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Immunohistochemical Study of Hexose Transporters GLUT-2 and GLUT-5 in Birds Gastrointestinal Tract

Piret Hussar[1], Florina Popovska-Percinic[2], Tõnu Järveots[3], Ilmārs Dūrītis[4], Martin Kärner[5], Katerina Blagoevska[2] and Lazo Pendovski[2]

1. Institute of Anatomy, University of Tartu, Tartu 50411, Estonia

2. Faculty of Veterinary Medicine, Ss. Cyril & Methodius University in Skopje, Skopje 1000, Republic of Macedonia

3. Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu 51014, Estonia

4. Faculty of Veterinary Medicine, Latvian University of Agriculture, Jelgava LV-3004, Latvia

5. Faculty of Science and Technology, University of Tartu, Tartu 51014, Estonia

Abstract: The hexoses glucose, galactose and fructose serve as important dietary energy sources in animals and glucose plays a central role in energy homeostasis within eucaryotic cells. As relatively little is known about patterns of hexose transporters expression in birds gastrointestinal tract, the aim of the study was to examine glucose transporters 2 (GLUT-2) and glucose transporters 5 (GLUT-5) expression in stomach and duodenal epithelium of two different species—broilers (*Gallus gallus domesticus*) and ostriches (S*truthio camelus* var. *domesticus*) chicken. Materials from the two parts of gastrointestinal tract were collected from six 7-day-old female broilers and six 7-day-old female ostriches. Specimens were fixed with 10% formalin, embedded into paraffin, cut into 7 μm thick slices, followed by immunohistochemical staining with polyclonal primary antibodies rabbit anti-GLUT-2 and rabbit anti-GLUT-5; the staining was carried out according to the manufacturers guidelines (IHC kit, Abcam, UK). The investigation showed that the staining for both antibodies was more intensive in the epithelial cells of stomach and duodenum of the 7-day-old broilers. In duodenal epithelium, goblet cells and brush border membranes were stained in both species, however the signal was stronger for GLUT-5 than GLUT-2. Staining for GLUT-2 and GLUT-5 occurred in different parts of gastrointestinal tract of 7-day-old ostriches, but was weaker compared to 7-day-old broilers, which showed that the gastrointestinal tract of 7-day-old female broilers was more developed for transportation of hexoses than 7-day-old female ostriches.

Key words: Birds gastrointestinal tract, GLUT-2, GLUT-5, hexose.

1. Introduction

In birds, the stomach is comprised of two anatomically and functionally different parts—the glandular part (*pars glandularis*, *s. proventriculus*) where gastric juice secretion takes place, and the muscular part (*pars muscularis*, *s. ventriculus*) where ingested food is subjected to mechanical action and chemical breakdown of nutrients continues. However, in 7-day-old ostriches chicken, the mucosa of the proventriculus (*tunica mucosa proventriculi*) is lined with a single columnar epithelial cells, which bending into the lamina propria of the mucosa (*lamina propria mucosae*) forms simple branched tubular glands known as superficial proventricular glands (*gll. proventriculares superficiales*). However, in 7-day-old hen chicken, the glands remain unbranched [1].

Bezuidenhout and Van Aswegen [2] and Illanes et al. [3] in their research about African ostrich stomach mucosa have found out that the structure of superficial glands varies from simple to branched tubular glands. On the other hand, in most species of birds, including broilers, the structure of the superficial proventricular glands is simple tubular without branching [4-7]. Moreover, comparing the intestinal crypts of the two birds species, according to the literature, the deepest

Corresponding author: Piret Hussar, Ph.D., research fields: human and veterinary histology, embryology and cytology. E-mail: piretut@gmail.com; piret.hussar@ut.ee.

intestinal crypts in the ostrich chicks between day 1 and day 45 of life are found in the ileum [8]; while in the chicks of *Gallus gallus domesticus*, the deepest crypts over the first day of life are observable in the duodenum [9].

