DISTRIBUTION AND CHARACTERIZATION OF THE GOBLET CELLS IN THE OSTRICH SMALL INTESTINE DURING THE PRE-AND POSTHATCH PERIOD

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Abstract. The distribution of the goblet cells has been widely studied both in mammals and in birds, mainly hens; however, the functional studies of cells, especially in growing birds of different species are still topical. The aim of this research was to determine the density of the goblet cells in the mucosa of the ostrich small intestine pre hatch and during the first months of life as well as to differentiate the goblet cells by the chemical composition of mucopolysaccharides. In the research, 42 ostriches of both sexes raised in Latvia were used, including six embryos obtained on the 38th incubation day and 36 chicks of age 1, 3, 7, 14, 30 and 60 days post hatch, distributed in groups of 6 birds in each group. The length of each small intestine segment was measured (mm). Histological samples of tissue (0.5-1x1cm) were taken from the small intestine: the medium segments of the duodenum, jejunum and ileum.

For overall histological assessment, the tissue samples were deparaffinized, hydrated and stained with haematoxylin and eosin stain complying the standard methods. By applying the histochemical reactions, the the goblet cells were differentiated by the qualitative composition of mucopolysaccharides into cells containing acid (AB+), neutral (PAS+) and mixed (AB/PAS+) mucopolysaccharides. The density of the goblet cells was determined in 10 villi of each preparation, each segment of the small intestine for each individual. The density of the obtained cells was calculated per 1mm^2 of the median longitudinal section of a villus.

The data obtained in the study were statistically processed by SPSS 17.5 software programme.

The density (number) of the goblet cells of the ostrich small intestine per one area unit of mucosa from the 38th day of embryonic development until the age of 30 days tended to decrease in all segments of the small intestine. On day of hatch, the largest density of the goblet cells per 1 mm^2 of mucosa was observed in the duodenum, in turn at 60 days of age - in the ileum. Differences of the density of goblet cells and the proportional division were observed depending on the chemical composition of mucopolysaccharides in different segments of the small intestine of ostrich chicks. The obtained results characterize both the quantitative and qualitative differences of mucopolysaccharides (mucus) secretion that is possibly connected with various specific roles of the small intestine segments in the processes of nutrients absorption.

Keywords: ostrich chicks, small intestine, goblet cells, mucopolysaccharides.

STRUČIŲ PLONŲJŲ ŽARNŲ TAURINIŲ LĄSTELIŲ PASISKIRSTYMAS IR SAVYBĖS PRIEŠ IŠSIPERINT IR IŠSIPERĖJUS

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Santrauka. Taurinių ląstelių pasiskirstymas žinduolių ir paukščių (dažniausiai vištų) organizme yra plačiai nagrinėtas, tačiau įvairių rūšių paukščių šių ląstelių funkciniai tyrimai vis dar yra labai aktualūs. Šio tyrimo tikslas – nustatyti taurinių ląstelių tankumą stručių plonosios žarnos gleivinėje prieš išsiperėjimą ir pirmaisiais gyvenimo mėnesiais, jas diferencijuoti pagal mukopolischaridų cheminę sudėtį. Tyrimui panaudoti 42 abiejų lyčių Latvijoje auginami stručiai. Tarp jų buvo šeši embrionai, paimti 38-tą inkubacijos dieną, ir 36 vienos, trijų, septynių, keturiolikos, trisdešimties ir šešiasdešimties dienų paukščiai. Išmatuotas (mm) kiekvienas plonosios žarnos segmentas. Audinio pavyzdžiai (0,5–1x1cm) paimti iš dvylikapirštės, tuščiosios ir klubinės žarnos dalies vidurio.

