

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Morphometric Characteristics Of Bovine Rumen Epithelium In Postnatal Ontogenesis.

Valentina Mikhaylovna Shpygova*, Olga Vladimirovna Dilekova, Andrey Nikolaevich Kvochko, Vladimir Anatolyevich Meshcheryakov, and Viktor Vasilyevich Mikhaylenko.

Stavropol State Agrarian University, Zootekhnicheskiy lane 12, Stavropol, 355017, Russia.

ABSTRACT

The aim of the research was to study the morphometric parameters of the epithelium of the papillae of the rumen of cattle in postnatal ontogenesis. The histological and morphometric methods have been used to study material from 90 cattle of black-and-white cattle taken from animals of six age groups: newborns, 1 month, 3 months, 6 months, 18 months, and 4-5 years. As morphometric parameters, the thickness of the epithelium of the papillae of the mucous membrane of the rumen of the stomach of cattle was determined in the region of the connective tissue papillae of the own plate of the mucous membrane of the scar and in the region of the epithelial ridges of the interpapillary zones, as well as their ratio. It has been established that the thickness of the epithelial crests of the interpapillary zones increases significantly from birth to six months and from eighteen months to 4-5 years. With age, the ratio of the thickness of the epithelium over the connective tissue papillae of the lamina propria of the mucous membrane to the thickness of the epithelial crests of the interpapillary zones is halved and is 1: 1.23 (81.42%) in newborns and 1: 2.47 (40.49%) in Lactating cows 4-5 years. This ratio is determined by us for wide papillae (leaf-shaped, semi-oval, double and tripartite). Under the epithelium of the papillae is a thick capillary network, forming with it microvascular-epithelial complexes. The diameter of the subepithelial vessels varies greatly depending on the age, physiological condition of the animal and the shape of the papillae of the scar.

Keywords: digestion, multi-chamber stomach, scar, mucous membrane, epithelium, calves, cattle.

**Corresponding author*

INTRODUCTION

The digestive apparatus of ruminants is capable of extracting nutrients from coarse feeds rich in fiber (Hofmann, 1989). This unique ability is known to be provided by the special structure of the mucous membrane of the pre-stomachs, covered with a stratified squamous epithelium, which can be as high as 0.05 mm and populates them microbiota (Pelagalli, 2007). In newborn animals, the epithelium is not thorny. The age-changing type of nutrition contributes to deep adaptive restructuring of structures and processes in a multi-chamber stomach (Lima, 2015). In the rumen (the largest first chamber of precancer in adult animals), up to 70% of the dry matter of the diet is digested without the participation of digestive enzymes (Penner, 2011; Cavalcanti, 2014). The cicatricial microbiota is fully formed by the totality of all groups of microorganisms by the 10-week age, however, the full morphological and physiological stabilization of the functions of the digestive tract of ruminants, characteristic of adult animals, occurs by 8-10 months (Shevelev, 2003).

Nutrient absorption mainly depends on the structure of the mucous membrane, the degree of vascularization and the area of contact with the food mass (Kristensen, 2007). An increase in the surface of the mucous membrane of the scar contributes to the formation of epithelio-connective papillae of various shapes. Volatile fatty acids (VFA) in ruminants from the rumen enter the blood and lymph by the cellular method, which is confirmed by the visualization of short-chain fatty acids in the expanded intercellular spaces using electronic histochemistry (Shevelev, 2001).

In the process of evolution in ruminants between the epithelium and the underlying stroma there is a close relationship, manifested by recurring morphofunctional units, consisting of abundantly vascular connective tissue of the papilla and the epithelial layer.

The study of the patterns of postnatal morphogenesis of epithelio-connective tissue formations of the anterior mucous membrane will allow an adequate assessment of its functional state and judge the degree of completeness of feeding and maintenance of animals.

The goal is to study the morphometric parameters of the epithelium of the papillae of the rumen of cattle in postnatal ontogenesis.

