

PERINATAL ONTOGENESIS OF GASTRIC MUCOSA IN THE OSTRICH (*STRUTHIO CAMELUS VAR. DOMESTICUS*)

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Abstract. There are several unique features in the anatomy of the stomach in African ostrich in comparison with other birds. The goal of this study was to determine changes in the main morphometric parameters and histological features of gastric mucosa in ostrich chickens from the 38th day of embryonal development until 60 days of age. For the study, 6 embryos (38th day of development) and 36 chicks (1, 3, 7, 14, 30, and 60 days post hatching; 6 chicks per time point) of both sexes were obtained from African ostrich farm in Latvia during May - October, 2009. Tissue samples were investigated in Preclinical Department, Faculty of Veterinary Medicine. The total area of proventricular mucosa (cm²) and surface area of deep glands (cm²) was measured. Tissue sections for histological assessment were stained with: hematoxylin and eosin and alcian-blue pH 2.5 - periodic acid - Schiff reaction. Thickness of proventricular and ventricular mucosa, depth of proventricular superficial glands as well as surface area of parenchyma occupied by deep glands were measured in histologic sections. As the chick age increased, there were changes in the ratio of areas occupied by proventricular superficial and deep glands with relative decrease in area occupied by deep glands. Proventricular deep glands developed rapidly after hatching; at the age of 30 days deep glands histologically resembled glandular structure of adult birds. Gastric mucosal epithelial cells of 38 days old ostrich embryos and of just hatched ostrich chicks contained neutral, acidic, and mixed mucopolysaccharides. Meanwhile, in the pyloric region of ventriculus acidic mucopolysaccharides predominated.

Key words: ostrich, proventricular glands, histochemistry, ontogenesis.

Introduction

Stomach of African ostrich (*Struthio camelus* var. *domesticus*), similarly to stomach in other birds, consists of two compartments: glandular part or proventriculus (*pars glandularis*), and muscular part or ventriculus (*pars muscularis*); however, there are several unique features in the anatomy of gastrointestinal tract of ostrich that are related to adaptation of this species to life in the climatic conditions characteristic to natural geographical distribution (desert) and relatively high content of fiber in ration (Sales, 2006). In comparison with other bird species, ratites (*Ratites*) have well developed proventriculus which in adult African ostrich is 2-2.5 times larger than ventriculus (Порчевскы, 2007). Within the proventriculus of ratites there is a region of deep glands (*gll. proventriculares profundis*) that produces hydrochloric acid and pepsin, and a region of superficial glands (*gll. proventriculares superficiales*) that produces secretion containing mucopolysaccharides (Cho et al., 1983; Порчевскы, 2007; Bezuidenhout and Wan Aswegen, 1990; Catroxo et al., 1997). In African ostrich, deep glandular region occupies a relatively small portion of the proventricular mucosa, and among ratites this relative deep gland area is comparatively the smallest (Cho et al., 1983; Cooper and Mahroze, 2004).

Histology of gastric mucosa of hens and chickens had been studied in detail in the middle of the past century; however, there is relatively scant research regarding this subject in ostriches. Several authors (Bezuidenhout and Wan Aswegen, 1990; Illanes et al., 2006; Wang et al., 2007) have described histology of gastric mucosa in African ostrich starting from 2 months of age until adulthood; however, there are no data regarding gastric mucosal development during perinatal period. Thus, the goal of this study

was to determine changes in the main morphometric parameters of gastric mucosa as well as to determine histological characteristics of gastric mucosa in ostrich embryos and chickens from 38th day of embryonal development until 60 days of age.

Materials and Methods

For the study, six African ostrich embryos (38th day of development) and 36 chickens (1, 3, 7, 14, 30, and 60 days post hatching; 6 in each age group) representing both sexes were used. Ostrich eggs were obtained and incubated at ostrich farm „Ozolīņi AB” located in Jekabpils region in Latvia during May-October, 2009. Starting from the 4th day post hatching, the chickens were fed commercial ostrich chicken feed *Strus Premium - Strus 1*. Feed and water were supplied *ad libidum*.