Norint išsamiai histologiškai įvertinti, audinių pavyzdžiai buvo deparafinuoti, hidruoti ir nudažyti hematoksilino ir eozino dažais laikantis standartinio metodo. Su histocheminių reakcijų pagalba nustačius kokybinę mukopolisacharidų sudėtį, taurinės ląstelės suskirstytos į ląsteles, užpildytas rūgščiais (AB+), neutraliais (PAS+) arba mišriais (AB/PAS+) mukopolisacharidais. Kiekvieno tirto paukščio taurinių ląstelių tankumas nustatytas kiekvienos dalies audinio pavyzdžio dešimtyje žarnos gaurelių. Ląstelių tankumas skaičiuotas 1 mm^2 žarnos gaurelių medianinio išilginio pjūvio.

Tyrimo duomenys apdoroti kompiuterine SPSS 17.5 programa.

Nustatyta, kad nuo 38-tos dienos embrioninėje fazėje iki 30-tos dienos po išsiperėjimo taurinių ląstelių tankumas (skaičius) plonosios žarnos ploto vienete mažėjo visuose jos segmentuose. Išsiperėjimo dieną daugiausia taurinių ląstelių 1 mm^2 gleivinės pastebėta dvylikapirštėje žarnoje, o 60-tą gyvenimo dieną – klubinėje žarnoje. Taurinių ląstelių kiekis ir pasiskirstymas skyrėsi priklausomai nuo įvairių stručiukų plonosios žarnos segmentų mukopolisacharidų cheminės sudėties. Tyrimo rezultatai rodo, kad kokybiniai ir kiekybiniai mukopolisacharidų (gleivių) skirtumai galimai susiję su specifiniu kiekvieno plonosios žarnos segmento vaidmeniu maisto medžiagų įsisavinimo procese.

Raktažodžiai: stručiukai, plonoji žarna, taurinės ląstelės, mukopolisacharidai.

Introduction. The goblet cells (mucocytes) producing secretion that contains mucopolysaccharides, have an important protective role against chemical irritants and microorganisms (barrier function) as well as transporting role of substances between the intestinal lumen and epithelial microvilli (Sgambati et al., 1996; Uni, Smirnov et al., 2003; Montagne et al., 2004; Plaisancie, 2006; Wang, Peng, 2008). The distribution of these cells has been widely studied both in mammals and in birds, mainly hens; however, the functional studies of the goblet cells, especially in growing birds of different species are still topical.

In mammals, two subtypes of the goblet cells ultrastructure are found depending on the placement of secretion in the cell – *granular* and *combined* mucocytes. The secretion of the granular type cells make globules filled with dense secretory granules, but in the combined type of mucocytes, the secretion occurs in a wide cisternal space made of endoplasmic reticulum. In addition, each type is divided depending on the maturity degree – *immature* (new) mucocytes (*oligomucocytes)* with little amount of secretion content in the cell, and *mature* mucocytes (*goblet cells)* with abundance of secretion in the cell cytoplasm (Cheng, 1974a). Mucopolysaccharides molecules are combined in large granules enclosed by a membrane and are located in the cytoplasm of the goblet cells. Mucin releases from the apical surface of the goblet cells (Sheahan, Jervis, 1976). The cell mucopolysaccharides are classified as neutral and acid subtypes. In the acid mucopolysaccharides, there are sulfatised and non-sulfatised groups (Sheahan, Jervis, 1976).

The chemical changes of mucopolysaccharides may affect significantly the biological activity of digestive enzymes, body immune defence, barrier function of intestinal mucosa, and substance transportation through the membranes of enterocyte microvilli. It is well known that in most mammals the chemical composition of mucopolysaccharides synthesized in the digestive canal changes during the lifetime and depends on the animal age, hormonal activity (glycocorticoids, insulin) and the feed fed (Alen, 1981; Forstner et al., 1995; Biol-N'garagba, Louisot, 2003; Plaisancie, 2006). During ontogenesis, the goblet cells develop in the way of mitosis from pluripotent stem cells at the base of the mucosa crypts or poorly specialized cells, so called oligomucocytes, at the basal parts of the crypts (Cheng, 1974b; Uni, 2006).

