent glucose transporters – SGLT family. The second group includes the facilitated diffusion transporters – GLUT family. GLUT family expression has been well described in mammalian cells. However, there is a lack of information about localization of glucose transporters in the GIT of birds.

The main aim of this thesis is to investigate the most abundant facilitated diffusion glucose transporters GLUT-1, GLUT-2 and GLUT-5 in different parts of the gastrointestinal tract of 30 days old ostriches, namely inside the superficial gland zone of proventriculus, duodenum

and the terminal zone of ileum.

1. REVIEW OF LITERATURE

1.1 The ostrich

Ostriches (*Struthio camelus var. Domesticus*), the only member of the *Struthionidae*, are the largest flightless birds from the *Ratitae* family ("the running bird") (Waugh E. E. *et al.*, 2007). Over 100 years it has been bred on farms of South Africa for feathers, leather, meat, eggs and oil. Today ostriches are farmed all over the world (Adams J., Revell B. J., 1998).

1.1.1 Use of the ostrich in farming

Ostriches produce red meat, which contains less fat (1,29 g fat per 100 g raw meat) than chicken, and particularly less cholesterol (57 mg cholesterol per 100 g raw meat). Compared with beef ostrich meat has half the calories. With such qualities ostrich meat is a healthy choice (Shanawany M. M., 1995; Cooper R. G., Horbanczuk J. O., 2002). Ostrich egg compared with egg of hen, which is widely represented in the market, has higher unsaturated-saturated fatty acid ratio and lower cholesterol content (1,2% vs 1,8%). Ostrich egg is also very resistant to breakage due to its strong shells, which is important in transportation (Sales J. *et al.*, 1996; Di Meo C. *et al.*, 2003). Cosmetic industry uses the ostrich oil in products like moisturizing creams, body lotions, soaps, lip balms, masks, because of its good skin penetration properties. These properties are due to the oleic acid (Omega-9), which has high similarity to human skin lipid. In food industry ostrich oil also is popular because of low cholesterol content (Shahrayar H. A., Lotfi A., 2012; Gavanji S., Larki B., Taraghian A. H., 2013). In medicine ostrich eyes are used in corneal research and are ideal human alternatives for transplants; tendons of the ostrich leg are also used in transplantation. Substance of ostrich brain is studied for treatment of Alzheimer's disease (Shanawany M. M., 1994; Cooper R., G.,

2007).

1.1.2 Problems in ostrich farming

Previously mentioned qualities make ostrich farming one of the most rapidly developing poultry industries in the XXI century. However, it should be noticed that there is still lack of information on anatomy of the ostrich, including morphology and physiology. Especially poorly is described the morphology of ostrich digestive system, where the small intestine plays an important role in absorption of most nutrients (carbohydrates, proteins, fat). This makes it difficult to study nutritional requirements of this species. Lack of knowledge in this field leads to high mortality rate and therefore prevents from ensuring profitable production (Iji P. A., 2005; Lavin S. R., 2008). According to the literature, mortality of ostrich chicken in farms is especially high until 30 days after hatching (mortality rate about 46 %) and only 50% of all chicks ever reach the age of four months (Cloete S. W. P. *et al.*, 2001). High mortality of ostrich chicken has been observed in farms throughout the EU (European Union) (Duritis I., 2011).

1.2 Digestive tract of the ostrich

The ostriches have a relatively large digestive tract, which includes (Fig. 1):

- beak, mouth and salivary glands,
- oesophagus,
- proventriculus (glandular stomach),
- ventriculus (muscular stomach),
- small intestine (duodenum, jejunum, ileum),
- large intestine (caeca, proximal, middle, distal colons),
- cloaca

(Brand T. S., Gous R. M, 2006).

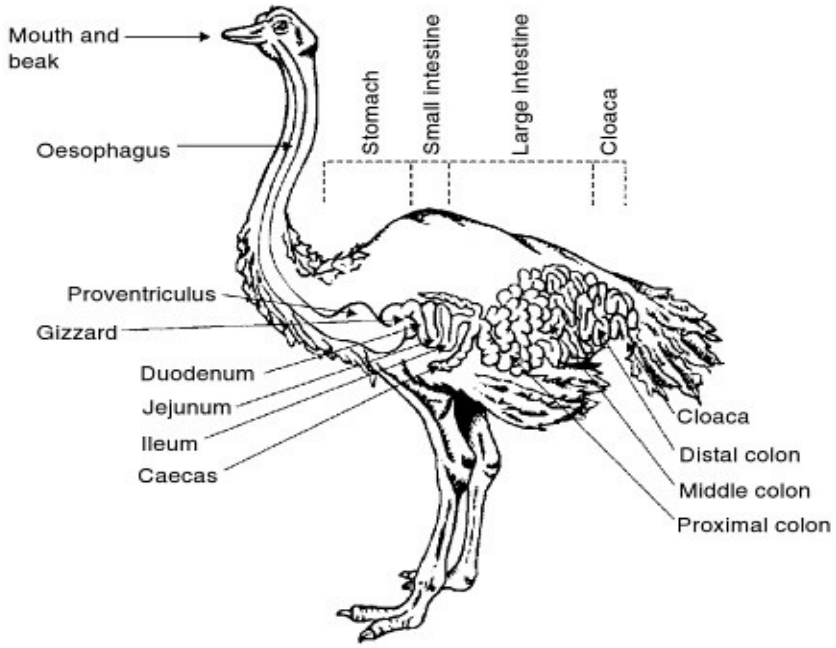

Figure 1. Illustration of the digestive system of the ostrich (Brand T. S., Gous R. M., 2006).

1.2.1 Histology of ostriches stomach

The stomach of the African ostrich (*Struthio camelus var. domesticus*) consists of two anatomically and functionally different parts – the glandular part (*pars glandularis,* s. proventriculus) and the muscular part (*pars muscularis,* s. ventriculus), which are separated by an isthmus (intermediate zone) (Fig. 2) (Duritis I., 2013). The proventriclus secretes enzymes that start digestive process. It is a short, thick-walled and spindle-shaped organ. The ventriculus (gizzard), is a muscular stomach with thick walls and horny interior epithelium. Its function is to break up and grind food (Shanawany M. M., Dingle J., 1999).

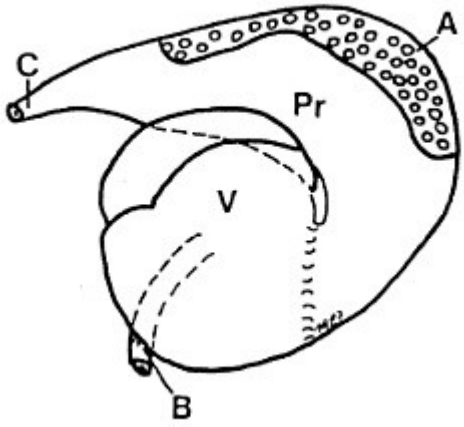

Figure 2. Stomach of the ostrich. Glandular area of the proventriculus marked with A, duodenum – B, oesophagus – C, proventriculus – Pr, ventriculus – V (Fowler M. E., 1991).

1.2.1.1 Proventriculus

The structure of the proventriculus differentiates into papillary and non-papillary regions. Non-papillary region is devided into folded and unfolded region. Papillary region wall contains *tunica mucosa*, *tunica muscularis* and *tunica serosa* (Tadjalli M. *et al.*, 2011). The *tunica mucosa* has parallel branched longitudinal folds with simple to branched tubular glands, which are extended into the *lamina propria* occupying the center of the mucosal folds (Bezuidenhout A. J., Van Aswegen G., 1990). Branched tubular glands are composed of numerous angular, polymorphic or rounded lobules arranged into small groups. Endocrine cells are located among the secreting cells of the deep proventricular glands. *Tunica muscularis* has outer longitudinal muscular layer and inner circular layer (Bezuidenhout A. J., Van Aswegen G., 1990). *Tunica serosa* is comprised of a single layer of mesothelial cells. *Muscularis mucosa* and submucosal glands are absent from the non-papillary region of the proventriculus. *Lamina propria* is usually combined with *tela submucosa* layers (Tadjalli M. *et al.*, 2011). *Tunica mucosa* with *tela submucosa* and *tunica muscularis* make the high folds. Epithelium is simple columnar cell layer and mucosal glands are simple branched tubular cells. *Tunica serosa* is a thick layer with a row of squamous cells, that make the mesothelium.

It is rich in blood vessels and nervous elements of *serous plexus* (Bezuidenhout A. J., Van Aswegen G., 1990; Tadjalli M. *et al.*, 2011).

1.2.1.2 Ventriculus

Ostriches have a well-developed muscular gizzard. Similarily to proventriculus, wall of the ostriches ventriculus consists of *tunica mucosa*, *tunica muscularis* and *tunica serosa*. The gizzard has thin, but hard *tunica mucosa*. It has simple columnar epithelium and some simple straight tubular glands, that secrete thin keratinoid lining (cuticule) in propria (Coles B., 2008). Cuticule layer is a surface that protects *mucosa* from the acid and pepsin, which are produced by the proventriculus (Zaher M. *et al.*, 2012). Like in papillary proventriculus, ventriculus has outer longitudinal and inner circular layers of *tunica muscularis*. Endocrine cells in the gizzard are located among the lining cells of the simple tubular glands. Similarly to proventriculus, *tunica serosa* of ventriculus is lined by mesothelium and surrounded by blood vessels, nerves and interstitial cells (Catroxo M. H. B. *et al.*, 1997).

1.2.2 Histology of ostriches small intestine

The small intestine is the longest section of the gastrointestinal tract (Fig. 3) that connects the stomach and the large intestine. The small intestine is divided into three structural parts: the duodenum is the first and the shortest section of the small intestine, second section is jejunum, and the last section – ileum.

