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MICROSCOPIC STRUCTURE OF THE GIZZARD OF THE BUDGERIGAR (MELOPSITTACUS UNDULATUS) OF DIFFERENT AGES

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The features of the histological structure and morphometric indicators of the microstructures of the gizzard (ventriculus gastris s. pars muscularis) of budgerigars of 9 age groups were determined: 1-, 3-, 7-, 14-, 21-day-old, 1-, 2-, 6-month-old and 1-year-old. It was confirmed that the wall of the gizzard consists of four membranes: mucous, submucosa, muscular and serous. The mucous membrane is formed by two layers: the lamina propria and the epithelial layer, the surface of which is covered with cuticle. The lamina propria, formed by loose fibrous connective tissue, contains densely located simple tubular weakly branched glands throughout its depth that produce keratin. The lamina propria is composed of a deep layer containing secretory departments of glands with an expanded base and a superficial layer containing villi that function as excretory ducts, along which liquid secretion moves from the depths of the secretory departments to the surface of the mucous membrane and which has the form of columns that are oxyphilically stained. The epithelial layer of the mucous membrane is represented by a single-layered single-row prismatic epithelium, the cells of which have a weakly oxyphilic cytoplasm and a basally located oval-shaped nucleus. The muscularis mucosae is not found in the composition of the mucous membrane. The submucosa formed by densely arranged bundles of mainly collagen fibers, connects the mucous and muscular membranes. The muscular membrane consists of a single layer, which is built of contractile elements of unstriated muscle tissue and thin fibers of connective tissue. Age-related features of morphometric indicators of microstructures of the budgerigar gizzard were an increase in the thickness of the cuticle, mucous and muscular membranes with age and a decrease in the depth of the glandular portions of the tubular glands. The greatest thickness of the cuticle was in parrots 6 months to 1 year old, and the greatest depth of the glands that produce it was in chicks 1-21 days old.

Keywords: *budgerigar, gizzard, histological structure, age-related features, morphometric indicators.*

МІКРОСКОПІЧНА БУДОВА М`ЯЗОВОЇ ЧАСТИНИ ШЛУНКУ ХВИЛЯСТОГО ПАПУЖКИ *(MELOPSITTACUS UNDULATUS)* РІЗНОГО ВІКУ

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Визначали особливості гістологічної будови і морфометричні показники мікроструктур м'язової частини шлунку (шлуночка шлунку) хвилястого папужки 9 вікових груп: 1-, 3-, 7-, 14-, 21-добового, 1-, 2-, 6- місячного і 1-річного віку. Підтверджено, що стінка м'язової частини шлунка складається з чотирьох оболонок: слизової, підслизової, м'язової і серозної. Слизова оболонка утворена двома шарами: власною пластинкою і епітеліальним шаром, поверхня якого вкрита кутикулою. Власна пластинка, що утворена пухкою волокнистою сполучною тканиною, на всій глибині містить щільно розташовані прості трубчасті слабо розгалужені залози, які продукують кератин. У складі власної пластинки виділено глибокий шар, що містить секреторні відділи залоз із розширеною основою і поверхневий шар, що містить ворсинки, що виконують функцію вивідних протоків, по яким переміщається рідкий секрет з глибини секреторних відділів на поверхню слизової оболонки і який має форму стовпчиків, що оксифільно забарвлюються. Епітеліальний шар слизової оболонки представлений одношаровим однорядними призматичним епітелієм, клітини якого мають слабо оксифільну цитоплазму і базально розташоване овальної форми ядро. М'язової пластинки в складі слизової оболонки не виявлено. Підслизова основа, що утворена щільно розташованими пучками переважно колагенових волокон, сполучає між собою слизову і м'язову оболонку. М'язова оболонка складається з одного шару, що побудований з скоротливих елементів непосмугованої м'язової тканини і тонких волокон сполучної тканини. Віковими особливостями морфометричних показників мікроструктур м'язової частини шлунку хвилястих папужок було збільшення з віком товщини кутикули, слизової і м'язової оболонок і зменшення глибини секреторних відділів трубкоподібних залоз. Найбільша товщина кутикули була у папужок 6-місячного – 1-річного віку, а найбільша глибина залоз, що його продукують, у пташенят 1-21-добового віку.