Investigating the chemical composition of mucopolysaccharides in various mammal species (mice, rats, hamsters, guinea pigs, rabbits, cats, dogs, and *Rhesus* monkeys) in different segments of the digestive tract, scientists have determined that in the stomach mucosa of most species mainly neutral mucopolysaccharides are produced, while in the intestines the acid mucopolysaccharides are prevailing. In addition, groups of sulfatised acid mucopolysaccharides are produced most often in the large intestine (Sheahan, Jervis, 1976; Sakata, Engelhardt, 1981). It should be mentioned that the changes of chemical composition of mucopolysaccharides in the digestive tract might indicate pathology of a particular organ in mammals. For instance, in humans the predominance of acid mucopolysaccharides in the stomach mucosa is frequently associated with adenocarcinoma development (Shah, Shrikhande, 1989).

Studies of the goblet cells in hens and turkeys show evidence that feeding conditions and microflora in the intestines also affect various parameters of mucopolysaccharides dynamics (Sharma, et al,1997; Uni, 2006;).

The aim of this research was to find out the density of the goblet cells in the mucosa of the small intestines of ostrich chicks pre hatch and during the first months of life as well as to differentiate the goblet cells by the chemical composition of mucopolysaccharides.

Material and methods. In the research, 42 ostriches of both sexes raised on the farm Ozolini AB (Jekabpils district), Latvia, were used, including six embryos obtained on the 38th incubation day and 36 chicks of age 1, 3, 7, 14, 30 and 60 days post hatch, distributed in groups of 6 birds in each group. Ostrich eggs were obtained from May to July 2008 and incubated in the incubator *Euro Elektronik KL-72S*.

On day 39 of incubation, the eggs were placed into the hatching chamber *Euro Elektronik KK-24S*, where chicks were kept also over the first 3 days of life. Starting from day 4, the chicks were placed in a heated box with the sand bedding. At this age they started to receive the commercial ostrich chick feed *Strus Premium - Strus 1** (*Feed ingredients, %: barley- 36,8; oats- 10; wheat- 18,2; wheat bran- 5; rapeseed oil- 3; chalk- 2; soy pellets- 22; Dolfos StrusMix PS-3). Feed and water were supplied *ad libitum*.

Before euthanasia, the birds of age groups of 7, 14, 30 and 60 days were taken off feed for 12 hours. After that, chicks were anaesthetized and then euthanized. Following euthanasia, each carcass was weighted on electronic scales Kern 442-512N (±1g) and subjected to necropsy for further examination. The length of each segment of the small intestine was measured (mm).

Histological samples of tissue (0.5-1x1cm) were taken from the small intestine: the medium segments of the duodenum, jejunum, and ileum. Before placing in fixative solution, the lumen content of each sample was flushed out from the mucosa with a warm 0.9% NaCl solution. Subsequently, the samples were fixed in 10% neutral formalin at room temperature for 48 hours, then dehydrated in the tissue processor (TISSUE-TEK II), and embedded into paraffin blocks according to a standardized histological preparation procedure of tissue (Carson, 1997; Kiernan, 2008).

Of each tissue sample, sections of 4-5 µm thick (microtome SLEE CUT 5062) were taken, floated on slides and dried for 24h at a temperature of 38ºC thus

preparing them for further histological processing.

For overall histological assessment, the tissue samples were deparaffinized, hydrated, and stained with haematoxylin and eosin stain complying the standard methods (Carson, 1997).