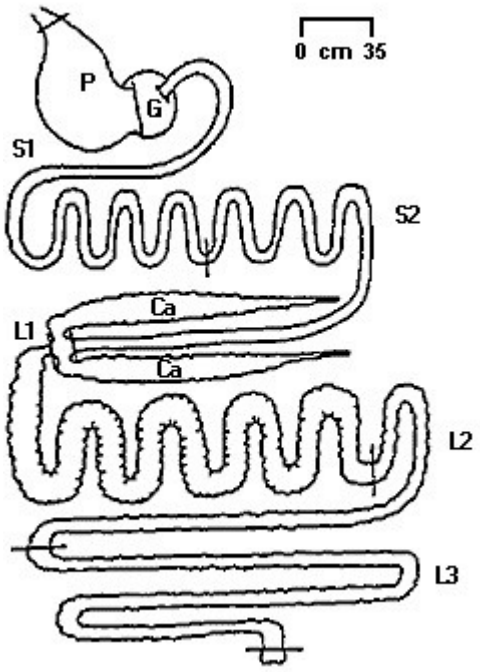

Figure 3. Gastrointestinal tract of an ostrich chick. Proventriculus marked with P, gizzard – G, small intestine – S1, caeca – Ca, sections of large intestine – L1-3 (http://www.ianr.unl.edu/pubs/animals/nf251.htm).

Ostriches duodenum is about 60-70 cm long. It has a „U"-shaped loop form and runs along the stomach almost to the pelvis. The length of the jejunum is approximately 4 m, and is located in the right half of the abdominal cavity. It does not form a loop, but in the middle owns a spiral shape. Ileum is the third part of the small intestine. It is located at the lower right corner of the duodenum, and links with the both sides of the cecum. It is 70-80 cm long, without loops and spirals (Bezudeinhout A. J., 1999).

The wall of all three segments of the small intestine is formed by *tunica mucosa*, *tunica muscularis* and *tunica serosa* (Igwebuike U. M., 2010).

Tunica mucosa is well-developed, there are a large number of crypts, villi and microvilli. Each villus has blood vessels. Outside of the villi are lined tiny projections – microvilli – which carry enzymes. Enzymes decay double sugars into simple saccharides, and complete

protein digestion. Each villus is lined by a simple columnar epithelium. Most of these cells are enterocytes (intestinal absorptive cells) with a brush border of microvilli (major barrier to the microbiota and pathogens), mucus producing Goblet cells (that protect epithelium from digestive enzymes) and endocrine cells (0,5-1%) (Karcher D. M., Applegate T., 2008; Zaher M. *et al.*, 2012). Crypts (of Lieberkuhn) are invaginations between villi. Their epithelium is similar to the epithelium of the villi, but the brush border is 2-3 times thinner. The highest concentration of villi is marked in the jejunum (Горбачева М. В., 2012). The length of intestinal villi decreases in the caudal direction. In duodenum and in the beginning of jejunum they are long, but in ileum they are significantly shorter and near the cloaca the villi almost completely disappear (Bezuidenhout A. J., Van Aswegen G., 1990). The length of microvilli is longest on the top of the villus and shortest on the crypt (Karcher D. M., Applegate T., 2008). The number of Goblet cells increases in the caudal direction, whereas in duodenum they are very rare. Paneth cells are not present (Aitken R. N. C., 1958; Порческу Г. С., 2007). *Tunica submucosa* is thin and narrow with few blood vessels.

Tunica muscularis is represented by outer longitudinal and inner circular smooth muscle layers. First layer is irregularly developed: in some places it is represented by two or three rows of smooth muscle cells, however in others it may be absent. Inner circular layer of *tunica muscular* is considerably better developed. *Tunica muscularis* is thickest in the ileum and thinnest in the jejunum (Illanes J. *et al.*, 2006; Zaher M., 2012).

Tunica serosa is comparatively thin (Порческу Г. С., 2007).

1.3 Classification of glucose transporters

One of the main types of nutritiens are carbohydrates, which are stored as glycogen and are transported between tissues as glucose (blood sugar). Glucose is the major immediate source of energy for cells, tissues and organs (Hazelwood R. L., 1972; Stevens L., 1996), and glucose

transporters play a pivotal role in the transfer of glucose across epithelial cell layers that separate distinct compartments in organism (Takata K., 1996). Glucose is transferred from the lumen of the small intestine through intestinal epithelium and then via blood vessels into target cells. It is absorbed by two structurally and functionally different groups of transporters. Classical active absorption is mediated by the Na^+ sodium-glucose transporters (SGLTs) and diffusive absorption is provided by the glucose transporters (GLUTs) (Braun E. J., Sweazea K. L., 2008). These two types of glucose transporters exhibit different substrate specificities, kinetic properties and tissue expression profiles (Wood I. S., Trayhurn P., 2003).

1.3.1 Sodium-glucose transporters

Active SGLT transport is used for absorption and reabsorption of glucose from food in GIT and from the urine in the kidney (Augustin R., 2010). It transports glucose against concentration gradient by coupling glucose with Na^+, which is transported down its concentration gradient. SGLT-1 and SGLT-2 transport glucose-galactose and glucose, respectively, across the membrane. SGLT-1 transporter has high affinity but low capacity. SGLT-1 high expression is detected in small intestine (brush border of enterocytes, apical side of intestinal) and in kidney (proximal renal tubule cells) (Ferraris R. P., 2001; Harada N., Inagaki N., 2012). SGLT-2, conversely, has low affinity and high capacity. It is expressed in the apical membrane of renal proximal tubules cells of the kidney and is responsible for about 90% of renal glucose reabsorption (Jiang M. *et al.*, 2014).

1.3.2 Glucose transporters

The GLUT protein family belongs to the major facilitator superfamily (MFS) and are grouped into three classes of transporters (Fig. 4): Class I (GLUT-1, GLUT-2, GLUT-3, GLUT-4, GLUT-14) are glucose transporters, Class II (GLUT-5, GLUT-7, GLUT-9, GLUT-11) are fructose transporters, and Class III (GLUT-6, GLUT-8, GLUT-10 and GLUT-12, GLUT-13

(proton driven myoinositol transporter HMIT)) are structurally atypical transporters, which are poorly defined at present (Mueckler M., Thorens B., 2013). These transporters transport glucose down its concentration gradient by facilitative diffusion.

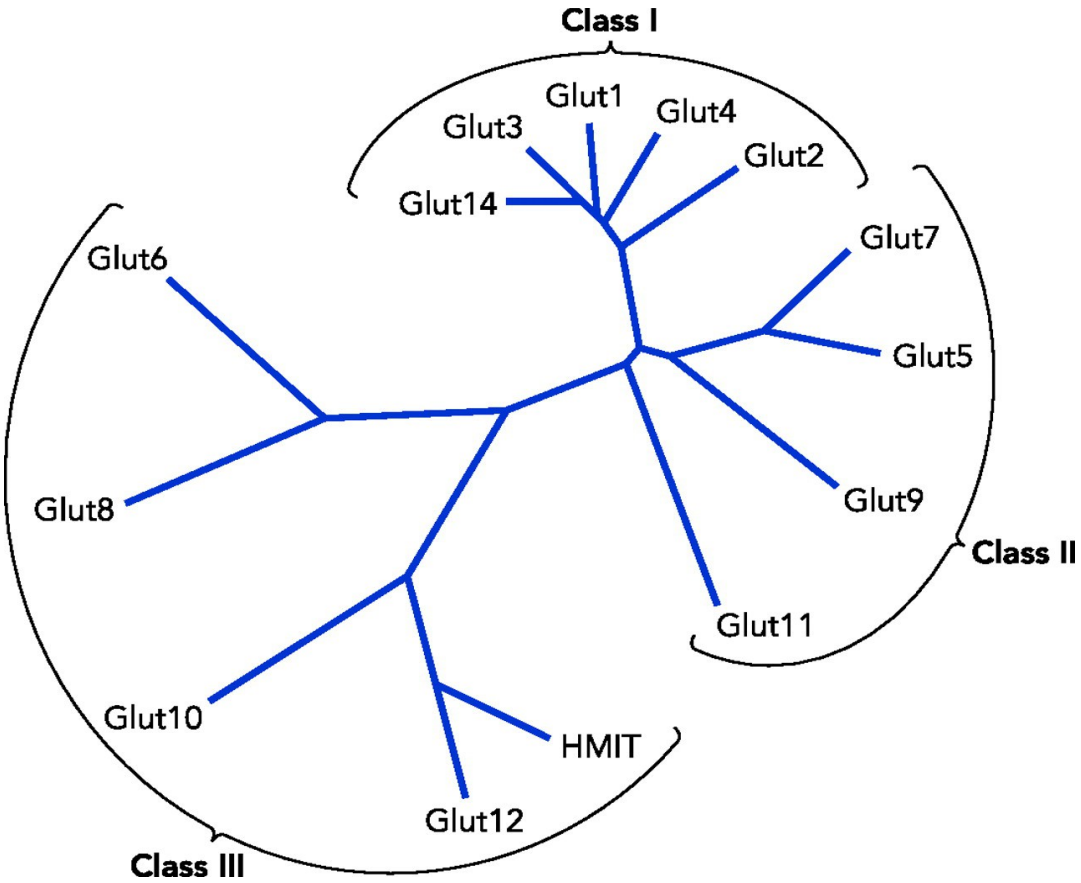

Figure 4. Three classes of the GLUT transporters (Manolescu A. R. *et al.*, 2007).

Diets of many animals change with seasons, and carbohydrate levels vary accordingly. But in comparison with dietary regulation of intestinal glucose transport in mammals, fish and amphibians, intestinal transport in birds does not change. This can be explained by the predominance of passive glucose transport, where glucose passes between cells, rather than through cells via active transport (paracellular pathway). However, such absorption is dependent on transepithelial concentration gradients (Ferraris R. P., 2001).

There are four most abundant transporters in the small intestine: SGLT-1, GLUT-1, GLUT-2 and GLUT-5 (Yoshikawa T. *et al.*, 2011).

1.3.2.1 Glucose transporter 1

Glucose transporter 1 (GLUT-1) is a high affinity glucose transporter with a Michaelis constant (Km) for glucose of 3-7 mM (Burant C. F., Bell G. I., 1992). Km value for GLUT proteins is the concentration of blood glucose at which transport into cell takes place at half its maximum rate. This Km value is below the normal blood glucose concentration, therefore the transporter functions at significant rate (close to maximal velocity) (Thorens B., 1996). GLUT-1 expression is mainly observed in erythrocytes and in the brain. In other tissues GLUT-1 expression level is decreased (Wood I. S., Trayhurn P., 2003). GLUT-1 is highly conserved among species. It has 12 putative hydrophobic transmembrane (TM) segments, which are separated by hydrophilic loops. N- and C-termini are located on the cytoplasmic side of the membrane. It has two large hydrophilic loops: one extracellular loop between TM domains 1 and 2, second cytoplasmic loop between TM domains 6 and 7 (Fig. 5) (Carruthers A. *et al.*, 2009).

