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Original Contribution

HISTOLOGIC AND MICROMETRIC STUDY OF THE PROVENTRICULUS AND GIZZARD OF THE WILD BRONZE TURKEY (*MELEAGRIS GALLOPAVO*)

D. Yovchev*

Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

The purpose of the present study was to investigate the histologic and micrometric study of the proventriculus and gizzard of the wild bronze turkey (*Meleagris gallopavo*). Thirty clinically healthy Bronze turkeys (15 females and 15 males) were used for the study. Twelve tissue samples (six from the proventriculus and six from gizzard) from the corresponding age group were used to prepare histological slides. The preparations were stained with hematoxylin (Erlich) - eosin and Masson's trichrome. The wall of the proventriculus was composed of four layers. They were *tunica mucosa*, *tunica submucosa*, *tunica muscularis* and *tunica serosa*. Mucosa had many grooves and plicae with simple columnar epithelium. The proventricular submucosa had compound tubuloalveolar glands which took the greatest part of the wall. The gizzard had *tunica mucosa*, *tunica muscularis* and *tunica serosa*. Compound tubular glands were found in the mucosa. On the mucosal surface was observed a thick keratinized layer, which was produced by the glands. In *lamina propria mucosae* were found compound tubular glands which opened on the mucosal surface by a short neck. The glands in the grooves were more branched, compared to those localized in the folds. *Lamina muscularis mucosae* was not found.

Key words: bronze turkey, proventriculus, gizzard, glands

INTRODUCTION

The digestive organs of the birds develop intensively in the first days after hatching, and no significant changes in their structure are observed until sexual maturity (1-5).

Structurally, the digestive system of birds has morphological characteristics that are not observed in mammals, such as lack of teeth and soft palate. In this way, the birds receive the maximum amount and nutrients in a short time, while the food contents are quickly evacuated to the caudal parts of the digestive system. These features are biologically related to the flight process. The intensity of development of the digestive tract in birds is species-specific and is decisive for their development. Faster development of the digestive tract is observed in birds with high growth (6).

After hatching, the digestive organs undergo significant development, as the intestinal segments develop more intensively than other organs of the digestive system (7).

The digestive system of birds shows anatomical and physiological features that are species specific. They are conditioned by changing conditions in the biological habitat and diet (8, 9).

***Correspondence to:** *David Yovchev, Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria; e-mail: davidgospodinovyovchev@gmail.com*

The stomach of the domestic birds, including the turkey, is composed of the proventriculus (glandular part) and ventriculus, gizzard (muscular part) (10-13). Other authors report for three parts - proventriculus (glandular part), ventriculus (muscular part) and pyloric part. That is provided by the specifics of the diet (14-17).

The size and shape of the stomach depends on the diet. In carnivorous and fish-eating birds, the proventriculus and gizzard are extensible and difficult to be distinguished. When the nutrients are solid in the diet of granivorous, omnivorous, insectivorous and herbivorous birds, the proventriculus has thin walls and glandular structure, and the gizzard has developed muscles. In granivorous and insectivorous birds, the gizzard is the most developed due to the presence of cereal components in the diet (10, 18- 20).

The proventricular mucosa of the Japanese quail has numerous folds. *Lamina epithelialis mucosae* is composed of simple columnar epithelium. *Lamina propria mucosae* is the next layer, represented by loose connective tissue with lymphatic nodules and diffuse lymphocytic infiltrations. The glands are compound and tubuloalveolar, which form the greatest part of the wall's thickness. Between the glandular lobules is found loose connective tissue and smooth muscle cells. The gizzard is composed of three layers – *tunica mucosa*, *tunica muscularis* and *tunica serosa*. The tubular glands in the mucosa are compound (21).

In order to determine the ability of the stomach in birds to process and absorb nutrients, it is necessary to study the morphological changes that occurr in it during the development of birds. Some studies have focused on changes in the size of the stomach and the activity of digestive enzymes, while data on morphological changes in proventriculus and gizzard are scarce. The above facts give us reason to undertake the present study to investigate the morphological changes that occur in the proventriculus and gizzard in the Bronze turkey and their relationship with the ability of the stomach in these birds to process and absorb nutrients.

MATERIALS AND METHODS

Thirty clinically healthy Bronze turkeys (15 females and 15 males) were used for the study. The conducted researches were financed and included in the following scientific project: 01/2011 "Age, weight and morphometric examination of the organs of the middle and caudal part of the digestive system in the Bronze turkey". The experimental animals were purchased from " Mazalat "Southeast State Enterprise". They were bred in an aviary. The diet included animal protein, cereals, green plant mass, and vitamin-rich supplements. B, A and E. The birds were divided into five age groups (one-day, 7-day, 14-day, 28-day, 49-day-old). Each group consisted of three male and three female turkeys. The experiments were performed in strict accordance with the rules of the Ethics committee at Trakia University, Stara Zagora.