For identification of epithelial mucosubstances, the following histochemical reactions were applied: periodic acid-Schiff test (PAS) for identification of the neutral mucopolysaccharides; alcian blue at pH 2.5 (AB) for identification of the acid mucopolysaccharides; alcian blue at pH 2.5- periodic acid-Schiff test (AB/PAS) for determination of the mixed mucopolysaccharides (Carson, 1997; Kiernan, 2008). Additional sections of the tissue samples, obtained from embryos and 1 to 3 days old ostrich chicks before staining with PAS and AB/PAS tests, were held in 1% amylase solution at room temperature for 30 minutes in order to distinguish the potential intracellular glycogen from the neutral mucopolysaccharides (Luna L., Luna D., 1992; Tyler, 1994; Carson, 1997).

Histological assessment of the small intestinal wall

By using the histochemical reactions, the differentiation of the goblet cells by the chemical composition of mucopolysaccharides was carried out into cells containing acid (AB+), neutral (PAS+) and mixed (AB/PAS+) mucopolysaccharides. The density of the goblet cells was determined in 10 villi of each preparation, each segment of the small intestine and for each individual. The density of the obtained cells was calculated per 1mm^2 of the median longitudinal section of a villus.

Statistical processing of data

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 among 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). Pearson correlation test was applied for determination of the correlation among parameters (Arhipova et al., 2003).

Results and discussion. The density of the goblet cells in the mucosa of the small intestine per 1 mm^2 of the longitudinal section area median of a villus in the first month of life had a tendency to decrease; however, from day 30 to 60 differences were observed in the density of cells depending on the segment of the intestine (Fig. 1).

Wang and Peng (2008) have also established that in 45 days old ostrich chicks the density of the goblet cells in the small intestine mucosa is larger than that in chicks at the age of 90 and 334 days. That shows evidence that the density of the goblet cells decreases per area unit with the increase of the bird's age. Whereas in the chicks of hens, the density of the goblet cells in the small intestine mucosa increases per area unit in the first weeks of life (Uni et al., 2000; Uni et al., 2003a).

On day 38 of embryonic development, the largest amount of the goblet cells per area unit was established in the jejunum, the smallest – in the duodenum.

During the further development until the age of 7 days, the density of the goblet cells in the duodenum was changeable and ranged from 606±41.9 cells on the day of hatch to 374±30.4 cells in chicks aged 3 days. Starting from day 7 of life (467±76.4), the amount of the goblet cells in the duodenum per 1mm^2 of the mucosa area decreased relatively rapidly ($p<0,01$) to 68±15.1 cells at 60 days of age (Fig. 1).

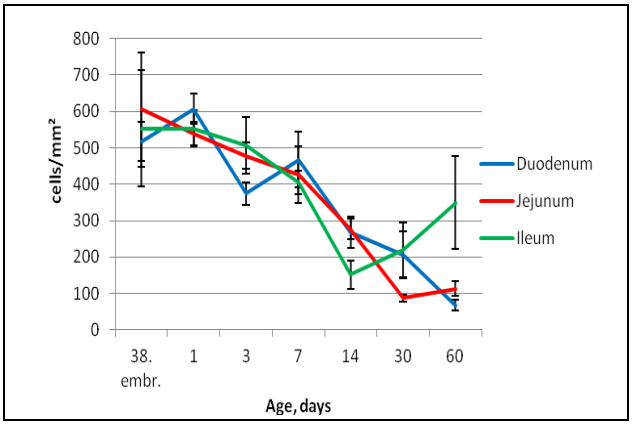

Fig. 1. **Density of the goblet cells** (±SEM) **mm² in the mucosa of the small intestine of the longitudinal section area median of a villus in the ostrich chicks from day 38 of embryonic development to day 60 of post hatch**

A similar tendency of the goblet cells was observed in the jejunum, where by the age of 30 days the density of the goblet cells was smoothly decreasing and it was more expressed from the 7th to 30th day of life ($p<0.05$). From the age of 30 days (87±8.8), the density of the goblet cells tended to increase in the jejunum mucosa until it reached on average 114±19.9 cells per 1mm^2 of the longitudinal section area median of a villus on day 60 of post hatch.