Ключові слова: *хвилястий папужка, шлуночок шлунку, гістологічна будова, вікові особливості, морфометричні показники.*

Introduction. Parrots are kept in captivity for their color, vocal abilities, longevity, behavior, and also for the purpose of preserving them as a biological species (Larcombe S. D. et al., 2015). They are known for their complex cognitive and communicative abilities, are social animals and require many interactions. The most famous and widespread psittacines include budgerigars (*Melopsittacus undulatus*), which are considered the best wild birds to keep in captivity at home (Pekmezci D. et al., 2020). In recent years, amateur breeding of parrots has contributed to their appearance in the wild in countries where they were not previously found. Thus, in less than two years, 2,675 specimens of parrots of 49 species were found in the wild in Poland. Moreover, they were most often observed in regions characterized by a larger population, higher income and a share of people with higher education (Ważna A. et al., 2024). It is known that exotic birds kept by humans as companion animals can be carriers of zooanthropozoonoses (Boseret G. et al., 2013; Yetismis G. et al., 2022). However, for

parrot owners, the main problem is their feeding. Most of them do not know how to feed their birds in a healthy way, and sometimes veterinarians and ethologists are faced with dramatic situations. It is believed that parrots in the wild in Australia, from which they have spread throughout the world, can balance their diets. According to Eggleston K. A. et al. (2019), in the wild in Australia, budgies feed mainly on seeds of grasses of the genus *Astrebla*, which are different from commercially available food. However, owners of companion birds often refuse to transfer them to complete pelleted food due to the difficulty of the transition (Cummings A. M. et al., 2022). At the same time, seed-based diets contain excess fat, low calcium to phosphorus ratios, are nutrient-deficient and monotonous. As a result, birds are fed a mixture of seeds, foods, and formulated diets (Brightsmith D. G., 2012). Balanced diets are needed, which are a key factor in maintaining the health of parrots (Orosz S. E., 2014).

Inconsistency of diet with natural needs leads to digestive diseases, which are common in parrots and often have similar clinical signs (Langlois I., 2003). A number of stomach diseases of both infectious and non-infectious etiology, including oncological ones, are common in parrots. Information on age-related features of the digestive system of both budgerigars and other parrot species is generally absent (Péron F. & Grosset C., 2014). As indicated by Eggleston K. A. et al. (2019), our knowledge of parrot nutrition is based on data from domestic chickens (*Gallus domesticus*), which became the basis for dietary recommendations for companion birds, which leads to a high risk of disease in them. The nutritional needs of young birds of different ages are even more unclear than for adult birds, and malnutrition among them is a common phenomenon. Currently, the issue of feeding, maintenance and medicine of old parrots is relevant (Lightfoot T. L., 2010; Reavill D. R. & Dorrestein G. M., 2010). There is no consensus among feed manufacturers regarding proper nutrition during parrot breeding (Cornejo J. et al., 2021). A basic understanding of the fine structure and physiology of the digestive organs is a necessary condition for the integration of pathophysiological and clinical signs associated with various disease processes, the compilation of feeding rations.

The aim of the study was to establish the features of the microscopic structure, determine the morphometric indicators of the microscopic structures of the muscular part of the stomach of the budgerigar (*Melopsittacus undulatus*) during the first year of the postnatal period of ontogenesis.

Materials and methods of research. The material for the preparation of histological preparations were samples of the gizzard of budgerigars of 9 groups: 1-, 3-, 7-, 14-, 21-day-old, 1-, 2-, 6-month-old and 1-year-old (n=5). The birds were kept and manipulated in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The diet of the parrots was a grain mixture, which included yellow, white and red millet, canary flax and sunflower seeds, oats. The diet included sand, as well as dried vegetables and fruits. In addition, a boiled chicken egg was periodically introduced into it. Fresh water was constantly available. After hatching, the female fed the chicks with "crop milk", and later with grain soaked in the wild.