Birds were anesthetized with intramuscular injection of 0.5 ml of 10% ketamine combined with 0.5 ml of 2% xylazine solution after which they were euthanized with intracardiac injection of 0.5 ml of pentobarbitol solution. Tissue samples were investigated in Preclinical Department, Faculty of Veterinary Medicine. Immediately after euthanasia, tissue samples for histological assessment were collected from the deep and superficial glandular regions of proventriculus, lateral walls of ventriculus and from pyloric region and were fixed in 10% neutral buffered formalin for 24-48 h in room temperature. The total area of gastric glandular part (cm²) and area of deep glands (cm²) was determined using planimeter method (Sokkia KP-90N).

Samples for histological analysis were dehydrated in the tissue processor (TISSUE-TEK II) and embedded in paraffin blocks. Tissue sections were cut (4-5 µm) and stained with hematoxylin and eosin

for morphological assessment (Carson, 1997). For the identification of epithelial mucoid secretions, the following special stains (reactions) were used: periodic acid-Schiff reaction (PAS) for identification of neutral mucopolysaccharides; alcian blue with pH 2.5 (AB) for identification of acidic mucopolysaccharides, and alcian blue pH 2.5-periodic acid-Schiff reaction (AB-PAS) for identification of mixed mucopolysaccharides (Carson, 1997; Kiernan, 2008). Tissue sections that were obtained from embryos and 1, 3, and 7 days old chickens were incubated in 1% amylase solution for 30 min in room temperature prior to staining with PAS. This was done to eliminate interference of intracellular glycogen with staining for neutral mucopolysaccharides (Carson, 1997).

Measurements of proventricular and ventricular mucosal thickness (μm), the depth of tubules of superficial glands in the proventriculus (μm), and area of deep glandular portion of proventriculus (μm^2) were obtained using light microscope (Leica DM500B) and microscopic image analysis program Image-Pro Plus 6.1. The thickness of gastric mucosa was measured at 30 sites in each tissue sample taking measurement from the apical aspect of epithelial cells on the mucosal surface to submucosal layer (5 fields, 6 measurements taken in each field). The depth of tubules within superficial glands was measured from the basal aspect of epithelial cells at the base of the glands to the apical aspect of the epithelial cells on the surface taking measurements of 30 tubules which were optimally oriented (the entire tubule visible in the tissue section). The area of deep glands was measured in 5 fields of view in each sample and compared with the total area included in 5 fields of view. Measurements were averaged to get mean parameter value for each chicken (Microsoft Excel, 2007), and results were evaluated for statistical significance using SPSS 11.5 with ANOVA Post Hoc data analysis tool.

Results and Discussion

In all birds used in this study, gastric proventricular and ventricular mucosa was covered with koilin (*Cuticula gastrica, s.coilin*), which in embryos and 1-3-day old chicks was bright green, rather thin and easily separable from mucosa, while in older birds it was thicker and green-yellow. M. M. Shanawany (1996) indicated that dark brown-green pigment in koilin comes from bile reflux from duodenum into stomach. It is reported that 7-12-week old ostrich chicks similarly to other birds have duodenogastric reflux (Degen et al., 1994). Results of our study show that bile secretion and its flow into duodenum and retrograde into gastric ventricular and proventricular compartments occurs as early as 38th day of embryonal development.

Examining glandular mucosa under the koilin, grossly visible is raised, well delineated region of deep glands which is spanning from the entrance of esophagus extending caudally along dorsolateral wall of stomach; within this region well visible are papillae (*papilla proventricularis*) with the central opening for

deep glandular ducts. Well delineated region of deep glands as a unique feature of ratites is mentioned by P. Cho et al. (1983) who point out that specifically in African ostrich proventriculus is developed the best (2-2.5 times larger than ventriculus) (Порческу, 2007); however, deep glandular area is smallest in comparison with other species comprising only 25% of total proventricular mucosal area.