In the ileum, the density of the goblet cells tended to decrease from day 38 of embryonic development (553±158.7) until the 14th day of life (151±39.6). During the following period, the goblet cells tended to increase in density until the day 60 of post hatch it had reached 349±127.7 cells per 1mm^2 of the longitudinal section area median of a villus (Fig. 1). Consequently, at the age of 60 days the largest density of the goblet cells was in the ileum, which complied with a generally accepted opinion that this parameter in the small intestine mucosa increases from the duodenum towards the ileum.

The total number of the goblet cells in the duodenum and jejunum was characterized by a strong negative correlation between the length of the relevant intestinal segments ($r > -0.7$; $p<0.001$) indicating that the density of the goblet cells reduces per area unit of the mucosa with the growth of the intestine. It is interesting to mention that such a correlation was not observed in the ileum that indicates a different development of this intestinal segment at a particular age.

It should be mentioned that other authors of similar investigations about ostrich chicks of various age have

established the largest density of the goblet cells on day of hatch in the jejunum (Wang and Peng, 2008). Whereas at the age of 45 days they have determined the largest density of the goblet cells in the ileum, which generally corresponds with results obtained in our studies.

Furthermore, in accordance with Wang and Peng (2008) studies, at the age of 334 days the density of the goblet cells per area unit of the small intestine mucosa is less than that at 45 days of age in all segments of the small intestine. These scientists have also noted that the density of the goblet cells increases in all parts of the small intestine mucosa during the time from day of hatch to day 45 indicating that secretion of mucus in the small intestine increases during this period. Results of our study, in turn, indicated the tendency of increase of the cell amount only in the jejunum and ileum segments in this period of ontogenesis.

In comparison with chicks of hens, on day of hatch the average density of the goblet cells per 1 mm^2 of the mucosa area ranges on average from 1000 cells in the duodenum, 1300 in the jejunum and to 2500 cells in the ileum (Uni, et al., 2003 b).

The results of the previous studies show that the density of the goblet cells in ostrich chicks differs from that of the broiler chickens (Uni et al., 2000; Uni et al., 2003a), in which the density of these cells in the small intestine mucosa increases from the duodenum towards the ileum.

On day 38 of embryonic development, enterocytes contained glycogen granules in all parts of the small intestine (Fig.2). On day of hatch, the glycogen granules were found only in the ileum mucosa in some birds, but three days post hatch, they were not found in any segments of the small intestine.

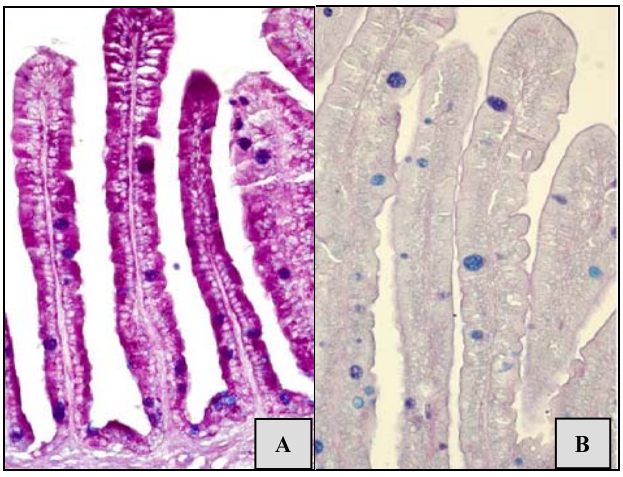

Fig. 2. **Section of the duodenum villi on day 38 of embryonic development demonstrates abundant presence of glycogen in the immature enterocytes** (A – without amylase; B – glycogen has disappeared after treatment with amylase)**, AB/PAS, 400X**

Analyzing the chemical composition of the goblet cells, it was established that the goblet cells containing the acid (AB+) and mixed (AB/PAS+) mucopolysaccharides predominated in all parts of the small intestine (Fig.3). The density of the cells containing neutral mucopolysaccharides was significantly lower in all segments of the small intestine ($p<0.05$) in all age groups of chicks (Fig.4).