Samples of the gizzard were taken from the same place in its middle part, fixed in 7-10% aqueous solution of neutral formalin. Histological preparations were prepared according to the classical histological technique. Paraffin sections were stained with hematoxylin and eosin, as well as Mallory. Histological preparations were studied and illustrations were made on a "*Jenamed 2*" microscope (Carl Seiss Jena, Germany). The size of the microscopic structures of the was determined using an ocular grid. The thickness of the muscular membrane of the was determined at the place of the greatest thickness of the thick muscles of the gizzard. The obtained digital indicators of the

structures were processed using a one-factor analysis of variance (ANNOVA) using the *Biostat LE 7.3* program with the definition of the arithmetic mean – M and its standard deviation – Sd. The significance of the difference in the values of the indicators between age groups was determined according to the Tukey test, where the difference was considered significant at $p<0.05$.

Research results. In the gizzard of the budgerigar, we identified four membranes: mucous, submucosa, muscular and serous. On the surface, the mucous membrane is covered with a cuticle. Two layers were identified in the composition of the mucous membrane: epithelial and lamina propria (Fig. 1). The muscularis mucosae in the composition of the mucous membrane was absent.

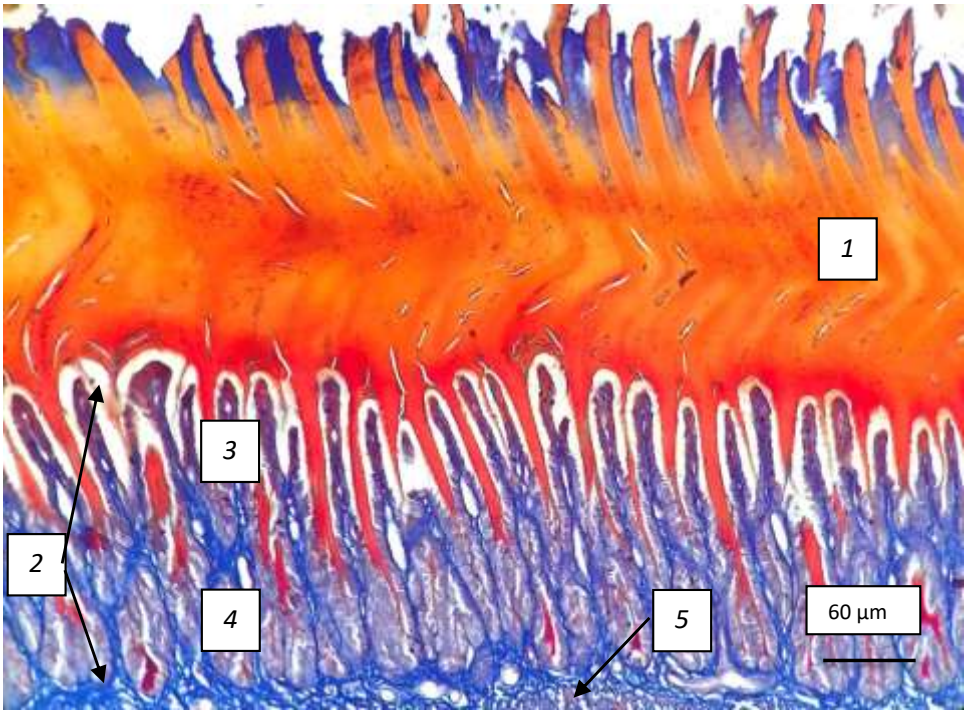

Fig. 1. **Gizzard of a budgerigar of 6 months of age. Histological preparation. 1 – cuticle; 2 – lamina propria of the mucous membrane; 3 – villi; 4 – glandular portions; 5 – submucosa; Mallory staining.**

Under the cuticle, in the composition of the lamina propria of the mucous membrane of the budgerigar's gizzard, a glandular layer was located. In the composition of this layer, its upper part, adjacent to the cuticle, contained villi (Fig. 2), and the lower part – glandular layer of branched tubular glands, consisting of two or three glandular portions (Fig. 3). In the composition of the glandular portions in the form of thin strands, and in the space between the villi in the form of wide homogeneous strands, there was a secret from which the cuticle was formed. The lower part of the glandular portions was expanded.