Proventricular mucosal area rapidly increases as ostrich chicks grow older, and between 30 and 60 days of age it increases in size 2.5 times ($p<0.001$); meanwhile the area of deep gastric glands increases slower – between 30 and 60 days of age its size has only doubled ($p<0.001$). Unproportion growth of both gastric mucosal regions is underscored also by the relative size measurements of deep gastric gland region. For example, in newly hatched chickens relative portion of deep gastric glands comprises 22% of the total area of proventricular mucosa and during maturing it decreases to 14% ($p<0.01$) at 60 days of age (Table 1).

Table 1
Macromorphometric measurements of gastric proventricular (*pars glandularis*) mucosa in ostrich chickens of various ages (mean $\pm$ SD)

Age, days	Mucosal area, cm^2	Region of deep glands	
		Area, cm^2	Relative area, %
38 (embryos)	24.7 $\pm$ 7.2	5.4 $\pm$ 1.3	22.5 $\pm$ 3.7
1(chicks)	32.1 $\pm$ 4.3	7.1 $\pm$ 1.8	22.0 $\pm$ 4.2
3(chicks)	43.6 $\pm$ 6.7	8.8 $\pm$ 1.6	20.1 $\pm$ 2.0
7(chicks)	74.0 $\pm$ 8.7	14.4 $\pm$ 1.8	19.6 $\pm$ 2.5
14(chicks)	131.3 $\pm$ 29.9	22.7 $\pm$ 3.8	17.8 $\pm$ 3.4
30(chicks)	183.5 $\pm$ 40.3	30.8 $\pm$ 6.0	17.0 $\pm$ 2.4
60(chicks)	459.6 $\pm$ 63.7	64.6 $\pm$ 9.8	14.0 $\pm$ 0.8

The mucosa of the proventricular superficial glands is folded, and the wall is thinner than the wall in deep glandular region.

Koilin layer covering ventricular mucosa is thicker, mucosa underneath it is smooth, and crypts are hardly visible. A.J. Bezuidenhout and G. Wan Aswegen (1990) and G.S. Porchesku (2007) (Порческу, 2007) described that in adult ostrich crypts are grossly easily seen and that they contain ducts for tubular glands producing secretion which becomes koilin. In the pyloric region, mucosa is folded and covered with a thinner koilin layer.

Mucosa of **proventriculus** (*tunica mucosa*) is covered by a single layer of columnar epithelium which is covering folded lamina propria (*lamina propria*) thus forming simple tubular glands – superficial glands (*gll. proventriculares superficiales*) of proventriculus (Bezuidenhout and Wan Aswegen, 1990; Illanes et al., 2006; Порческу, 2007).

On the 38th day of embryonal development, tubules of the superficial glands are short, and the epithelium

lining them is low columnar but at the base of the glands – cuboidal. The nuclei of the cells are located basally while apical portion of the cytoplasm contains many mucous-filled granules which react positively with neutral, acidic and mixed mucopolysaccharides. The number of mucous-filled granules increases in the direction of the neck of the glands, and single cell often contains granules with several types of mucopolysaccharides.

On the day of hatching, tubules of the superficial glands are deeper (in comparison with 38th day of hatching), and with increasing age, their length also increases. Beginning with day 3 of age, branching of isolated glands is noted, but on day 14 of age branching is seen in many glands, their lumina are wider and they produce secretion that consists of neutral and acidic mucopolysaccharides forming koilin layer on the surface. Cells that are closer to the neck of the glands mostly contain neutral mucopolysaccharides while the cells located deeper in the glands contain acidic and mixed mucopolysaccharides. Similarly, in domestic chickens, neutral and acidic mucopolysaccharides are detected in the epithelium of superficial glands of proventricular mucosa (Jamroz et al., 2006).

It needs to be mentioned that in most birds, including domestic birds, architecture of superficial glands is simple and branching is not observed (Catroxo et al., 1997; Samuelson, 2007; Aughey and Frye, 2001; Bacha, and Bacha, 2006; Попчевскы, 2007); however, studies of other (Bezuidenhout and Wan Aswegen, 1990; Illanes et al., 2006; Wang et al., 2007) indicate that in ostrich superficial gland architecture varies from simple to branching tubular glands. Thus, results of our study are in agreement with previously published reports.