The tasks are to investigate the thickness of the epithelial layer in different zones of the papillae of the scar and describe the dynamics of their correlations in the age aspect.

MATERIALS AND METHODS

90 cattle stomachs of black-and-white breed taken from animals of six age groups were studied: newborns, 1 month, 3 months, 6 months, 18 months and 4-5 years. Slaughter of calves aged from 1 day to 3 months was carried out with a scientific and production purpose. All manipulations were carried out on the slaughterhouses of the Stavropol Territory in compliance with Directive 2010/63 / EU of the European Parliament and the Council of the European Union of September 22, 2010 on the protection of animals used for scientific purposes. Slaughter of clinically healthy animals of older age groups was carried out in order to obtain livestock products. To obtain histological sections, pieces of the wall of the cicatrix with papillae were fixed in 10% neutral buffered formalin solution, condensed in paraffin, papillae transverse sections 5 μ m thick were prepared, stained with hematoxylin and eosin for morphological studies and Van Gieson for identifying connective tissue. As morphometric parameters, the thickness of the epithelium of the papillae of the mucous membrane of the rumen of the stomach of cattle was determined in the region of the connective tissue papillae of the own plate of the mucous membrane of the scar and in the region of the epithelial ridges of the interpapillary zones, as well as their ratio. The obtained morphometric parameters were processed by the method of variation and inductive statistics.

RESULTS AND DISCUSSION

Epithelio-connective tissue formations - the nipples of the rumen mucosa in newborn calves are formed and covered with a multi-layered flat non-squaring epithelium, in which three layers of cells are clearly visible: the basal, spinous and flat cells with thin long "rod-shaped" nuclei. The layer of basal cells are located in one row in the region of the papillae of the lamina propria of the mucous membrane and one or two rows in

the region of the epithelial crests of the interpapillary zones. Its thickness is from 15 to 45% of the thickness of the epithelium, which depends on the shape of the papillae. The narrower the papillae of the mucous membrane of the scar, the smaller the thickness of their epithelial layer.

On the transverse section, the basal and prickly layers slightly project into the loose connective tissue of the papilla, forming epithelial combs, between which are the outgrowths of the lamina propria of the mucosa, forming the connective papillae of the own lamina of the mucosa (Figure 1a).

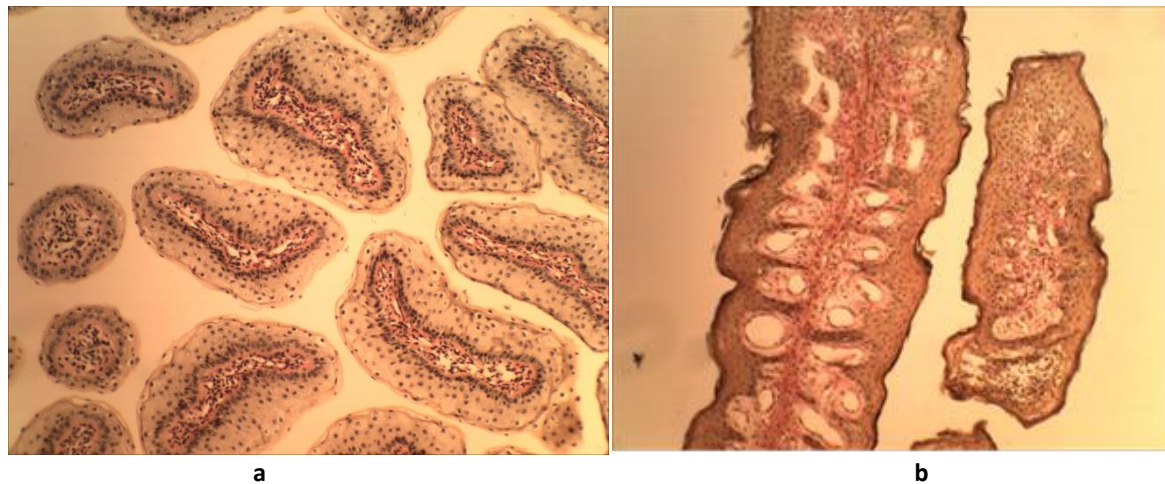


Figure 1: Transverse section of the papillae of the mucous membrane of the rumen of cattle. A) female, age 1 day) dilated venules, Female, 4 years. Coloring by Van Gieson. Increase x 80.