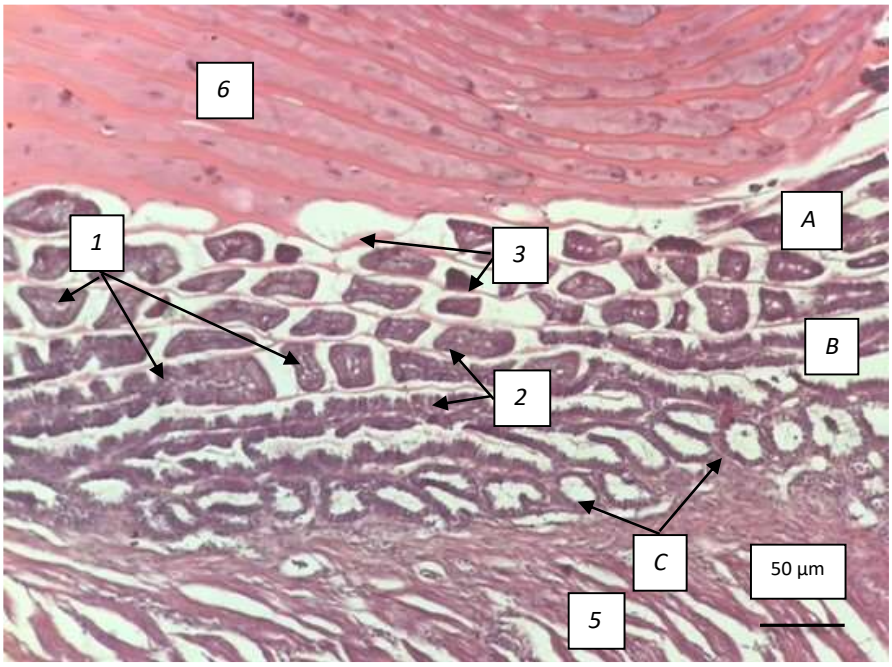

Fig. 2. **Glands of the lamina propria of the mucosa of the gizzard of a 21-day-old budgerigar in transverse and longitudinal sections. Histological preparation. A – transverse section of villi; B – longitudinal section of villi; C – transverse section of glandular portions; 1 – core of villi; 2 – epitheliocytes of villi; 3 – glandular secretion; 4 – submucosa; 5 – muscular membrane; 6 – cuticle; stained with hematoxylin and eosin.**

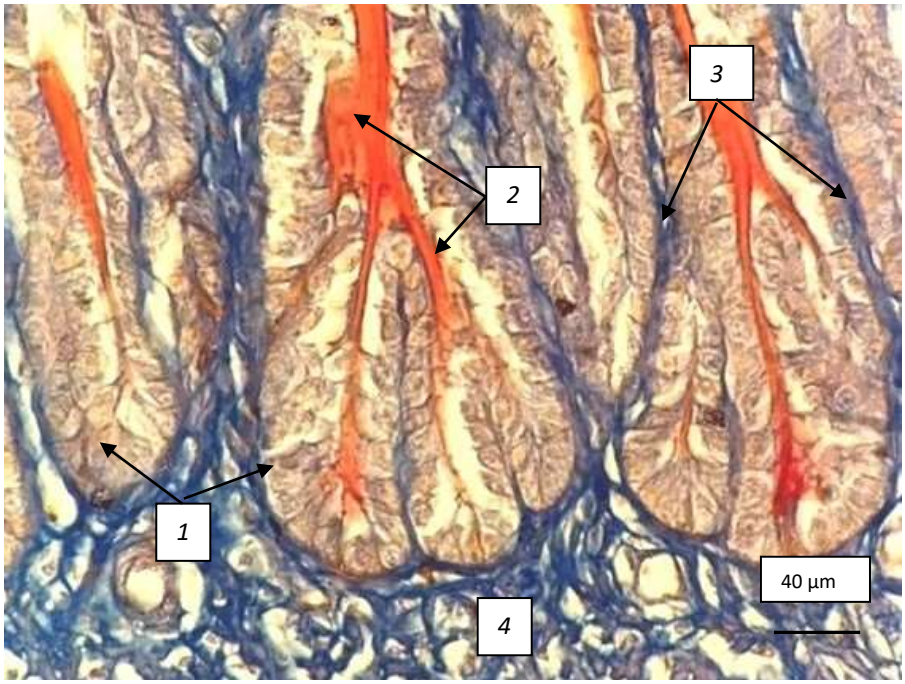

Fig. 3. **Gizzard of a 2-month-old budgerigar. Histological preparation. 1 – glandular portions; 2 – glandular secretion; 3 – loose fibrous connective tissue of the lamina propria; 4 – submucosa; Mallory staining.**

In 1-3-day-old budgies, despite the presence of secretion between the strands of cells that formed the glands, there was no clear division into glandular portions and excretory ducts in the glandular layer (Fig. 4), which indicates active processes of morphogenesis.