The depth of superficial glands in the proventriculus of adult ostrich varies around 742 μm , but the width is 22 μm (Попчевскы, 2007). Results of our research show that in ostrich chicks the depth of superficial glands in the proventriculus, similarly to mucosal thickness in this region, increases proportionally with

increasing age of chickens. At the age of 60 days, the depth of gland is $342.4 \pm 50.2 \mu\text{m}$, which is significantly increased in comparison with the 38th day of embryonal development and day of hatching ($p < 0.01$), while the thickness of mucosa is $701.0 \pm 151.0 \mu\text{m}$ ($p < 0.05$) (Figure 1). Epithelium lining glands becomes taller, and low columnar to cuboidal cells are only observed at the base of the glands.

In the dorsolateral wall of the proventriculus there are located deep glands (*gll. proventriculares profundi*). In adult birds they are composed of many rounded, angular or polymorphic lobules which are arranged in clusters. Each lobule is composed of tightly packed tubules which through tertiary or secondary ducts open into the central cavity of the gland. Central cavity of the gland is lined by one type of epithelium – single layer of cuboidal or low columnar epithelial cells which produce hydrochloric acid and pepsin (Randall and Reece, 1996; Bezuidenhout and Wan Aswegen, 1990; Samuelson, 2007; Rossi et al., 2005).

On the 38th day of embryonal development, these tubuloalveolar gland clusters already represent largest part in the entire gastric wall in the corresponding region. Glandular groups consist of poorly developed rounded polymorphic lobules separated by connective tissue fibers and smooth muscle cells. In the periphery of the lobules there are short, poorly developed tubules which connect to short, poorly developed tertiary ducts followed by secondary ducts which open into the center of the lobule. Secondary ducts as well as central cavity are lined by a single layer of low columnar epithelium that does not contain mucous-filled granules. Glandular tubules are separated by cuboidal glandular epithelial cells with large round vesicular nuclei and granular cytoplasm. The papillary ducts (primary ducts) through which the central cavities of groups of lobules open onto the surface are lined by a single layer of columnar epithelium; the apical portions of these cells contain abundant mixed mucopolysaccharide-filled granules. Between groups of glands there is a relatively thick

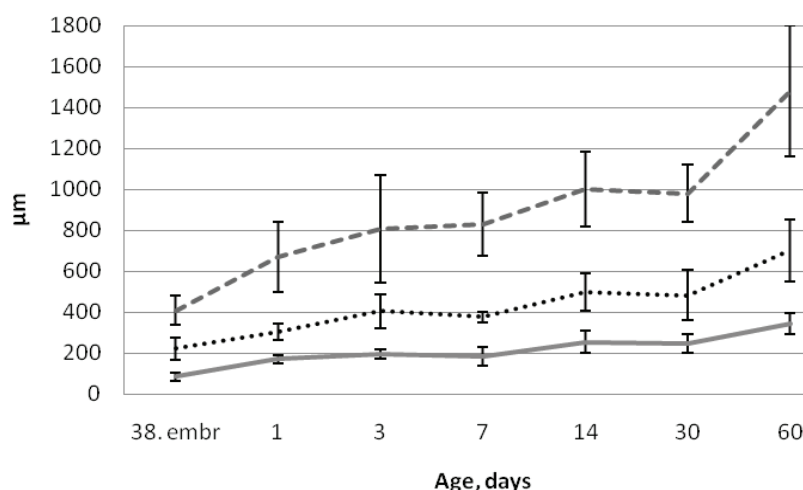


Figure 1. Thickness of gastric mucosa and depth of superficial glands in μm (mean $\pm$ SD) in ostrich chickens from the 38th day of embryonal development until 60 days of age: ---- *pars muscularis*; *pars glandularis*; — superficial glands.

connective tissue layer with abundant blood vessels and nerve fibers as well as individual mesenchymal cells and loose connective tissues. Smooth muscle cells are also observed.