The thickness of the epithelium above the papillae of the lamina propria of the mucous membrane in this age group is minimal (table). The vessels are mainly capillaries with a diameter of 3 to 8 microns.

In calves, by the age of one month, the thickness of the epithelial crests of the interpapillary zones increases by 1.3 times, the thickness of the epithelium above the papillae of the lamina propria of the mucous membrane does not significantly change. The ratio of their thickness in this age group is reduced by 18%. On the cross-section, the basal and prickly layers more prominently protrude into the loose connective tissue of the papilla. The subepithelial vessels of the connective tissue papillae are also predominantly capillaries with a diameter of from 5 to 10 microns. On the surface cells of the epithelium with foci, an oxyphilic nuclear-free substance is found.

Table 1: Morphometric parameters of the epithelium of the papillae (n = 15)

Options	Animal age					
	Newborns	1 month	3 months	6 months	18 months	4-5 years
Epithelial thickness NLP	52,97±10,49	53,19±6,40	53,24±8,52	53,93±9,11	54,43±14,79	54,86±9,96
ECIZ thickness	65,06±10,59	83,86±14,10*	106,92±15,81*	114,69±24,74*	116,69±18,47	135,48±18,98*
The ratio of NLP to ECIZ	1: 1,23	1: 1,58	1 : 2,01	1 : 2,13	1 : 2,14	1: 2,47
%	81,42%	63,43	49,80	47,02	46,65	40,49

Note: the statistical significance of differences with earlier age: * p <0.05
 NLP - nipples of the lamina propria; ECIZ - epithelial crests of interpapillary zones.

In calves of three months of age, the thickness of the epithelial crests of the interpapillary zones increases by 1.27 times, the epithelium is multi-layered flat and keratinizing. Papillae own plate mucous

membranes are well defined. The ratio of the studied parameters in this age group is reduced by 13.6%. The epithelial vessels are predominantly capillaries. An oxyphilic nuclear-free substance is located on the surface cells in a thin continuous layer.

By six months of age, the thickness of the epithelial crests of the interpapillary zones slightly increases but reliably (7%). The papillae of the lamina propria of the mucous membrane is well pronounced, embossed. The ratio of the parameters studied in this age group decreases by 2.8% , with signs of desquamation.

At eighteen months of age, the studied morphometric parameters and their ratios are stable.

By the age of 4-5 years in the lactating females, the thickness of the epithelial crests of the interpapillary zones increases significantly by 16.1%. Subepithelial vessels, greatly dilated (Figure 2). Their diameter varies considerably from 19.08 μm to 95.12 μm , depending on the age, functional state of the animal and the shape of the papillae (Figure 2b).