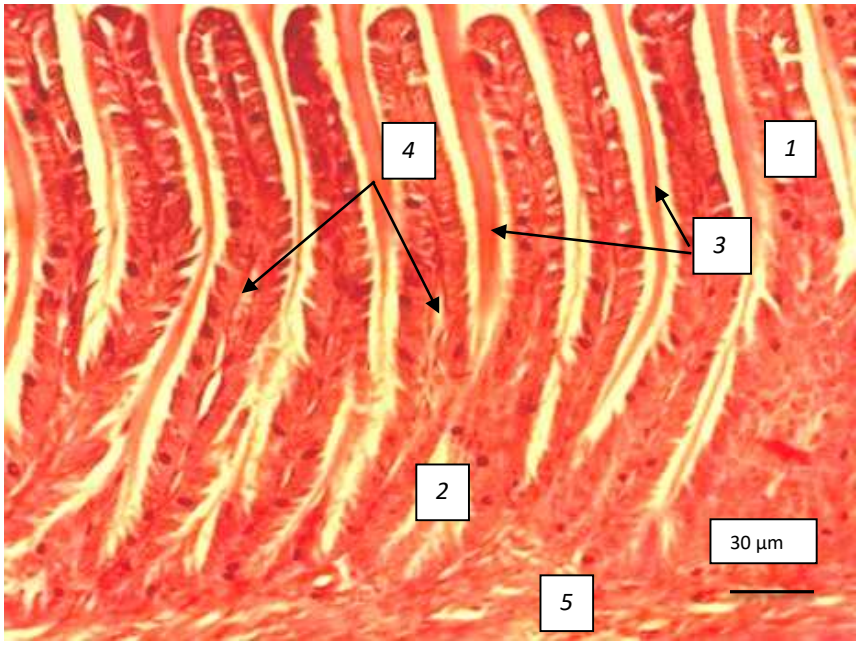

Fig. 4. **Glands of the lamina propria of the the gizzard mucous membrane of a budgerigar of 3 days of age. Histological preparation. 1 – villus; 2 – glandular portions; 3 – glandular secretion; 4 – loose fibrous connective tissue of the core of the villi; 4 – submucosa; staining with hematoxylin and eosin.**

The thin serous membrane of the budgerigar gizzard consisted of a thin connective tissue plate and mesothelium. Sometimes ganglia of the nervous plexus were found under the serous membrane.

The core of the villi and the space between the secretory sections of the glands were built from loose fibrous connective tissue, in which its cells and fibers, as well as small blood vessels, were found. The core of the villi and the space between the secretory sections of the glands were built from loose fibrous connective tissue, in which its cells and fibers, as well as small blood vessels, were found. Epitheliocytes of both the villi and the glandular portions had a prismatic shape, basophilic cytoplasm and a round-shaped nucleus with clearly visible grains and pockets of heterochromatin and one or two nucleoli.

Between the mucous and muscular membrane of the budgerigar's gizzard, in the form of a wide layer, consisting mainly of a dense plexus of connective tissue fibers, was the submucosa. In its composition, both blood capillaries, arterioles and venules were found. Macroscopically, on the transverse section of the gizzard, on its opposite sides, the thick muscles of the stomach – *m. crassus cranioventralis* and *m. crassus caudodorsalis* – were clearly distinguished by their convex shape, which were connected to each other by two thin connective tissue plates of intermuscular tendons – *centrum tendineum*. Microscopically, the muscular membrane, which was formed by the thick muscles of the stomach, consisted of one layer, which had a circular orientation of bundles of smooth muscle tissue, which were separated from each other by thin connective tissue fibers.