On the day of hatching, lobules are more prominent and the amount of connective tissues between lobules is smaller. Tubules of the glands are relatively short with narrow lumen. Glandular epithelium forms longer rows of cells thus forming tubules. Between the rows of epithelial cells, capillary endothelium prominently stands out.

Beginning with day 7, smooth muscle fibers predominate between lobules, but between the groups of glands - mostly loose connective tissues, large blood vessels, and nerve plexuses are seen. Tubules that form the lobules of the glands are longer and their lumina wider. The borders between lobules are well delineated by smooth muscle fibers. The outline of the lobules is multiangular. Tertiary ducts are short. In this age group, in the epithelium that lines lumen of the lobules a few neutral mucopolysaccharide - containing granules are noted.

Beginning with day 14, tubules of the glands are well developed. Cross-section of the lobule is mainly occupied by tubules lined by cuboidal glandular epithelium. The central cavity of the lobule with tertiary and secondary ducts are present in the center of the lobules.

At the age of 30 and 60 days, there is a small amount of connective tissue and many blood vessels between glandular lobules. In each cluster of glands there are many variably sized lobules. Long, well developed tubules continue as tertiary and secondary ducts. Between the rows of glandular epithelium there are wide tubular lumina. The cytoplasm of epithelial cells lining central cavity of glands and primary ducts contain neutral polysaccharides. In this age group, the histology of deep glands already resembles deep glands of adult birds.

A.J. Bezuidenhout and G. Wan Aswegwn (1990) noted that mucous-containing granules are only present in the epithelium lining primary ducts; however, the

results of our study show that beginning from day 7 of age, neutral mucopolysaccharides containing granules are present in a few epithelial cells that line central cavities of glands and the number of these cells increases with age.

The measurements of deep glandular parenchyma relative to the entire glandular area (Figure 2) show that proportion of deep glandular area markedly increases between the 38th day of embryonal development and the day of hatching ($p < 0.05$) as well as between 3rd and 14th day of age ($p < 0.05$), which is the time when the birds start to eat on their own and begin to consume feed intensively. At 60 days of age, deep glands already occupy 92% of glandular area. Mucosal muscle layer within the region of superficial glands is rather poorly developed but within deep glandular region it surrounds lobules of the deep glands.

Within the gastric ventricular mucosa, similarly to other birds, there are simple as well as branching tubular glands (*gll. ventriculares*) which extend into mucosal lamina propria and open onto the mucosal surface in the wide crypts (Bezuidenhout and Wan Aswegen, 1990; Randall and Reece, 1996). In an adult ostrich, 8-28 tubular glands open into a single crypt (Попрецьк, 2007). The base of the glands is formed by a single layer cuboidal epithelium (basal cells) with large, round vesicular nuclei and pale cytoplasm. Glandular tubules are lined by low prismatic epithelial cells (chief cells) with round or oval nuclei. These cells produce mucoid secretion. Cytoplasm of these cells distally from the nucleus is filled with poorly staining small secretory granules. The surface of the mucosa and crypts is covered by a single layer of prismatic epithelium (surface epithelial cells). Apical portion of the cells is filled with rough eosinophilic secretory granules which closer to the nucleus stain basophilic. On the surface of the crypts, exfoliated cells are present as well as cells with degenerative features. Within basal aspect of the glands there are small numbers of scattered pyramidal cells (entero-endocrine cells) (Bezuidenhout and Wan Aswegen, 1990).