Twelve tissue samples (six from the proventriculus and an six from gizzard) from the corresponding age group were used to prepare histological slides. The resulting tissue samples were fixed in 10% aqueous formaldehyde solution (Merck KGaA, Darmastadt, Germany). After fixation, they were washed under running water, dehydrated in ascending ethanol series, clarified in xylene and embedded in paraffin. Cuts with a thickness of 5 to 7 μm were made using a rotary microtome YD-335A (J. Y. M. A. Ltd., China). The preparations were stained with hematoxylin (Erlich) - eosin and Masson's trichrome (22).

The obtained preparations were observed with a light microscope - VDN-200M (LUMENLAB, China), and the results were documented using a digital CMOS camera.

The results were interpreted in accordance with the histological terms terms (23).

The statistically obtained data (mean ± standard deviation (SD) for each age group) were processed with one-way ANOVA, using a software StatView v. 4.53 for Windows (Abacus Concepts, Inc). The differences between the mean values of the age groups were equated by several gamma LSD tests. The differences were significant at $P < 0.05$.

RESULTS

Histology of the stomach of the post-hatching male Japanese quail

Proventriculus:

The wall of the proventriculus was composed of four layers. They were *tunica mucosa*, *tunica submucosa*, *tunica muscularis* and *tunica serosa*. Mucosa had many grooves and plicae with simple columnar epithelium. *Lamina propria mucosae* was constructed by loose connective tissue where were simple tubular glands.

The second type of the proventricular glands were compound tubuloalveolar and they took the greatest part of the wall. They were found in *tela submucosa*. The glands were composed by polymorphic lobules, between which were observed connective tissue septa. Each lobule had many tubules which unified to form a central, secondary duct. Several secondary ducts formed a primary duct, which opened into a prominent mucosal papilla. The papilla's mucosa possessed folds and grooves. The covering epithelium of the folds had columnar cells and that of the grooves was composed of shorter cells. The wall of the primary duct had columnar cells which passed on the rest part of the proventricular wall **(Figure 1).**

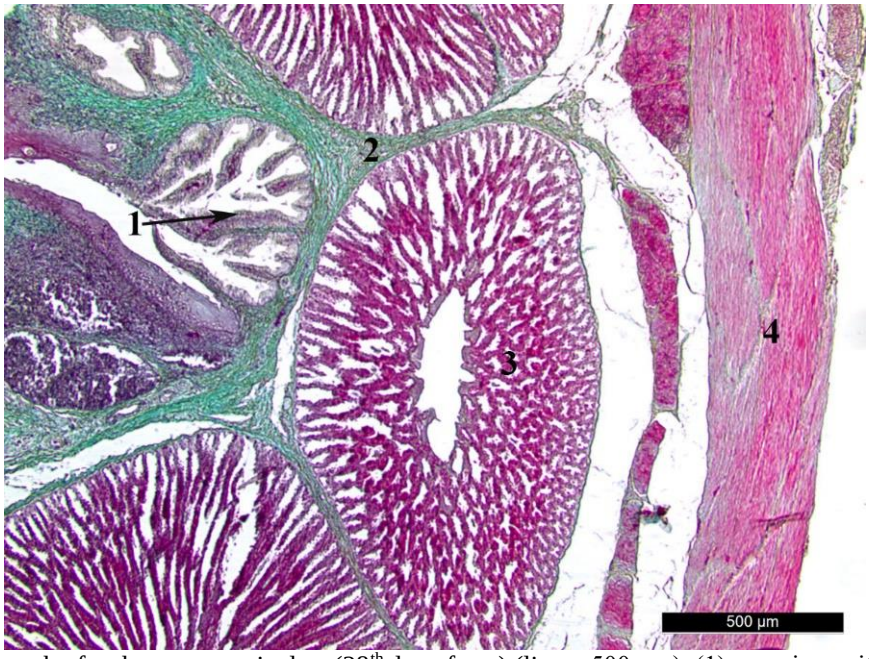

Figure 1. Photomicrograph of turkey proventriculus. (28th day of age) (line – 500 µm). (1) covering epithelium of the primary duct; (2) *tela submucosa*; (3) proventricular gland; (4) *tunica muscularis*. Masson's trichrome stain.

Gizzard: The gizzard had *tunica mucosa*, *tunica muscularis* and *tunica serosa*. Compound tubular glands were found in the mucosa. *Tunica mucosa* composed many folds and grooves. The covering epithelium was columnar type with oval nucleus in the basal part of the cells. The cytoplasm of the cells was basophilic.