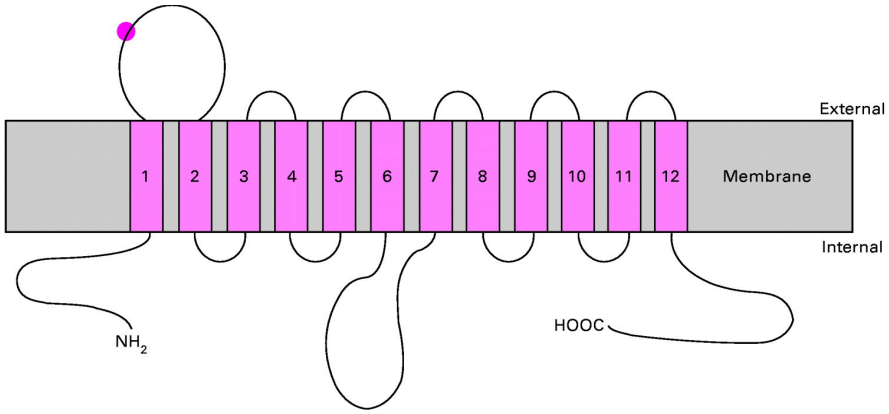

Figure 5. Schematic representation of Class I GLUT family members in the membrane (Tetaud E. *et al.*, 1997).

1.3.2.2 Glucose transporter 2

Glucose transporter 2 (GLUT-2) is a low affinity glucose transporter with uniquely high Km – 17 mM, but it is a high affinity glucosamine transporter with Km – 0,8 mM (Uldry M. *et al.*, 2002). It can also transport galactose, mannose and fructose with low affinity. GLUT-2 is highly expressed in hepatocytes, pancreatic beta cells, intestinal absorptive epithelial cells and basolateral surface of kidney (Mueckler., 1994). GLUT-2 has similar structure to GLUT-1 because they both belong to Class I glucose transporters (Fig. 5). The only difference is that QLS (Gln-Leu-Ser) motif in Helix7, which is highly conserved in GLUT-1, is not present in GLUT-2. QLS has high substrate specificity on the transporter and its lack can explain the high affinity for glucosamine. In GLUT-2 QLS motif is replaced by HVA (His-Val-Ala) motif (Seatter M. J. *et al.*, 1998; Zhao F.-Q., Keating A. F., 2007).

1.3.2.3 Glucose transporter 5

Glucose transporter 5 (GLUT-5) is a high affinity fructose transporter, which is expressed mainly in the small intestine and spermatozoa, and is also present in brain, kidney, adipocytes and muscle (Mueckler M., 1994). Km value for fructose for GLUT-5 is 6 mM. GLUT-5 exhibits no glucose transport activity in human, but has limited transport activity in rats (Augustin R., 2010).

1.4 Glucose transporters in gastrointestinal tract

Small intestine is the main site for absorption, where epithelial cells absorb glucose, galactose and fructose from the intestinal lumen and then export sugars into blood. Firstly, absorption is dependent on active transport in the apical brush border, then the absorbed glucose, galactose or fructose move out of enterocytes by passive transport (Thorens B., 1993).

SGLT-1 is expressed in the apical membrane of duodenum and ileum and transports glucose

and galactose by sodium cotransport (Mueckler M., 1994). First of all, dietary glucose gets across apical membrane of the enterocyte by SGLT-1, and then by GLUT-2 exits across the basolateral membrane. In addition to glucose, GLUT-2 transports fructose, but its concentration against a gradient is not important (Kellett G. L., Brot-Laroche E., 2005).

GLUT-5 is located at the brush border membrane of the enterocyte and expressed mainly in the jejunal region of the small intestine and in the terminal part of ileum (Augustin R., 2010). GLUT-1 is expressed in glandular stomach and in the small intestine where its concentration is insignificant (Yoshikawa T. *et al.*, 2011).

2. EXPERIMENTAL PART

2.1 Aim of the study

The aim of the study was to perform immunohistochemical (IHC) analysis of GLUT-1, GLUT-2, GLUT-5 glucose transporters in different parts of ostriches gastrointestinal tract: glandular part of proventriculus, duodenum and terminal zone of ileum.

2.2 Matherials and methods

2.2.1 Materials

Samples for routine histological and immunohistochemical (IHC) analyses were obtained from six ostriches of 30 days after hatching.

The Ethical Committee of Latvian University of Agriculture has approved the study as ostriches were raised in farms of Latvia.

2.2.2 Methods

Current study was performed with following methods of histological treatment of biological objects: sample fixation, paraffin-embedding, microtome cutting and staining of sample slides with hematoxylin and eosin. IHC was made by labeled streptavidin-biotin method (LSAB).

2.2.3 Tissue sample preparation

2.2.3.1 Fixation

All six samples of fresh tissues were removed from ostriches gastrointestinal tract (proventriculus, duodenum and ileum) and processed with 10% neutral buffered formalin

(NBF) in order to prevent tissue drying out, autolysis and infection with bacteria and fungi.

2.2.3.2 Embedding

In order to prevent distortion of the tissue during sectioning, the samples were paraffin-embedded and fixed in formaldehyde. Firstly, tissues were rinsed with PBS (Phosphate Buffer Saline) until fixative was completely removed. Then tissues were dehydrated, as paraffins are insoluble in water. Dehydration was reached by immersion of the sample in increasing concentrations of ethanol (alternatively, acetone can be used) 10 minutes in each:

- 50% ethanol for 10 min
- 70% ethanol for 10 min
- 80% ethanol for 10 min
- 95% ethanol for 10 min
- 100% ethanol for 10 min, (three times)

Afterwards, tissue samples were immersed in ethanol-xylene solution, and then in 100% xylene to remove residual ethanol:

- 2:1 ethanol:xylene for 10-15 min
- 1:1 ethanol:xylene for 10-15 min
- 1:2 ethanol:xylene for 10-15 min
- 100% xylene for 10-15 min (three times)

Finally, fixed and dehydrated tissue samples were placed in paraffin at a temperature of 60^0C:

- 2:1 xylene:paraffin for 30 min
- 1:1 xylene:paraffin for 30 min
- 1:2 xylene:paraffin for 30 min
- 100% paraffin for 1-2 h
- 100% paraffin for 24 h

After hardening into solid blocks of wax the samples were ready for microtome cutting.

2.2.3.3 Microtome cutting

Sections were trimmed into the frustum form and sliced with microtome at 7 μm. Thin sections of the samples were placed on microscope slides.

2.2.3.4 Deparaffinization and rehydration

Poor staining of the sections may be caused by paraffin. Therefore, before staining the slides were deparaffinized in xylene and rehydrated in ethanol:

- xylene for 5 min (three washes)
- 100% ethanol for 10 min (two washes)
- 95% ethanol for 10 min (two washes)
- dH_2O for 5 min (two washes)

2.2.3.5 Endogenous peroxidase activity block

In order to avoid non-specific background staining, the tissues were treated with hydrogen peroxidase:

- treat with 3% H_2O_2 for 10 min
- wash in buffer (two times)

In the present study to dilute hydrogen peroxidase TBS was used.

2.2.3.6 Antigen retrieval

In this study formalin-fixed tissues were used that mostly require retrieval step before IHC staining. It is necessary because of methylene bridges formation in fixation stage, which cross-links proteins and therefore hides antigenic sites. In the present study heat-induced

epitope retrieval technique (HIER) was used. Slides were immersed in 10 mM Tris/1 mM EDTA pH 9.0 buffer, heated that causes the protein cross-links to break and open the original protein structure:

- hold at sub-boiling temperature for 18 min
- cool at room temperature (RT) for 30 min
- wash slide in buffer (three times)

2.2.4 Tissue sample staining

2.2.4.1 Immunostaining

2.2.4.1.1 Epitope blocking

In order to prevent the nonspecific binding of the antibodies all epitopes on tissue sample should be blocked. Protein Block was used for 5 minutes on samples at RT, thereafter the sections were washed in a buffer.

2.2.4.1.2 Antibody applying

After washing antibodies are applied. Polyclonal rabbit antibodies Rabbit anti-GLUT-1, Rabbit anti-GLUT-2 and Rabbit anti-GLUT-5 (Abcam, U.K.) served as the primary antibodies. As the secondary antibody Biotinylated Goat Anti-Polyvalent was used, provided by the manufacturer in Immunohistochemistry kit (Abcam, U.K.):

- primary antibody (dilution 1:1000) (diluted in TBS with 1% BSA (Bovine Serum Albumin)) apply in moisture chamber at $+37^0$C degrees for 30 min
- wash in buffer (four times)
- secondary antibody (ready-to-use antibody by manufacturer) apply at RT for 10 min
- wash in buffer (four times)

2.2.4.1.3 Avidin-biotin method

Avidin and streptavidin are tetrameric proteins with a very high affinity for biotin, which can be conjugated to antibodies.

There are two avidin-biotin methods: the avidin-biotin complex (ABC) method and the labeled streptavidin-biotin (LSAB) method (Fig. 6). They are tehnically similar:

1. unlabeled primary antibody,
2. biotinylated secondary antibody,
3. complex of avidin-biotin peroxidase or enzyme-streptavidin.

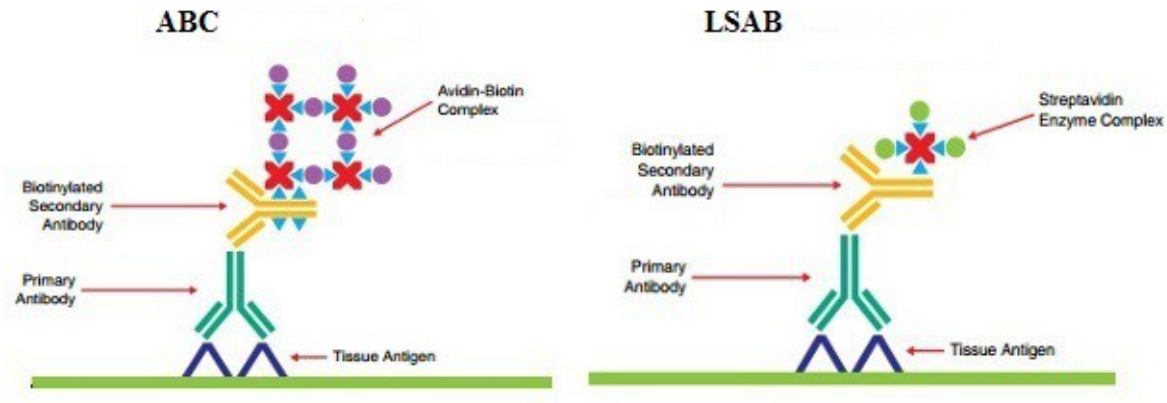

Figure 6. Avidin-biotin methods: avidin-biotin complex (ABC) and labeled streptavidin-biotin (LSAB) methods (Petersen K., Pedersen H. C., 2013).