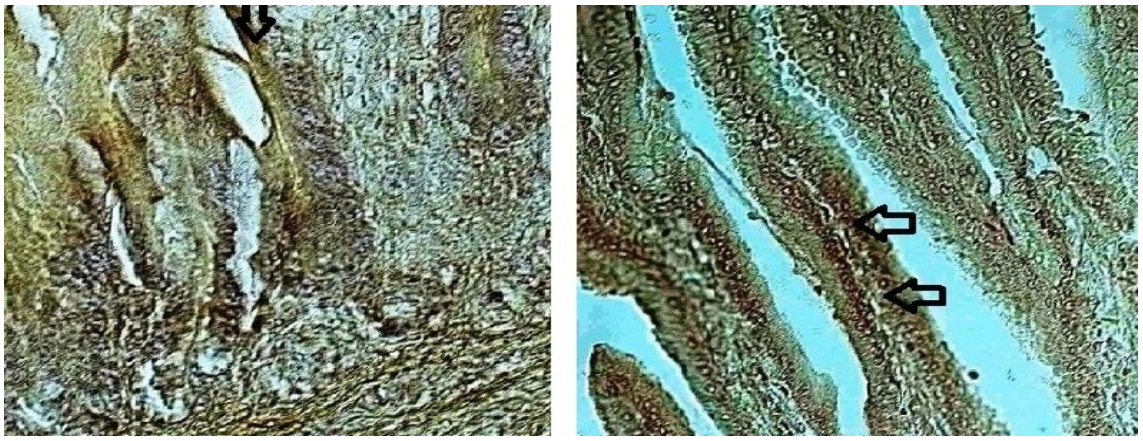
Glucose transporter (GLUT) proteins play a key role in the transport of monosaccharides across cellular membranes, blood sugar regulation and tissue metabolism [10]. Although the patterns of GLUT expression have been well characterized in mammals, relatively little is known about patterns of GLUT expression in bird gastrointestinal tract. The aim of this study was to examine GLUT-2 and GLUT-5 expression in stomach and duodenal epithelium of two different species—broilers (*Gallus gallus domesticus*) and ostriches (S*truthio camelus* var. *domesticus*) chicken.

2. Materials and Methods

Materials from glandular stomach and duodenum were collected from six 7-day-old female broilers and six 7-day-old female ostriches. Specimens were fixed with 10% formalin, embedded into paraffin and then cut into 7 μm thick slices. The slices were deparaffinized with xylene and rehydrated in a graded series of ethanol. Endogenous peroxidase activity was blocked with 3% H_2O_2 and the sections were stained with immunohistochemistry kit (Abcam, UK), according to the manufacture guidelines. Polyclonal rabbit antibodies GLUT-2 and GLUT-5 served as primary antibodies (Abcam, UK). Biotinylated secondary antibody and streptavidin-conjugated peroxidase were used for detection, with 3,3' diaminobenzidine (DAB) as chromogen. Nuclei were counterstained with Harris hematoxylin. Negative controls contained antibody diluter (Dako, S0809) instead of primary antibodies. Rat liver tissue sections for identifying GLUT-2 and human kidney tissue sections for GLUT-5 were used as positive controls, which was available for comparision on Abcam antibody producer's homepage (http://www.abcam.com) as examples for the antibodies immunohistochemistry on paraffin-embedded tissues (IHC-P).

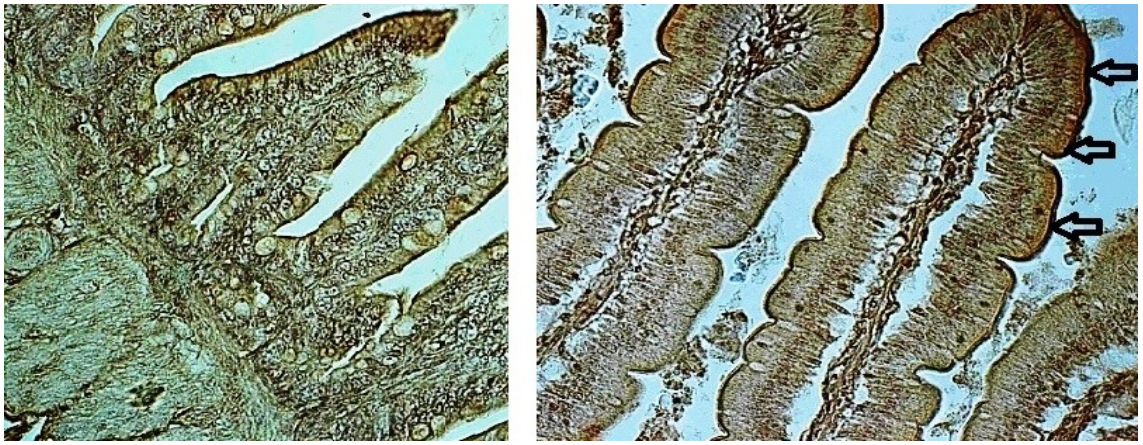
3. Results and Discussion

In both broilers and ostriches, the epithelial cells of the glandular stomach were stained weakly for GLUT-2 and GLUT-5 antibodies (Figs. 1a and 1b). In duodenal epithelium, some goblet cells and brush border membrane were stained in both species, but the signal was stronger for GLUT-5 than GLUT-2 (Figs. 2 and 3). Comparatively, the staining was more intensive in duodenal epithelial cells of 7-day-old broliers than ostriches, especially in brush border membranes of duodenal epithelial cells and some nuclei of the enterocytes (Fig. 3b).