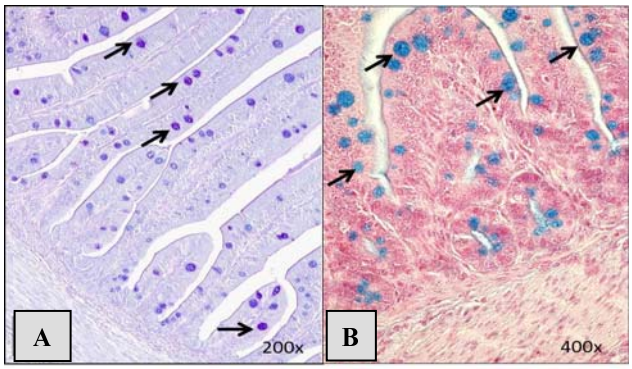

Fig. 3. **Section of the duodenum mucosa in ostrich chicks stained with AB/PAS -A demonstrates the goblet cells containing the mixed** (arrow) **and AB - B acid** (arrow) **mucopolysaccharides**

On the 38th day of embryonic development, in the duodenum of all goblet cells predominated AB+ cells (51.0±11.6%) and AB/PAS+ cells (43.0±9.58%), and their proportion was significantly larger ($p<0.05$) than PAS+ cells (6.2±2.7%) (Fig.4).

On day of hatch, in the duodenum the proportion of the PAS+ goblet cells increased (12.1±2.7%) and reached its maximum in the observed period of ontogenesis. Starting from one-day age to the 14th day the proportion of the goblet cells tended to increase and reached its maximum (64.3±12.8%) throughout the observed period (Fig.4).

During the all observed period of ontogenesis, predominance of AB+ and AB/PAS+ goblet cells in the jejunum and ileum was also established (Fig.4). However, the largest proportion of PAS+ cells was observed on the 38th day of embryonic development (15.3±5.0% and 21.8±6.7%, respectively, out of the total goblet cell density). The proportion of AB+ cells in the jejunum and ileum (Fig.4), gradually increased from the 38th day of embryonic development (46.0±9.7%), and at the age of 7 days it reached the maximum throughout the observed period. It makes 84.5±3.7% and 70.3±4.8%, respectively, out of the total density of the goblet cells. In the next period, AB/PAS+ and PAS+ cell proportion tended to increase in these regions of the small intestine (Fig.4).

Uni, et al. (2003b) research on the development of the small intestine of chicks has established that the mucopolysaccharides producing cells appear in the small intestine mucosa from day 17 of embryonic development.

Summarizing the results obtained in our research, a conclusion may be drawn that from day 3 to day 7 of life the increase of AB+ cell proportion is pronounced in all parts of the small intestine, but in the following period it reduces again on the account of the increase of AB/PAS+ cell proportion. A significant increase of the AB+ cell proportion possibly might be connected with the start of ostrich chicks feeding from the third day of life.