In LSAB method (unlike in ABC) the enzyme reporter is directly conjugated to streptavidin. Epitopes are more easily tagged, because the formed complexes are smaller. Streptavidin is less-prone to nonspecific binding, because it is not glycosylated like avidin (Саркисов Д. С., Перов Ю. Л., 1996).

In this study biotinylated secondary antibody and streptavidin-conjugated peroxidase were used for detection using DAB (3,3'-diaminobenzidine) as chromogen which gives the brown

coloration for positively stained cells:

- streptavidin peroxidase apply at RT for 10 min
- wash in buffer (four times)
- 20µl DAB chromogen add to 1 ml of DAB substrate, mix and apply on tissue for 10 min
- wash in buffer (four times)

2.2.4.2 Hematoxylin and eosin staining

To make cells microscopically visible, tissue samples were stained with hematoxylin and eosin (H&E). Hematoxylin acts as a basic dye with purplish blue colour. Eosin acts as an acid dye and structures appear in red-pink (Gamble M., 2008).

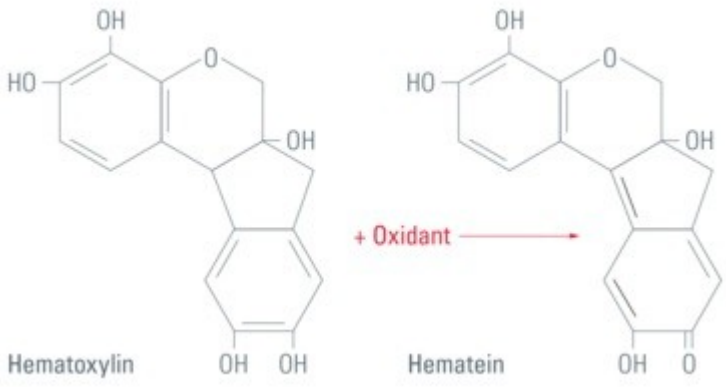

Figure 7. Hematoxylin oxidation (Myers R., 2011).

Hematoxylin does not bind to the tissues, but it can be oxidized to form hematein (Fig. 7). The latter does bind and gives purple colour to tissues because of quinoid ring structure (Exbrayat J.-M., 2013).

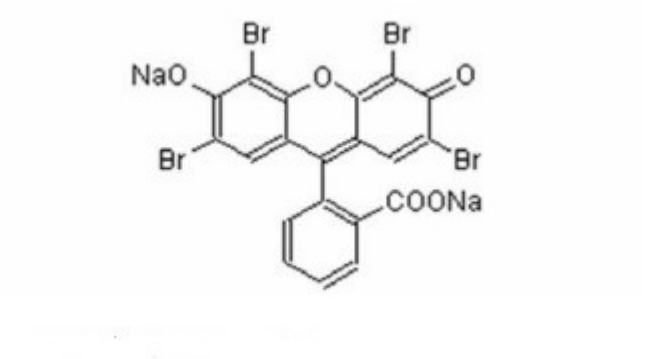

Figure 8. Eosin Y (eosin yellowish). Tetrabromo derivate of fluorescein. The most often used eosin (of two eosin forms) (Gill G. W., 2010).

Staining protocol:

1. immerse in Harris hematoxylin for 5 min
2. wash in running tap water for 5-10 min
3. rinse with dH_2O
4. differentatiate with acid alcohol (0,3%) for 1-3 sec
5. rinse with water
6. immerse in eosin for 1-2 min
7. rinse with water
8. dehydrate in alcohol (95% and 2x100%) for 2 min each
9. clear with xylene for 1-2 min
10. mount with Canada Balsam (1-2 drops)
11. cover with cover slides

Identical tissue samples were used for negative control, which did not contain primary antibodies.

The stained sections were examined with a light computer microscope (Leica DM 2500). Photomicrographs were taken by digital camera (Leica DFC 320R) attached to the microscope.

2.3 Results and discussion

2.3.1 Hematoxylin-eosin method

The histological structure of ostriches proventriculus, duodenum and ileum was studied by H&E method. In all observed parts of gastrointestinal tract 3 layers were noticed: *tunica mucosa*, *tunica muscularis* and *tunica serosa*.

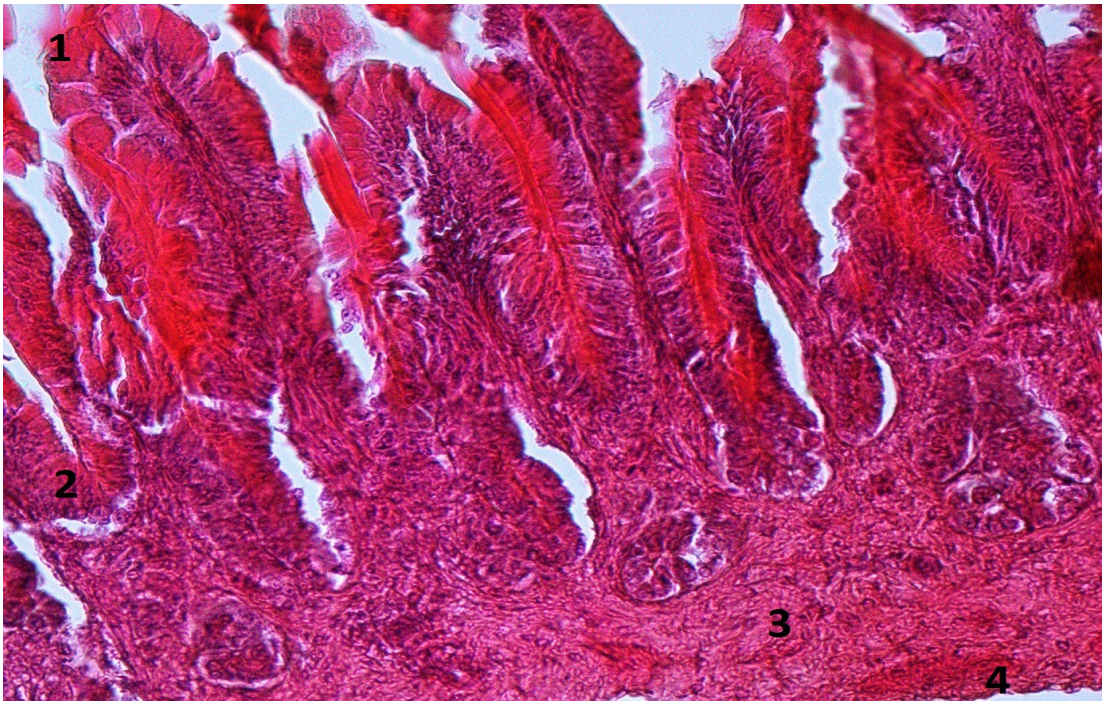

Figure 9. Superficial epithelium of *tunica mucosa (T. mucosa)* of 30 days old ostriches proventriculus (H&E, 200x): *epithelium superficiale* (1), *lamina propria s. glandularis mucosae* (2), *lamina muscularis mucosae* (3), *tela submucosa* (4).

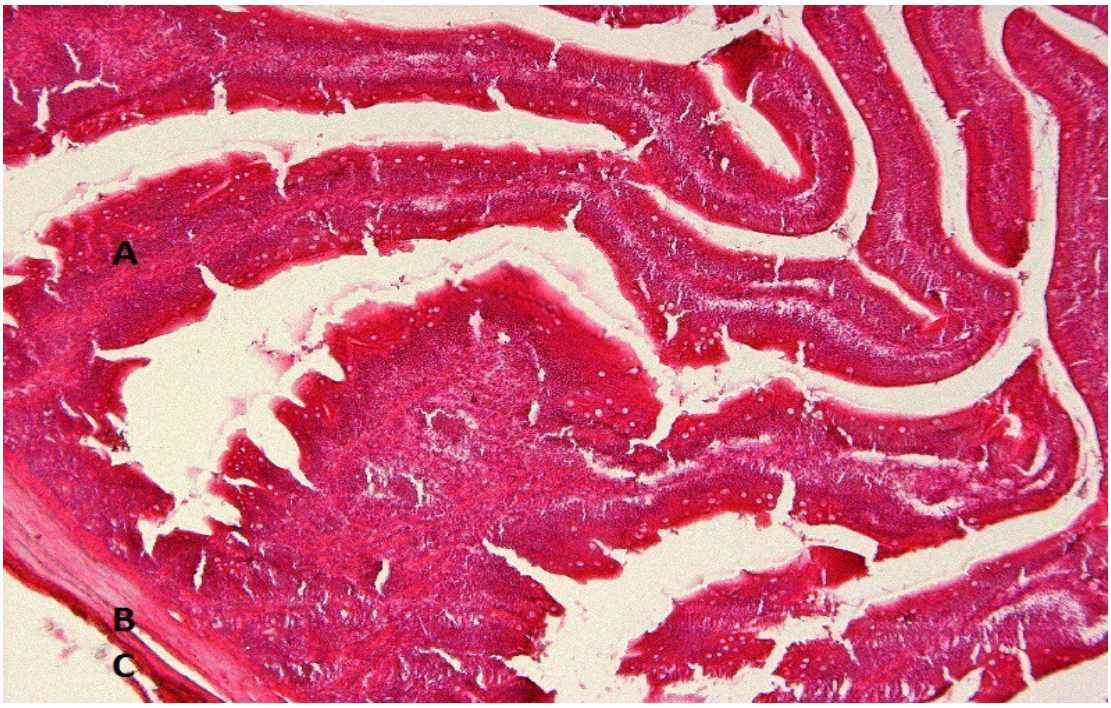

Figure 10a. *Tunica mucosa* (A), *tunica muscularis* (B), *tunica serosa* (C) of 30 days old ostriches duodenum (H&E, 100x).