(a) **(b)**

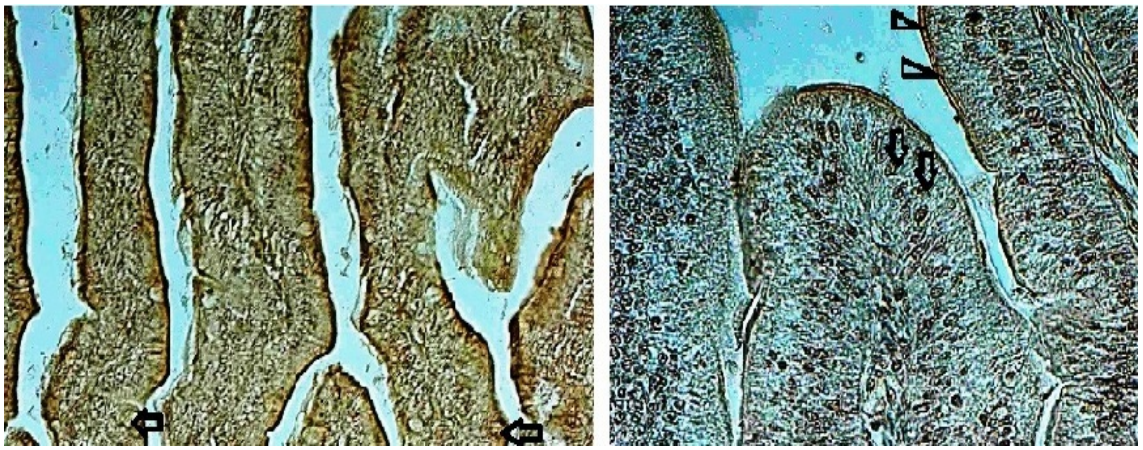
Fig. 1 Glandular stomach: (a) epithelial cells weakly stained for GLUT-2 in 7-day-old ostriches proventriculus, 200×; (b) epithelial cells weakly stained for GLUT-5 in 7-day-old broiler chickens, 200×.
Arrows pointing to the epithelial cells.



(a) (b)

Fig. 2 Staining for GLUT-2 in duodenal epithelium: (a) weakly stained duodenal epithelium of 7-day-old ostriches, 400×; (b) duodenal villi of 7-day-old broiler chickens, 400×.

Arrows show the brush border membranes strongly stained for GLUT-2.



(a) (b)

Fig. 3 Staining for GLUT-5 in duodenal epithelium: (a) duodenal epithelium of 7-day-old ostriches, 200×; (b) duodenal epithelium of 7-day-old broilers, 400×.

Arrows in Fig. 3a indicate goblet cells stained in spots for GLUT-5; arrows in Fig. 3b indicate some enterocyte nuclei and arrowheads show brush border membraines stained strongly for GLUT-5.

In the study, the expression of hexose transporters GLUT-2 and GLUT-5 in the epithelium of the glandular stomach and duodenum was comparatively investigated in two different species—broilers (*Gallus gallus domesticus*) and ostriches (S*truthio camelus* var. *domesticus*) at the same age.

The GLUT family consists of 14 members and is divided into three major classes, based on sequence homology and substrate selectivity [11-13]. GLUT-2, a low-affinity transporter which is capable of recognizing fructose, glucose and galactose, is involved in a bidirectional manner, mainly in fructose uptake in the basolateral membrane of the intestinal epithelial cells [14]. Moreover, fructose is transported passively across membranes by a member of the facilitative GLUT family, named GLUT-5 [15-19].

The vectorial transport of hexoses from the lumen to the interstitial space is a two-step process. The first uptake of glucose and galactose through the apical brush border is catalyzed by a Na+/D-glucose co-transporter (SGLT1), whereas uptake of fructose is catalysed by the GLUT5-fructose carrier. Secondarily, the diffusion of glucose, galactose and fructose in the intestinal tissue in close proximity to blood capillaries

is catalysed by GLUT-2 [20, 21].

Fructose is transported across the apical membrane by GLUT-5, for which it is highly specific in whole intestine [22]. Since plasma fructose is negligible in comparison with plasma glucose, only a facilitative transporter is necessary. In addition to regional expression patterns, GLUT-5 gene expression appears to be tightly regulated by developmental, nutritional, hormonal and circadian influences [13].

Once in the enterocyte, glucose crosses the basolateral membrane into the circulation, via the facilitative transporter GLUT-2. In contrast to GLUT-5, GLUT-2 transports both glucose and fructose, providing a common exit pathway from the enterocyte [23].

The present investigation showed that the staining for both antibodies was comparatively more intensive in the duodenal epithelial cells of the 7-day-old broilers than in the same region of 7-day-old ostriches chicken. These experimental results may indicate that the gastrointestinal tract of ostriches in the first week after hatching is not yet entirely able for transportation of carbohydrates [24].

4. Conclusions

The study provides valuable informations about the immunolocalization of GLUT-2 and GLUT-5 in 7-day-old broilers and ostriches gastrointestinal tract. When the staining for both antibodies in both species occurred to be stronger in duodenal epithelium than the epithelial cells of the glandular stomach, the duodenal epithelium of 7-day-old broilers was stained comparatively stronger than the epithlium of 7-day-old ostriches.

Based on results of these study, the authors concluded that the gastrointestinal tract of 7-day-old female broilers is more developed for transportation of hexoses than 7-day-old female ostriches.

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