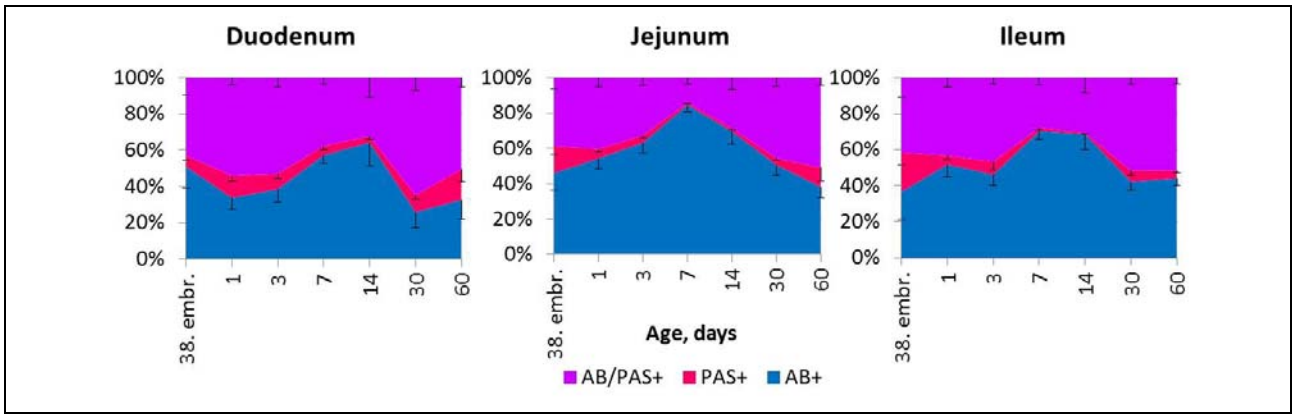

Fig. 4. Proportion of the goblet cells containing acid, neutral, and mixed mucopolysaccharides (%±SEM) **in the small intestine mucosa of ostrich chicks from day 38 of embryonic development to day 60 of post hatch**

Scientists are of the opinion that the acid mucin possesses better protective qualities against the bacterial translocation in the mucosa because it is relatively resistant to the glycosidase and protease of bacteria (Fontaine et al., 1996; Robertson, Wright, 1997). Taking into account this fact and our obtained results, we may draw a conclusion that the gastro-intestinal tract becomes contaminated when the chick starts consuming feed, and the particular type of mucopolysaccharides provides a greater resistance of the intestinal mucosa.

Unfortunately, there is lack of data of similar studies on the chemical composition of mucopolysaccharides in the goblet cells of the intestinal mucosa of ostrich chicks. However, Uni et al. (2003a) investigations on the development of intestinal mucosa in broiler chickens have established that the goblet cells contain only acid mucopolysaccharides during their embryonic development, but from day of hatch to day 7 of life the density of cells containing the acid and neutral mucin is similar. It should be mentioned that the authors have ignored and not differentiated the cells containing both types (mixed) of mucopolysaccharides the proportion of which is of great importance in ostrich chicks, as it is seen in our studies.

Conclusions. The density of the goblet cells in the small intestine mucosa of ostrich chicks per area unit from the 38th day of embryonic development to the age of 30 days of life tended to decrease in all segments of the small intestine. On day of hatch, the largest density of the goblet cells per 1 mm^2 of mucosa was observed in the duodenum, and on day 60 post hatch – in the ileum.

On day 38 of embryonic development, in all segments of the small intestine enterocytes were not fully specialized (contained glycogen granules), but on day of hatch the epithelium of the small intestine was completely specialized in the proximal and medial part of the small intestine. However, in the distal part (in the ileum) the process was completed later from the first to third day of life.

From day 3 to day 7 of life, the pronounced increase of the proportion of AB+ cells in all segments of the small intestine was possibly connected with the start of ostrich chicks feeding.

In ostrich chicks, differences were observed in both the density of the goblet cells and in proportional division in different segments of the small intestine depending on the chemical composition of mucopolysaccharides. The obtained results characterize both the quantitative and qualitative differences of mucopolysaccharides (mucus) secretion that is possibly connected with specific roles of the small intestine segments in the processes of nutrients absorption.

The role of mucopolysaccharides secretion in absorption of substances and provision of the mucosa resistance in various stages of ontogenesis, as well as not established mechanism of mucopolysaccharides secretion is the reason for further investigations to find out these issues.

Taking into consideration that the large intestine constitutes more than 50% of the total length of intestines in ostrich chicks (Duritis, Mugurevics, 2011) and intensive secretion and absorption of volatile fatty acids take place in this part of gastro-intestinal tract (Sales, 2006; Clench, Mathias, 1995), similar studies should be also carried out on this part of the digestive canal.

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