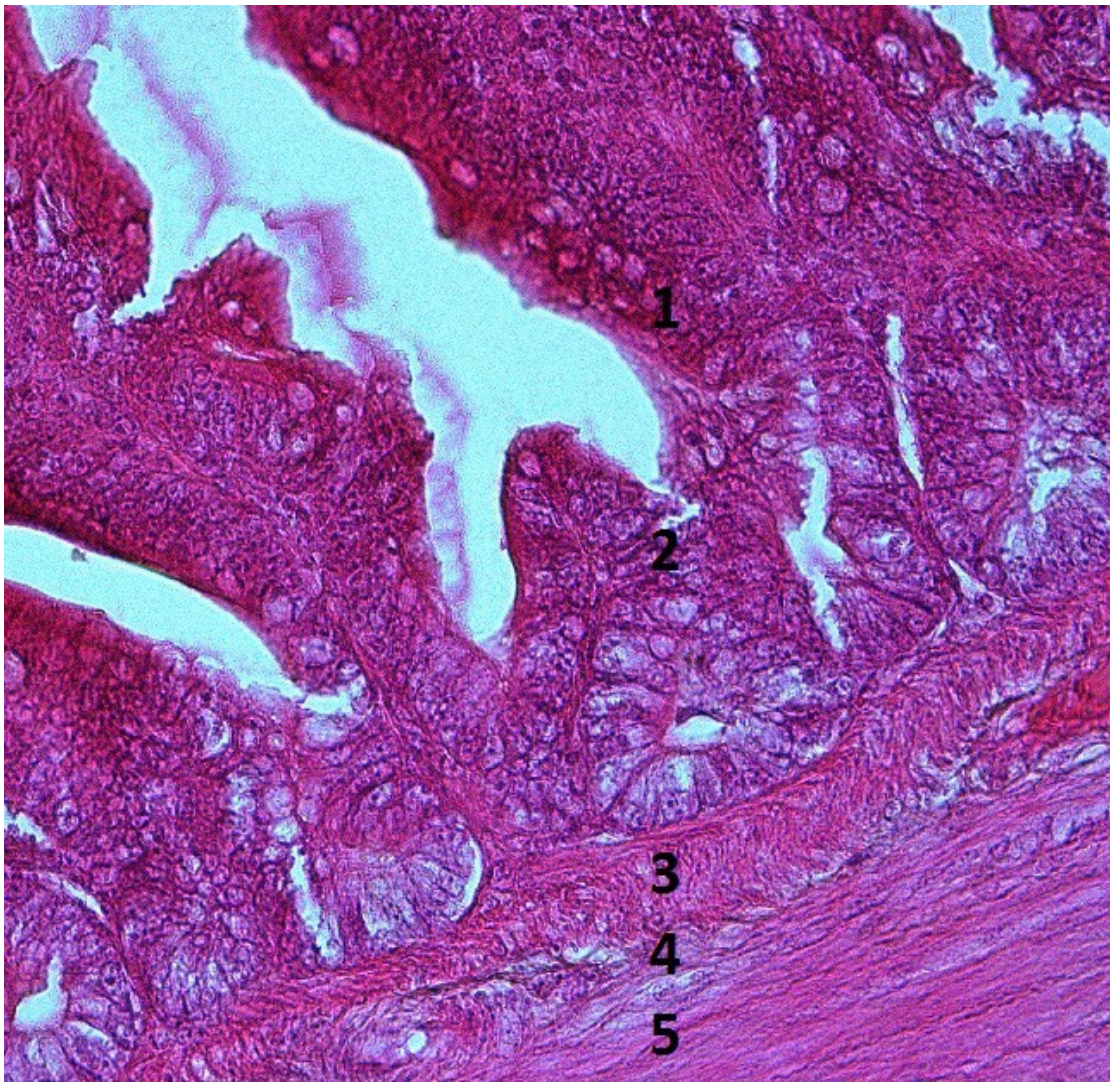

Figure 10b. *Tunica mucosa* and *tunica muscularis* of 30 days old ostriches duodenum (H&E, 400x). *T. mucosa*: *epithelium superficiale* (1), *lamina propria s. glandularis mucosae* (2), *lamina muscularis mucosae* (3), *tela submucosa* (4). *T. Muscularis: stratum circularis* (5).

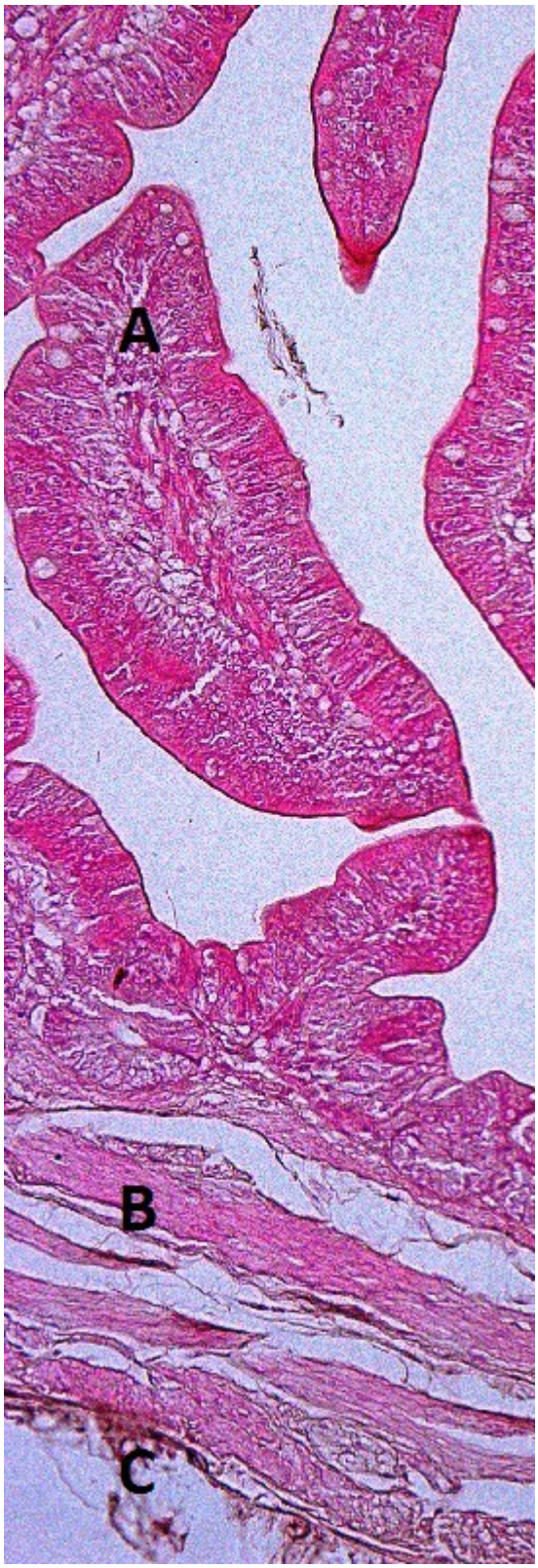

Figure 11. *Tunica mucosa* (A), *tunica muscularis* (B), *tunica serosa* (C) of 30 days old ostriches ileum (H&E, 200x).

2.3.2 Immunohistochemistry

2.3.2.1 Immunohistochemistry staining for glucose transporter 1

Strong positive staining for GLUT-1 was detected in the epithelial cells of terminal zone of ileum (Fig. 12), in comparison with duodenum and proventriculus (Fig. 13 and Fig. 14), where epithelial cells were stained weaker. Goblet cells in ileum and duodenum were unstained.

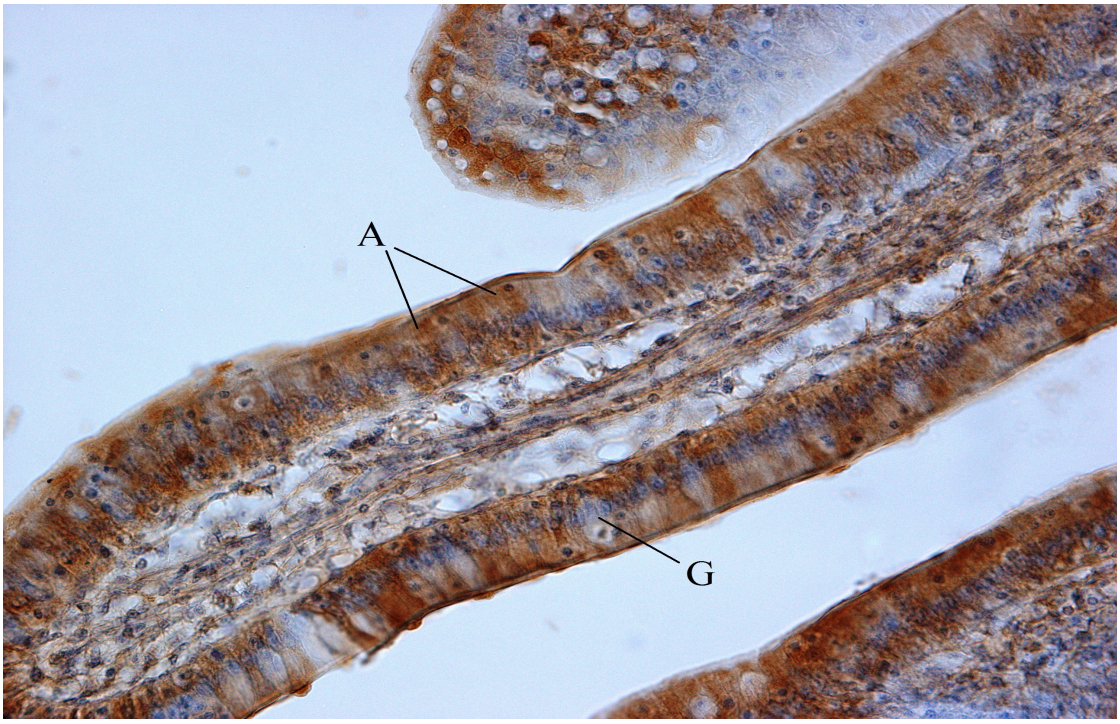

Figure 12. Photomicrograph of IHC showing strongly positive staining for GLUT-1 in 30 days old ostriches absortive cells of ileal mucosa (400x). Absorptive cells marked with A, Goblet cell – G.