According to the results of morphometric studies, the smallest value of the cuticle thickness of the gizzard was established in chicks of 1 day of age (Table 1). With age, it gradually increased and reached the largest value – 470.6±40.0 μm at 1 year of age. During the first month, it increased by 25.7%, during the second – by 22.1%. During the first month, during the first week, it increased by 118.3%, during the second – by 6.4%, during the third – decreased by 6.5%, during the fourth – increased by 3.9%.

Table 1

Morphometric parameters of the cuticle, mucosa and submucosa of the gizzard of budgerigars 1-day-old - 1-year-old (μm, M±Sd, n=5)

Age	Mucosa thickness	Cuticle thickness	Submucosa thickness	Muscular membrane thickness
1 day	271,6±28,7^{bc}	140,2±11,2^{a}	18,6±1,1	3171,2±324,7^{a}
3 days	357,2±23,3^{c}	174,6±19,2^{ab}	25,2±0,8	4031,3±297,6^{b}
7 days	287,8±26,8^{bc}	306,0±26,7^{b}	28,2±1,1	4337,2±316,3^{bc}
14 days	265,2±18,3^{b}	325,6±37,3^{bc}	29,4±1,1	5326,4±402,3^{cd}
21 days	275,0±25,9^{bc}	304,4±27,8^{bc}	28,6±1,1	5626,4±381,7^{cd}
1 month	217,4±13,1^{a}	316,4±29,2^{bc}	29,2±1,8	6251,1±406,5^{d}
2 months	219,8±23,5^{ab}	386,2±27,0^{c}	26,4±0,9	6591,6±424,7^{d}
6 months	227,6±25,1^{ab}	455,8±31,1^{d}	34,9±3,7	5046,8±322,2^{c}
1 year	236,1±19,1^{ab}	470,6±40,0^{d}	37,7±2,0	5384,1±340,8^{cd}

The smallest value of the height of the villi in the glandular layer of the gizzard mucosa – 96.0±15.4 μm was found in 1-month-old parrots, the largest – 152.4±11.7 μm in 3-day-old birds (Table 2). At the same time, the largest values of the depth of the glandular portion were found in 1-day-old parrots – one month old, the smallest – in parrots aged 2 months to 1 year. Moreover, the maximum value of this indicator – 204.8±14.2 μm was found in 3-day-old birds, the minimum – 95.6±9.3 μm in 6-month-old parrots.

In general, the highest values of the thickness of the gizzard mucous, which consisted mainly of glands, were characteristic of birds 1-21 days old. The maximum value of this indicator – 357.2±23.3 μm was established in 3-day-old chicks. In older birds, the thickness of the glandular layer of the mucous was smaller and amounted to 217.4±13.1 – 236.1±19.1 μm.

The thickness of the muscular membrane was the smallest in 1-day-old chicks, and then it increased until 2 months of age. In parrots 6 months and 1 year of age, its thickness was smaller. Moreover, during the first month it increased by 97.1%, and during the second – by 5.4%. During the first month, during the first week, it increased by 36.8%, during the second month, by 22.8%, during the third month, by 5.6%, and during the fourth month, by 11.1%.

Compared with 1-day-old birds, at 1-year-old birds, the thickness of the cuticle of the gizzard was 235.7% greater, the mucous membrane was 13.1% less, the submucosa was 102.7% greater, and the muscular membrane was 69.8% greater.

The thickness of the submucosa of the gizzard wall was the smallest in 1-day-old budgerigars and was 18.8 μm, the largest in 6-month-old and 1-year-old birds and was 35.3 and 37.5 μm, respectively. In the period from 3 days to 2 months of age, it was 25.0-29.4 μm.