On the mucosal surface was observed a thick keratinized layer, which was produced by the glands. In *lamina propria mucosae* were found compound tubular glands which opened on the mucosal surface by short neck. The glands in the grooves were more branched, compared to those localized in the folds.

The glands were composed by cuboid or low columnar epithelial cells with rounded nuclei in their basal part and basophilic cytoplasm. *Lamina muscularis mucosae* was not found. *Tunica muscularis* had circular smooth muscle tissue, which formed mainly the wall of the gizzard. In addition, the smooth muscle tissue formed two layers: outer longitudinal layer and inner oblique layer. A tendinous layer was under the muscle layer. It was with parallel orientated each other collagen fibers **(Figure 2).**

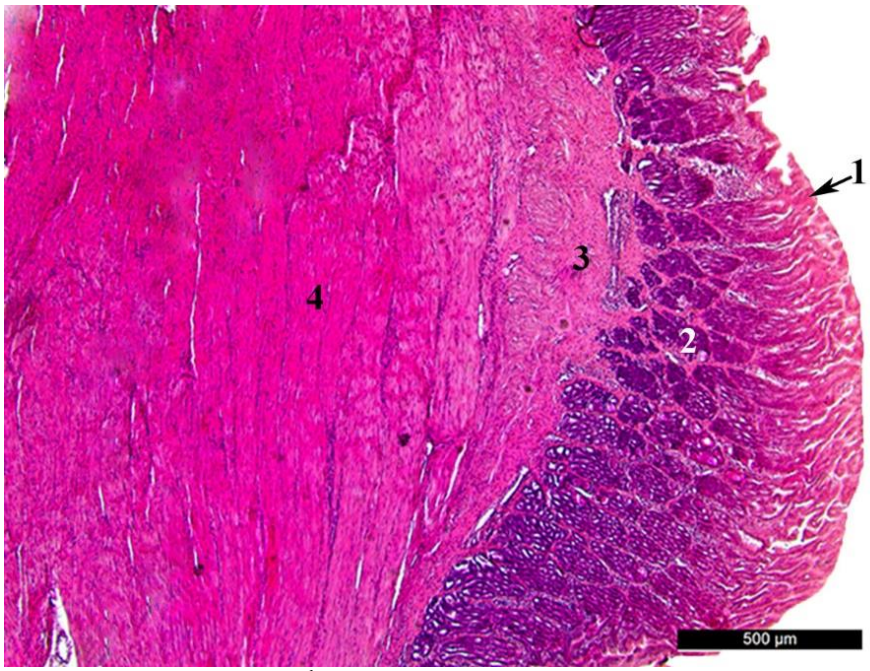

Figure 2. Photomicrograph of turkey gizzard. (14th day of age) (line – 500 µm). (1) *lamina epithelialis mucosae*; (2) gizzard's glands; (3) *lamina propria mucosae*; (4) *tunica muscularis*. Hematoxylin / Eosin staining.

Post-hatched morphometric parameters of the proventriculus and gizzard in the Bronze turkey

The values of the studied micromorphometric parameters were compared between two successive age groups. The thickness of the proventricular mucosa's folds and the height of the mucosal epithelium increased significantly till 7th day of age while the height of the glandular epithelium increased till 14th day of age. The thickness of proventricular *tunica submucosa* increased significantly after 7th day of age and that of *tunica muscularis* after 14th day of age. During the whole period of the investigation were not observed statistically significant differences in the development of *tunica serosa* **(Table 1).**

Table 1. *Micrometric parameters of the structures in proventriculus of the Bronze turkey in age aspect.*

AGE (day)	Thickness of the mucosal folds in proventriculus (µm)	Height of the epithelium in the mucosa of proventriculus (µm)	Height of the glandular epthelium of proventricuclus (µm)	*Tunica submucosa* (µm)	*Tunica muscularis* (µm)	*Tunica serosa* (µm)
1	214.16±27.52[a]	12.68±3.08[a]	7.08±1.08[a]	909.16±20.51[a]	96.15±5.67[a]	14.48±1.98[a]
7	567.68±21.14[b]	23.68±2.16[b]	8.64±1.12[a]	1145.84±38.16[a]	120.64±6.74[b]	15.38±2.96[a]
14	598.34±17.28[b]	26.48±1.98[b]	10.48±1.18[b]	1406.88±37.14[b]	148.38±10.12[b]	17.64±2.04[a]
28	690.43±38.42[b]	27.93±2.11[b]	10.96±1.15[b]	1616.99±40.13[b]	325.68±19.48[c]	18.76±4.05[a]
49	784.38±32.15[b]	28.15±2.14[b]	12.07±2.05[c]	2268.74±86.68[c]	492.54±15.64[c]	20.14±3.05[a]

The columns without common superscript indicated significant differences between two successive age groups ($P < 0.05$).