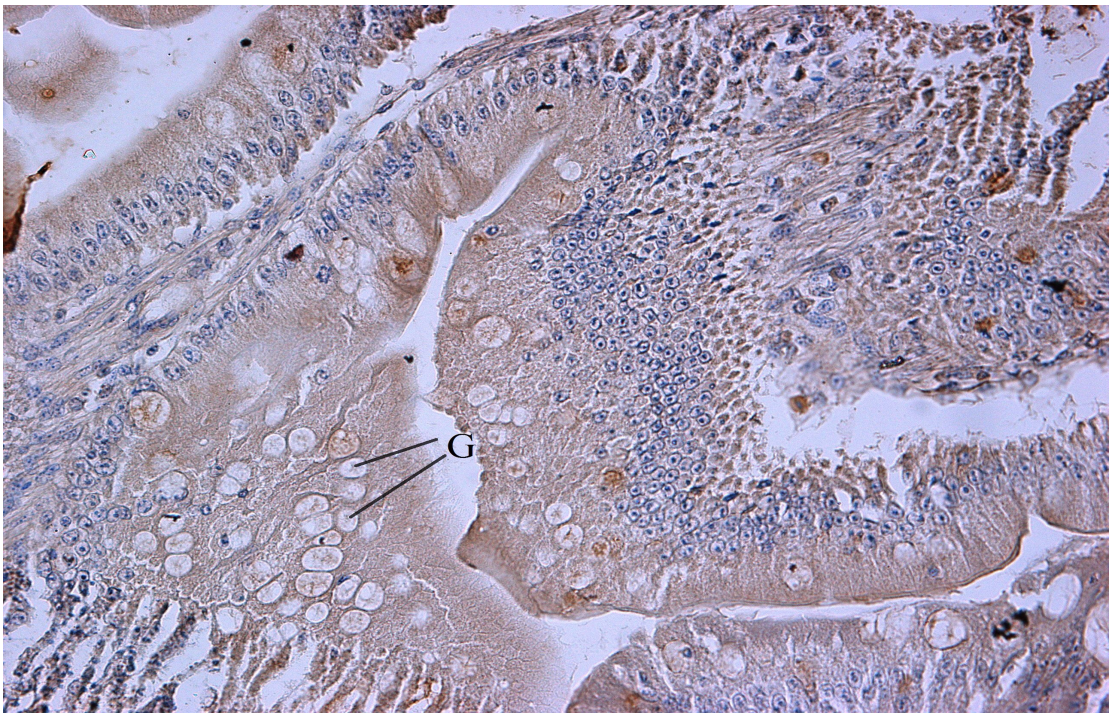

Figure 13. Photomicrograph of IHC showing weak staining for GLUT-1 in 30 days old ostriches duodenum (400x). Goblet cells – G.

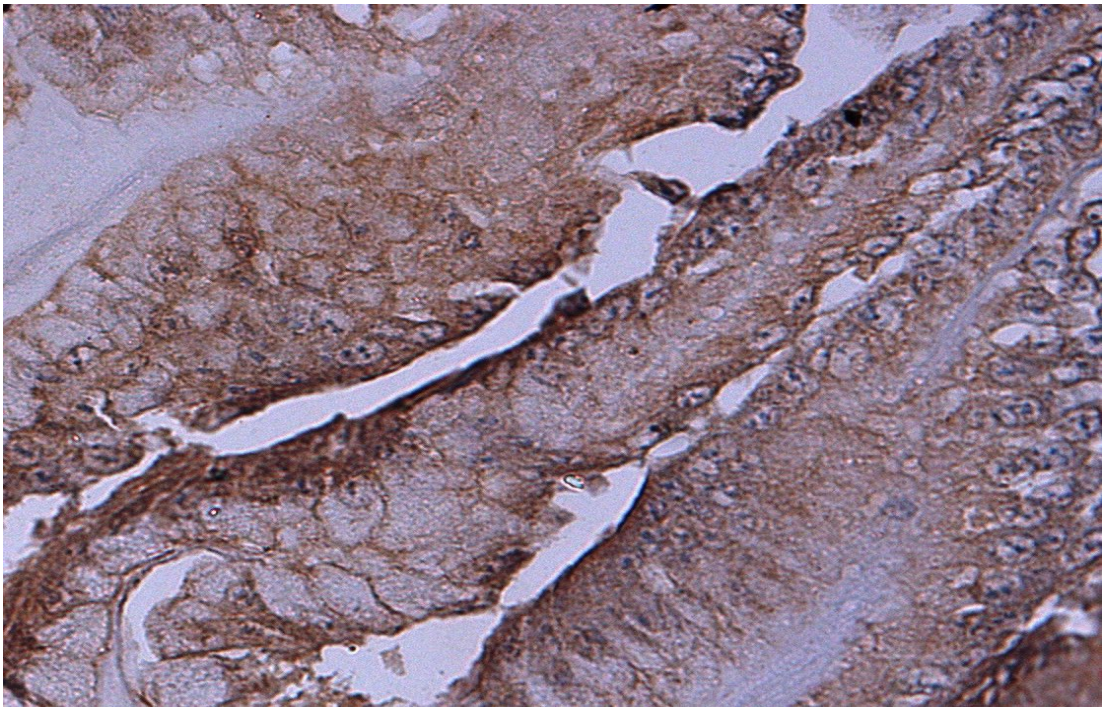

Figure 14. Photomicrograph of IHC showing weak staining for GLUT-1 in 30 days old ostriches proventriculus (400x).

2.3.2.2 Immunohistochemistry staining for glucose transporter 2

Brush border membranes of enterocytes and also Goblet cells in duodenum and ileum stained strongly positive (Fig. 15 and Fig. 16). In proventriculus epithelial cells and glandular cells in the bottom of the crypts stained also strongly positive (Fig. 17).

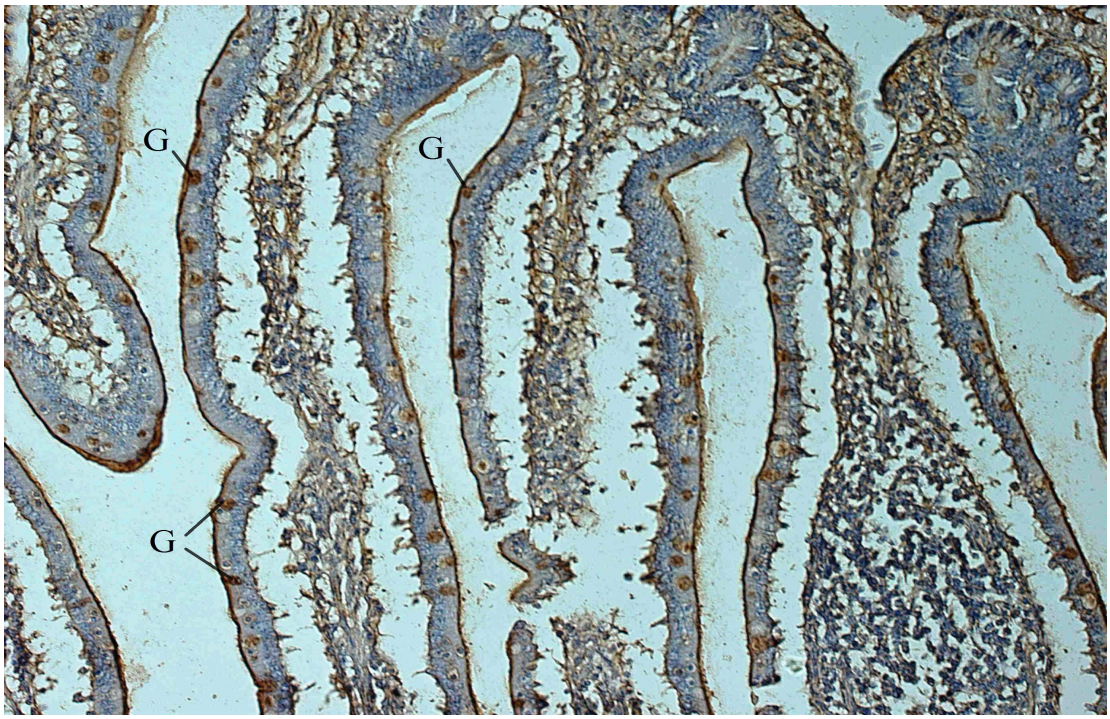

Figure 15. Photomicrograph of IHC showing staining for GLUT-2 in 30 days old ostriches ileal mucosa (200x). Goblet cells – G.

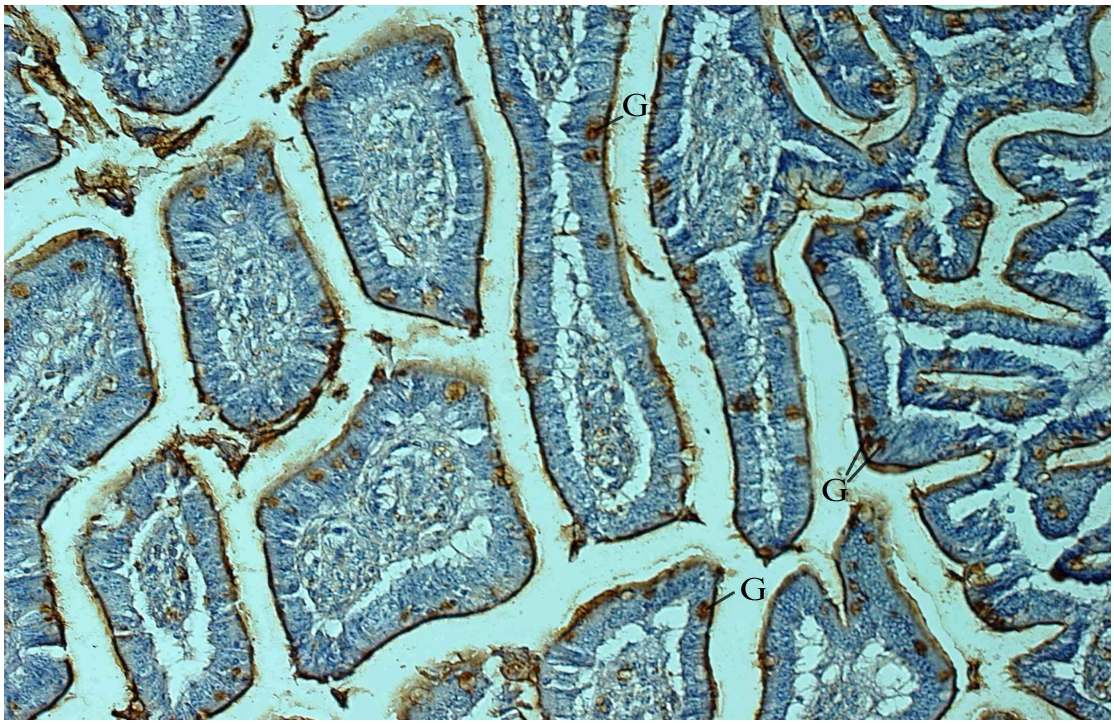

Figure 16. Photomicrograph of IHC showing staining for GLUT-2 in 30 days old ostriches duodenal epithelium (200x). Goblet cells – G.