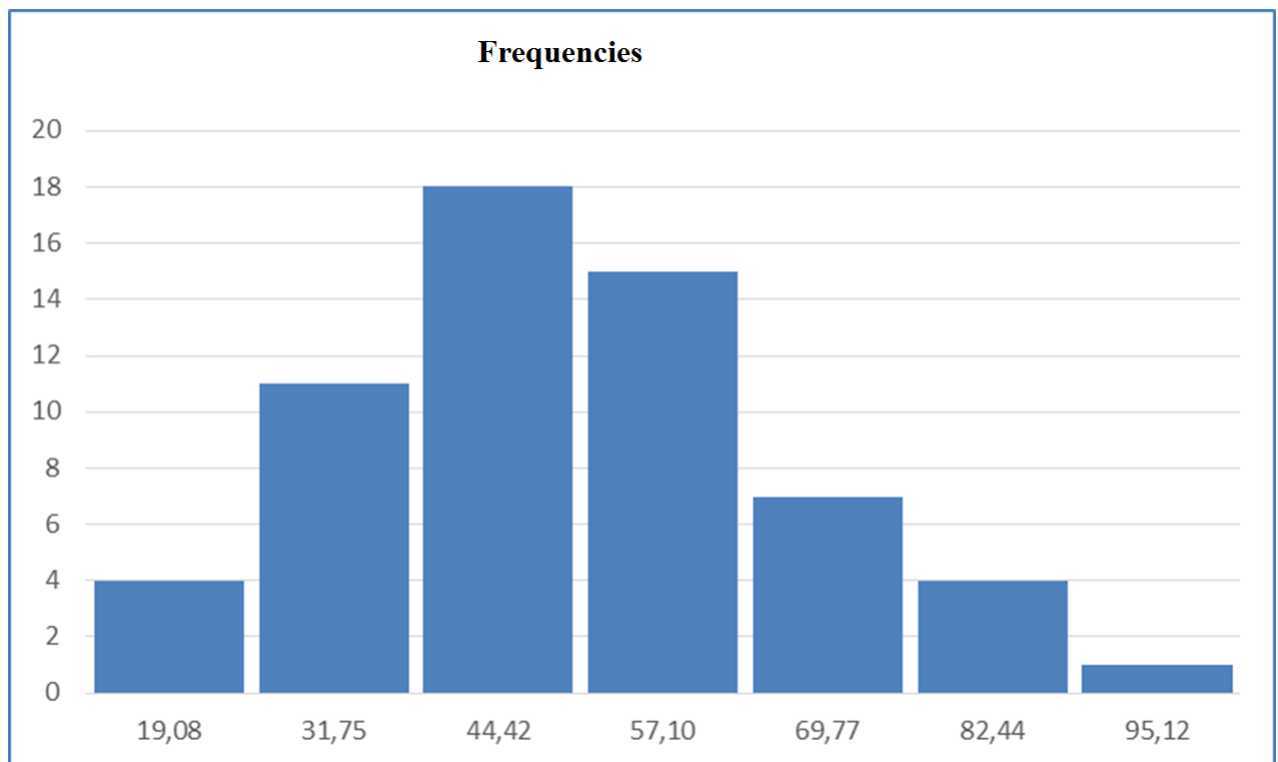


Figure 2: Distribution of diameters of venules in the papillae of the lamina propria of the mucous membrane of the cicatrix of the stomach of cattle (n = 60). Female, age 4 years (μm).

Of particular importance in the morphological characteristics of stratified squamous epithelium is the ratio of the thickness of the epithelium over the region of the connective tissue papilla to the thickness of the epithelial layer in the interpapillary zone, which is important in assessing the functional state of the scar mucosa. In the scientific literature, there is no information on the detailed description of the morphometric parameters of the epithelium of the anterior gastroes. This ratio was considered in a study of the human esophagus (Geboes, 1978; Bykov, 2006; Sabri, 2007), where the authors determined that the epithelium thickness over the connective papillae is about one-third (approximately 33%) of the maximum layer in the interpapillary zone. According to our data, this ratio in the epithelium of the rumen of cattle is not static. With age, it decreases by half from 81.42% in newborns to 40.49% in adult animals (lactating cows). This ratio is determined by us for wide papillae (leaf-shaped, semi-oval, double and tripartite). Under the epithelium of the papillae is a thick capillary network, forming with it microvascular-epithelial complexes.