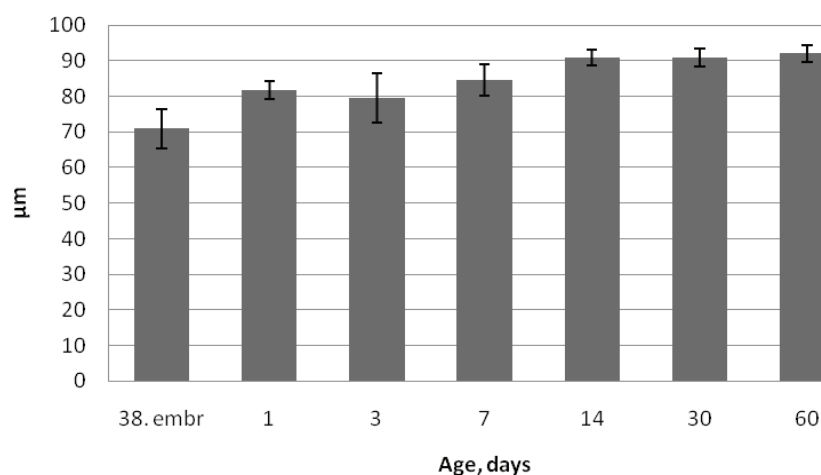


Figure 2. Proportion of deep glandular parenchyma (μm^2) relative to $100 \mu\text{m}^2$ of glandular mucosal area (mean $\pm$ SD) in ostrich chickens from the 38th day of embryonal development until 60 days of age.

Our research shows that in ostrich chicks on the 38th day of embryonal development glandular tubules are poorly developed, they are short, convoluted, and open onto mucosal surface as a single tubule or in groups up to three. Superficial epithelial cells contain mainly neutral mucopolysaccharides, but cells that contain acidic and mixed mucopolysaccharides are also observed. In the direction toward pyloric region, the epithelium covering surface contains relatively numerous cells producing acidic mucopolysaccharides, but in the transitional zone between stomach and duodenum mucosa forms folds which are covered by a single layer columnar epithelial cells that contain abundant amount of mixed and neutral mucopolysaccharides. Within mucosal lamina propria there are convoluted tubular glands formed by low columnar to cuboidal epithelium with large round nuclei (*gll.pyloricales*). Similar histological structure is described in hens (Aitken, 1958), in which numerous gastrin and somatostatin-positive cells are seen (Aksoy and Cinar, 2009; Rawdon et al., 1999).

Secretion produced by glands within the ventricular mucosa contains mostly neutral mucopolysaccharides, but secretion produced in pyloric region contains mostly acidic mucopolysaccharides. With increasing age glandular tubules become longer, and at 14 days of age they almost reach submucosa (*tela submucosa*); the volume of glands in the pyloric glandular transition zone increases. With increasing age there are no substantial changes in the epithelial cell mucopolysaccharide content and in the qualitative content of secretion.

The thickness of mucosa within ventriculus (Figure 1) in a newly hatched chick composes

668.9±170.0 µm, which is twice thicker than gastric wall within proventriculus (305.4±40.6 µm). At two months of age it is significantly increased and in the ventricular part it is 1480.8±318.4 µm ($p<0.001$), but in the proventricular part- 701.0±150.9 µm ($p<0.01$). G.S. Porchesku (Порческу, 2007) indicates that in adult ostrich mucosal thickness in the various regions of ventriculus varies from 0.5 to 1.5 mm.

Conclusions

1. As the chick age increases, there are changes in the ratio of areas occupied by proventricular superficial and deep glands with relative decrease in area occupied by deep glands.
2. Proventricular deep glands develop rapidly after hatching and at the onset of feed consumption; at the age of 30 days deep glands histologically resemble glandular structure of adult birds.
3. Gastric mucosal epithelial cells of 38-day old ostrich embryos and of just hatched ostrich chicks contain neutral, acidic and mixed mucopolysaccharides, but with increasing age there is increase in the relative proportion of neutral mucopolysaccharides; meanwhile, in the pyloric region of ventriculus acidic mucopolysaccharides predominate.
4. Granules of neutral mucopolysaccharides are observed in the epithelial cells lining central cavity of deep glands of 7-day old chicks and there is trend for these granules to increase with increasing age.
5. On the 38th day of embryonal development, ventricular mucosa is twice thicker than proventricular mucosa, and this ratio is maintained as the ostrich chicks grow older.

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