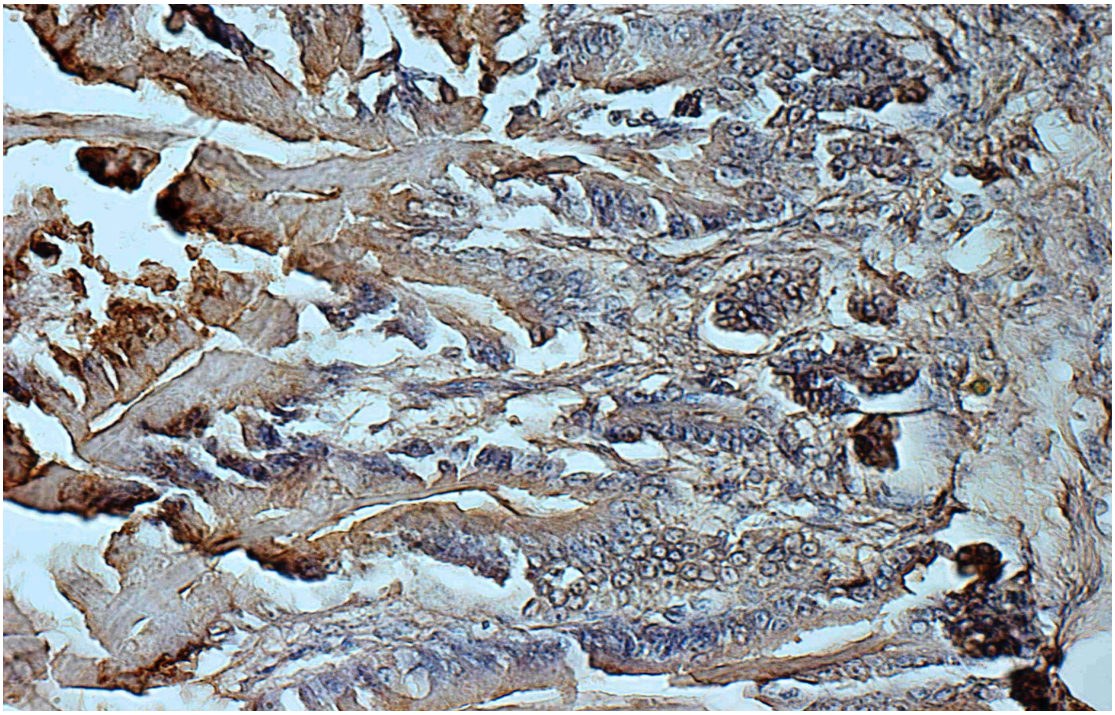

Figure 17. Photomicrograph of IHC showing staining for GLUT-2 in 30 days old ostriches proventriculus (400x).

2.3.2.3 Immunohistochemistry staining for glucose transporter 5

In 30 days old ostriches ileal epithelium the brush border membranes and Goblet cells stained moderately for GLUT-5 (Fig. 18). Goblet cells and brush border membranes of duodenal mucosa stained strongly positive (Fig. 19), and the epithelial cells of the glandular proventriculus stained strongly positive (Fig. 20) for GLUT-5.

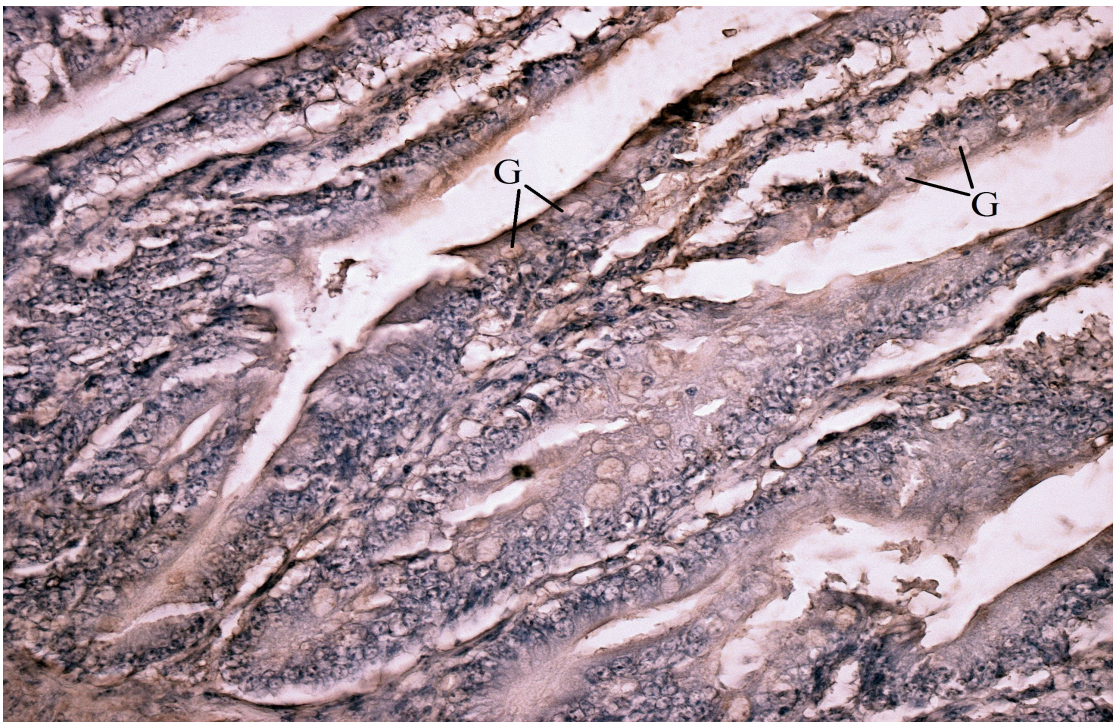

Figure18. Photomicrograph of IHC showing staining for GLUT-5 in 30 days old ostriches ileal epithelium (400x) Goblet cells – G.

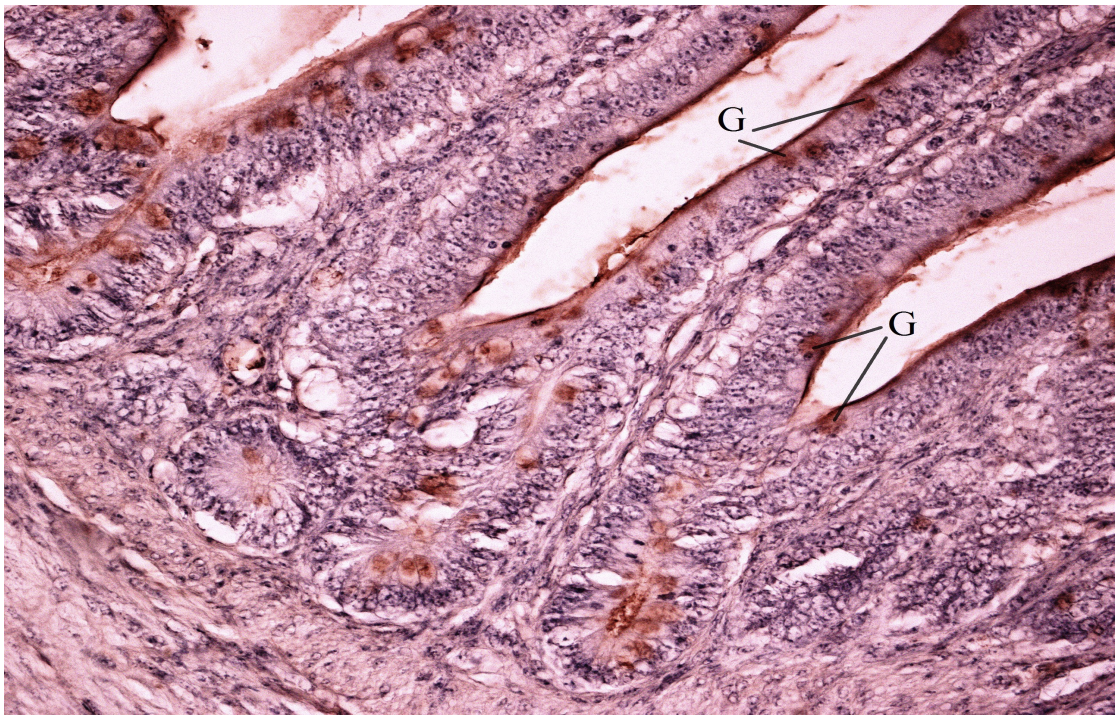

Figure 19. Photomicrograph of IHC showing staining for GLUT-5 in 30 days old ostriches duodenal epithelium (400x). Goblet cells – G.