Table 2

Morphometric parameters of the glandular layer of the gizzard of budgerigars 1 day old - 1 year old (μm, M±Sd, n=5)

Age	Height of villi	Depth of glandular portion	Width of glandular portion
1 day	108,4±19,0^{ab}	163,2±19,9^{cd}	35,5±2,9^{ab}
3 days	152,4±11,7^{b}	204,8±14,2^{d}	32,5±3,3^{ab}
7 days	103,2±17,9^{ab}	184,6±24,4^{cd}	35,3±3,9^{ab}
14 days	101,2±16,6^{ab}	164,0±18,2^{cd}	36,8±2,9^{b}
21 days	119,8±12,2^{ab}	155,2±13,9^{c}	42,6±2,2^{c}
1 month	96,0±15,4^{a}	121,4±9,6^{b}	37,0±3,8^{bc}
2 months	121,8±17,7^{ab}	98,0±13,8^{ab}	40,6±4,7^{bc}
6 months	132,0±11,8^{b}	95,6±9,3^{a}	32,4±3,8^{ab}
1 year	128,3±16,1^{ab}	107,8±9,0^{ab}	30,3±3,9^{a}

The largest values of the width of the glandular portion of the glands – 32.5±3.3 – 42.6±2.2 μm were determined in parrots aged from 1 day to 2 months, the smallest – 30.3±3.9 – 32.4±3.8 μm – in older birds.

Discussion. Knowledge of the anatomy and physiology of the gastrointestinal tract is necessary to determine the ability of a species of bird to use a particular type of feed, which can be used to create a nutritionally adequate diet. Understanding the factors that influence the choice of an appropriate diet allows poultry farmers, veterinarians and nutritionists to successfully keep and breed these birds (Koutsos E. A. et al., 2001).

In the gizzard of the budgerigars, we identified four membranes: mucous, submucosa, muscular, and serous, which is consistent with the data of Al-Saffar F. J. & Al-Samawy E. R. M. (2014), Beheiry R. R. (2018) regarding the structure of this organ in birds. The cuticle covering the mucous membrane from the surface is known to be a product of the activity of its glands (Bartels T. et al., 1998; Rossi J. R. et al., 2005). As indicated by Abumandour M. M. A. (2014), its thickness depends on the type of bird and food. Two layers were identified in the composition of the mucous: epithelial and lamina propria. We did not detect a muscularis mucosae. These facts are consistent with the information of Matsumoto F. S. et al. (2009); Beheiry R. R. (2018), Ahmed Y. A. et al. (2011) regarding its absence in budgerigars, domestic turkeys, quails and contradicts the data of Feder F. H. (1969), according to which it can be clearly seen in the wall of the gizzard of budgerigars, since it is separated from the muscularis mucosae by a layer of connective tissue. Al-Samawy E. R. M. et al. (2021) in the wall of the gizzard of the African grey parrot (*Psittacus erithacus*) observed the muscularis mucosae as a thinner layer of circularly arranged bundles of cells separating the lamina propria from the submucosa.

The lamina propria of budgerigar gizzard contained a glandular layer under the cuticle, which is consistent with the data of Rossi et al. (2005); Ahmed et al. (2011). The glandular layer is a tubular gland contained in the loose connective fibrous tissue of the lamina propria. It consists of an outer layer formed by villi that act as excretory ducts and an inner layer formed by the glandular portions of the glands. It should be noted that some researchers indicate that the outer layer of the lamina propria of the mucous membrane contains crypts, i.e., the immersion of the epithelial layer into the lamina propria (Zaher M. et al., 2012; Qureshi A. S. et al., 2017), or represents its folds (Rodrigues M. N. et al., 2012). However, as can be seen from Fig. 2, these are indeed villi, which is clearly visible on their longitudinal and transverse sections. According to the results of our studies, the

submucosa of the budgerigar's gizzard had the appearance of a wide layer formed by a dense plexus of connective tissue fibers. Given that this layer is constructed from a dense plexus of predominantly collagen fibers that had different directions, and, accordingly, a relatively small content of ground substance and cells, the connective tissue of the submucosa of budgerigar's gizzard can probably be classified as dense irregular. These data are consistent with the information of other researchers: in the yellow-blue macaw and the African gray parrot (*Psittacus erithacus*), it is constructed from dense connective tissue (Rodrigues M. N. et al., 2012; Al-Samawy E. R. M. et al., 2021), in the domestic turkey – from connective tissue formed mainly from collagen fibers (Beheiry R. R., 2018).