CONCLUSION

Conducted morphological and morphometric studies revealed the following patterns:

- the thickness of the epithelial crests of the interpapillary zones increases significantly from birth to six months and from eighteen months to 4-5 years;
- with age, the ratio of the thickness of the epithelium over the connective tissue papillae of the lamina propria of the mucous membrane to the thickness of the epithelial crests of the interpapillary zones decreases twice and is 81.42% in newborns and 40.49% in lactating cows 4-5 years;
- The diameter of the subepithelial vessels varies significantly depending on the age, physiological condition of the animal and the shape of the papillae of the scar.

REFERENCES

- [1] Bykov V.L., IseyevaYe.A. Functional morphology of the surface epithelium of esophageal tunica mucosa // *Morfologiya*. 2006. Vol. 129, № 3. P. 7–21.
- [2] Shevelev N.S., Grushkin A.G. Peculiarities of metabolism and morphofunctional structure of rumen mucosa in ruminant animals (review) // *Sel'skokhozyaistvennaya biologiya*. 2003. № 6. P. 15–21.
- [3] Shevelev N.S., Morozov I.A., Grushkin A.G. et al., Visualization of volatile fatty acids in the mucous membrane of sheep scar using electronic histochemistry, *Dokl. TSCA*, 2001. - T. 273, 2. - 43–46.
- [4] Geboes K., Desmet V. Histology of the esophagus // *Frontiers of gastrointestinal research (Front GastrointestRes)*. – 1978. – V. 3. – P. 1–17.
- [5] Cavalcanti L. F.L., Borges I., Vandenberg L. S. et al. Morphology of pre-stomach and ruminal papillae of growing Santa Inês female lambs under two nutritional schemes // *Pesquisa Veterinária Brasileira. Pesq. Vet. Bras.* 2014. Vol. 34, №.4. P. 374–380.
- [6] Hofmann R.R. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system // *Oecologia*. 1989. – Vol. 78. – P. 443–457.
- [7] Kristensen, N.B., Sehested J., Jensen S.K. et al. Effect of milk allowance on concentrate intake, ruminal environment, and ruminal development in milk-fed Holstein calves // *Journal of Dairy Science*. 2007. Vol. 90. – 4346–4355.
- [8] Lima R. F., Resende J. J. Ch., Costa S. F. et al. Morphological response of the ruminal and omasal mucosae to the variation in diet energy // *Ciênc. Agrotec., Lavras*. 2015. Vol. 39, № 6. P. 574–582.
- [9] Pelagalli, G.V. Morphological studies in the buffalo as a contribution to biotechnological methodologies in the animal production // *Journal of Animal Science*. 2007. Vol. 6, № 2. P. 184–193.
- [10] Penner, G.B., Steele M.A., Aschenbach J.R., McBride B.W. Ruminant Nutrition Symposium: Molecular adaptation of ruminal epithelia to highly fermentable diets // *Journal of Animal Science*. 2011. Vol.89, № 4. P. 1108 –1119.
- [11] Plaizier J.C., Krause D.O., Gozho G.N. et al. Subacuteruminal acidosis in dairy cows: The physiological causes, incidence and consequences // *Veterinary Journal*. 2008. Vol. 176, № 1. P. 21–31.
- [12] Sabri M. T., Hussain S. Z., Shalaby Th. M., Orenstein S. R. Morphometric Histology for Infant Gastroesophageal Reflux Disease: Evaluation of Reliability in 497 Esophageal Biopsies // *Journal of Pediatric Gastroenterology and Nutrition*, 2007 – Vol. 44 – Is. 1 – p. 27–34.
- [13] Shpygova V. M. Dilekova O. V., Mikhaylenko V. V., Meshcheryakov V. A., Pisarenko N. A. Organ-Specific Features Of The Terminal Bloodstream Of The Grid Of The Stomach Of Cattle // *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2018. Vol. 9, № 6. P. 1234–1238.
- [14] Swan, G.E., Groenewald H.B. Morphological changes associated with the development of the rumino-reticulum in growing lambs fed different rations // *Journal of Veterinary Research*. 2000. Vol. 67, № 2. P. 105–114.