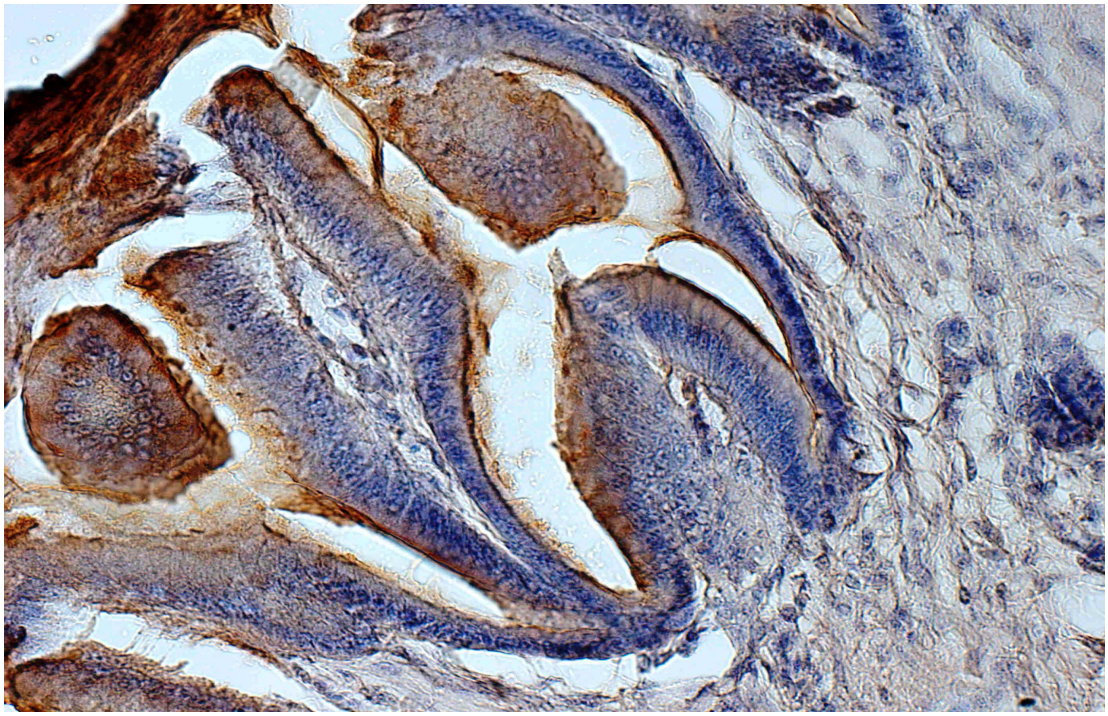

Figure 20. Photomicrograph of IHC showing staining for GLUT-5 in 30 days old ostriches proventriculus (400x).

In this study high affinity GLUT-1, low affinity GLUT-2 and fructose transporter GLUT-5 were used. GLUT-1 are found in almost every tissue, but their expression is higher in cells with high glycolytic activity. GLUT-2 – in tissues carrying large glucose fluxes. As a response to variations in metabolic conditions, the expression of these transporters is regulated by glucose and different hormones. Thus, because of their specific characteristics and regulated expression, the facilitated glucose transporters control fundamental aspects of glucose homeostasis (Thorens B., 1996). In this study stronger positive staining for GLUT-1 was detected in ostriches superficial gland zone of proventriculus. GLUT-2 is up-regulated at the brush border membrane, enhancing the capacity of glucose transport. In the present experiments brush border membranes and Goblet cells in the epithelium of the terminal zone of ileum were stained strongly positive for GLUT-2 that refers to the passive transport of glucose in this area. GLUT-5 transport fructose from the intestinal lumen into the enterocyte by facilitated diffusion due to high concentration of fructose in the intestinal lumen. In this study GLUT-5 was detected in the epithelial cells of proventriculus, in the brush border of enterocytes as well as in the Goblet cells in the ostriches small intestine indicating to the similar location of the glucose transporter as in small intestine of mammals.

Glucose transporters localization information is a prerequisite for understanding transepithelial transport of sugars, which is very important, considering that carbohydrates are the main energy source of food.

CONCLUSION

Glucose transporters play a crucial role in the transport of glucose across epithelial cell layers. Up to now there was a little information about the localization of glucose transporters in birds gastrointestinal tract.

In mammals regulation of intestinal glucose transport is active, whereas glucose transport in birds occurs predominantely by passive regulation.

The facilitated-diffusion GLUT family consist of 14 members divided into three major classes of which the most abundant transporters in gastrointestinal tract are GLUT-1, GLUT-2 and GLUT-5.

Each of them has a specific localization and expression, therefore specific role in glucose handling in different cell types to regulate metabolism.

GLUT-1 is a high affinity glucose transporter expressed in almost every tissue. GLUT-2 is a low affinity glucose transporter, which is found in tissues carrying large glucose fluxes such as intestine, kidney and liver. GLUT-5 is the main fructose transporter highly expressed in small intestine and spermatozoa.

Glucose transport from the lumen to the interstitial space is carried in two steps: foremost there is active glucose and galactose absorption through the apical brush border by a Na^+-coupled glucose transporter SGLT-1, whereas uptake of fructose is fulfilled by diffusive-facilitator GLUT-5. Then diffusion of glucose, galactose and fructose across the basolateral membrane is catalysed by GLUT-2.

In the present study facilitated-diffusion glucose transporter family members GLUT-1, GLUT-

2 and GLUT-5 were detected in different parts of ostriches gastrointestinal tract using immunohistochemistry method.

Positive staining for GLUT-1 was stronger in ostriches superficial gland zone of proventriculus and in epithelial cells of the terminal zone of ileum and in epithelial cells of duodenum. Besides proventriculus, brush border membranes of small intestine were positively stained for GLUT-2. GLUT-5 was found in the epithelial cells of proventriculus, in the brush border of enterocytes and in the Goblet cells of the small intestine. Compared to GLUT-2 the terminal zone of ileum was more intensively stained by GLUT-5.

The present investigation provided information concerning the localization of GLUT-1, GLUT-2 and GLUT-5 in ostriches gastrointestinal tract, which is a prerequisite for understanding transepithelial transport of sugars.

Glükoosi transporterid jaanalinnu seedetraktis

Aleksandra Rotmistrova

KOKKUVÕTE

Jaanalind *(Struthio camelus var. Domesticus)* on suurim lind, jaanalinnuliste seltsi ainus liik. Kuna jaanalinnud on väga kohanemisvõimelised linnud, on jaanalindude kasvatamine väljunud nende päriskodust Aafrikast üle maailma. Jaanalinnufarme paikneb üle maailma vähemalt 70-nes erinevas riigis alates külma kliimaga Alaskast kuni Kesk-Aafrika ekvatoriaalpiirkondadeni välja.

Jaanalindude kasvatamine on maailmas väga aktuaalne teema peamiselt rikkalike põllumajandussaaduste tõttu: jaanalinnu liha on hinnatud just madalaima rasva ja kolesterooli sisalduse tõttu. Jaanalinnurasval on mitmekülgne põletikuvastane toime ja nahka peetakse tugevuselt teiseks maailmas. Jaanalindudel on oluline roll ka meditsiinis: jaanalindude sarvkesta ja kõõluseid kasutatakse implantatsioonis, aju uuringud on seotud Alzheimer'i tõve raviga.

Jaanalindude kasvatamisel on aga märgatud probleemi nende elulemisega esimesel elukuul. Farmerite andmetel hukkub jaanalinnufarmides 46% vastkoorunud jaanalindudest esimese elukuu jooksul. Samas teaduslikke uurimustöid jaanalindude erinevate organsüsteemide, sealhulgas seedesüsteemi, arengu kohta vastaval perioodil on vähe. Võib oletada, et jaanalindude kasvatamisel on suur tähtsus õigel söötmisel, kus sahhariidid e. karbohüdraadid s.h. glükoos on peamiseks energia allikaks.

Glükoosi transportimisel üle seedeepiteeli osalevad kaks glükoositransporterite klassi: Na^+/D-sõltuv (SGLT1) ja lihtsustatud-diffusiooniga (GLUT). Kirjanduse järgi glükoosi transport

lindudel toimub peamiselt passivselt, milles osalevad GLUT-1, GLUT-2 ja GLUT-5.

Kuna glükoosi transporterite lokalisatsiooni kohta jaanalindude seedetrakti epiteelis praktiliselt andmed puuduvad, oli käesoleva uurimistöö eesmärgiks, kasutades immunohistokeemilist uurimismeetodit, määrata GLUT-1, GLUT-2 ning GLUT-5 lokalisatsioon jaanalindude seedetrakti epiteelis.

Uuringute tulemusena leiti epiteelirakkude positiivne värvimine GLUT-1 suhtes eesmaos, niude- ja kaksteistsõrmiksooles. GLUT-2 tugev positiivne värvumine ilmnes eesmao epiteelirakkudes ja peensoole epiteelirakkude mikrohattudes; GLUT-5 – eesmao epiteelrakkudes ja enterotsüütide mikrohattudes ning karikrakkudes.

Saadud katsete tulemused annavad olulist informatsiooni sahhariidide transepiteliaalse transpordi kohta jaanalindude seedetraktis.

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SUPPLEMENTS

PROTOCOL 1

Protocol for Mouse and Rabbit Specific HRP/DAB (ABC) detection IHC kit

Staining protocol

Deparaffinize and rehydrate formalin-fixed paraffin-embedded tissue sections:

a. incubate sections in three washes of xylene for 5 min each,

b. incubate sections in two washes of 100% ethanol for 10 min each,

c. incubate sections in two washes of 95% ethanol for 10 min each,

d. wash sections twice in dH_2O for 5 min each.

Add enough drops of Hydrogen peroxide Block to cover the sections. Incubate for 10 min.

Wash 2 times in buffer.

Perform appropriate pretreatment:

a. add the appropriate antigen retrieval buffer (10 mM Tris/1 mM EDTA pH 9.0) to the microwaveable vessel with slides,

b. place the vessel inside the microwave, maintain at a sub-boiling temperature for 18 min,

c. cool at room temperature for 30 min,

d. wash slide 3 times in buffer.

Apply Protein Block and incubate for 5 minutes at room temperature to block nonspecific backround staining. Wash 1 time in buffer.

Apply primary antibody and incubate for 30 min in moisture chamber at +37°C.

Wash 4 times in buffer. Apply Anti-Mouse and Rabbit and incubate for 10 min at room

temperature. Wash 4 times in buffer.

Apply Streptavidin Peroxidase and incubate for 10 min at room temperature.

Rinse 4 times in buffer. Add 20µl DAB Chromogen to 1 ml of DAB Substrate, mix by swirling and apply to tissues. Incubate for 10 min. Rinse 4 times in buffer.

Add enough drops of Hematoxylin to cover the section. Incubate for 1 min.

Rinse 7-8 times in tap water **. Add Mounting Medium to cover the section.

** If permanent slides are desired, immerse slides in dH_2O two times for 5 min, dehydrate sections as described in Hematoxylin-eosin staining protocol and thereafter mount coverslips.

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