We have identified one layer in the muscular membrane of the budgerigar gizzard, which is consistent with similar data for the yellow-and-blue macaw (Rodrigues M. N. et al., 2012), the domestic turkey (Beheiry R. R., 2018), and the common quail (Zaher M. et al., 2012). At the same time, according to Al-Saffar F. J. & Al-Samawy E. R. M. (2015), in the mallard (*Anas platyrhynchos*), the gizzard muscular membrane consists of three layers – a thin inner, a longitudinal outer and a very thick intermediate one, according to Al-Samawy E. R. M. et al. (2021) in the African grey parrot it consists of two thin layers, and in the common francolin (*Francolinus francolinus*) – of three.

According to the results of determining the size of the microscopic structures of the budgerigar gizzard, two stages of cuticle thickening were established: during the first week – by 118.6% and in the period from 2 months to 1 year of age – by 54.6%. Probably, the period from 1 day to 1 month of age, when the cuticle thickness was 140.2±11.2 – 325.6±37.3 μm and the period after 1 month of age and older, when it was 386.2±27.0 – 470.6±40.0 μm reflect the time periods of feeding with different food. After hatching and up to 1 month of age, the parrots are fed with the mother's "milk" and soaked grain in her will, older birds are fed with whole grain food. Accordingly, different types of food correspond to different cuticle thicknesses. The high value of the thickness of the mucous and the depth of the glandular portions in 3-day-old chicks – 357.2±23.3 and 204.8±14.2 μm, respectively, probably indicates a significant intensity of the process of synthesis of cuticle substances. Unlike the cuticle, the absolute and relative thickness of which increased with age, the thickness of the glandular layer of the mucous membrane, where it was formed, decreased. Moreover, within the glandular layer, the depth of the glandular portions decreased to a greater extent. Thus, this indicator in parrots 1-21 days old was 155.2±13.9 – 204.8±14.2 μm, and in 2 months. – 1 year old – 95.6±9.3 – 107.8±9.0 μm. Also smaller in 6-month-old parrots – 1-year-old age was also the width of such glandular portions of the stomach. It should be noted that up to 1 month of age, the index of the depth of the secretory departments had a greater value than the height of the villi, and after 1 month of age, the index of the height of the villi was greater, and the depth of the glandular portions was smaller. Unlike the glandular layer, the thickness of the submucosa gradually increased with the age of the birds and, compared with 1-day-old parrots, in 1-year-olds it was 102.7% greater. Compared with the mucous, the thickness of the submucosa in parrots of different ages was 5.8-9.6 times less. At the same time, according to Rodrigues M. N. et al. (2012), in the yellow-blue macaw it was thicker than the lamina propria. The thickness of the muscular membrane increased until 2 months of age, and was smaller in older birds thereafter.

Thus, the most intensive change in the parameters of the microscopic structures of the budgerigar gizzard occurred during the first weeks of the postembryonic period of ontogenesis, which is consistent with the data of Baltz A. P. & Clark A. B. (2001), Kubiak M. (2020) regarding the very short duration of the breeding season of budgerigars in Australia. A number of researchers have reported on the intensive nature of the growth of

the digestive organs, which outpaces the growth rate of the whole body in young domestic birds (Gille U. et al., 1999, Makhotina D. S. et al., 2020). It should be noted that changes in various morphometric indicators of the gizzard occurred asynchronously.

Conclusions.

1. The microscopic structure of the gizzard of budgerigars corresponds to the general patterns of its structure in other bird species. Its wall consists of four membranes: mucous, submucosa, muscular and serous. The mucous membrane consists of an epithelial layer and a lamina propria, which contains simple, weakly branched tubular glands, the secretion of which forms a cuticle.

2. The submucosa is located between the mucous and muscular membranes and is built of tightly intertwined bundles of mainly collagen fibers.

3. The muscular membrane of the budgerigar gizzard consists of a single layer, built of smooth muscle tissue. Its thickness increased up to 2 months of age, in older parrots it was smaller.

4. The histological structure of the wall of the gizzard of budgerigars 1 day old – 1 year old is characterized by significant changes in the parameters of its microstructures. Against the background of an increase in the thickness of the cuticle with age, the depth of the glandular portions of the glands that form it decreases.

The prospect of further research is to determine the microscopic structure of other digestive organs of budgerigars.

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