The thickness of the gizzard's glands increased till 7th day of age. In the same time the thickness of the keratinized layer and the height of the gizzard's glandular epithelium increased significantly till 28th day of age. *Tunica muscularis* in the gizzard increased intensively till14th day of age **(Table 2)**.

Table 2. *Micrometric parameters of the structures in gizzard of the Bronze turkey in age aspect.*

AGE (day)	Thickness of the glands in gizzard (μm)	Height of the glandular epithelium in gizzard (μm)	Thickness of the keratinized layer (μm)	*Tunica muscularis* (μm)	*Tunica serosa* (μm)
1	302.56±28.16[a]	6.14±2.08 [a]	137.68±29.16 [a]	1847.75±115.14 [a]	16.78±2.04 [a]
7	415.50±42.68[b]	7.24±1.94 [a]	156.86±34.12 [a]	1993.18±214.07 [a]	18.41±2.67 [a]
14	467.63±25.13 [b]	9.28±1.67 [b]	236.16±38.47 [b]	2345.28±217.18 [b]	17.34±2.35 [a]
28	483.24±28.12 [b]	14.38±2.38[c]	331.82±19.05 [c]	2456.52±198.58 [b]	19.68±3.65 [a]
49	552.68±28.13 [b]	17.66±2.44[d]	345.25±19.15 [c]	2508.65±208.58 [b]	21.86±4.06 [a]

The columns without common superscript indicated significant differences between two successive age groups ($P < 0.05$).

DISCUSSION

The results of the light microscopic study, by means of staining with Hematoxylin / Eosin and Masson's trichrome, presented better data on the histological structure of the wall of the proventriculus and the gizzard in Bronze Turkey. In addition, to identify the tissue layers that compose the wall of the proventriculus and the gizzard, we also studied the micrometric changes in some of the structures that compose the wall of these organs. According to us, these organs develop intensively in the first days after hatching. No changes in tissue structures were observed in sexually mature individuals. These data correspond to those found by some authors (1-4), who describe the development of the digestive organs in birds in age aspect.

The specific structure of the wall of the proventriculus and gizzard, the presence of four tissue layers in the wall of the proventriculus and respectively three layers in the gizzard, as well as mucosal folds, compound tubuloalveolar glands, papillary formations in the proventriculus and keratinous layer in the gizzard, are in a biologic relationship with the specific habitat and diet. The bronze turkey is characterized by high growth, due to which the digestive organs, incl. proventriculus and gizzard develop intensively. This thesis complements the scientific data for the specific age-related changes in the digestive tract of the birds, which are species specific and dependent on the biological environment and food components (6, 8, 9, 13).

The presence of two parts in the stomach of the Bronze turkey - proventriculus (glandular part) and ventriculus, gizzard - muscle part is due to the specific nutrients in the diet of the birds. That correspond to the published data for these organs (10-13).

Our data for the histological structure of the wall of the proventriculus and gizzard show that the proventriculus has thin walls and glandular structure, and the gizzard has well developed muscles and is better developed. That is due to the presence of cereal components in the diet determines the peculiarities in the structure of both organs (10, 13, 18-20).

We claim that the wall of the proventriculus is composed of four layers - *tunica mucosa*, *tunica submucosa*, *tunica muscularis* and *tunica serosa*, as the mucosa forms folds and grooves covered by a single layer of prismatic epithelium. At the same time, the glands are compound, tubuloalveolar, composed of polymorphic lobules. On the other hand, the muscular stomach consists *of tunica mucosa*, *tunica muscularis* and *tunica serosa*, as the mucosa is covered by a keratinized layer and also has folds. It should be noted that the glands in the grooves are strongly branched, unlike those in the folds, and *lamina muscularis mucosae* is missing. Therefore, the above results correspond to the data on the layered structure of the proventriculus and gizzard in the Japanese quail (21).

The histological structure in the wall of the proventriculus and gizzard in age aspect was determined, as well the changes of the micrometric parameters in the wall of these organs in the Bronze Turkey. We investigated the relationship between the variations of these parameters in the age aspect. The obtained data could be used as a basis to monitor the normal development of the proventriculus and gizzard, which is a marker for the normal growth of the birds.

REFERENCES

1. Mihaylov, R., Comparative investigations of the morphological features of the intestinal canal of the Japan quail (*Coturnix Japonica T. & Sch.*) and wild quail (*Coturnix coturnix L.*). Dissertation, Trakia University, Stara Zagora, Bulgaria, 2006.
2. Mihaylov, R. and Dimitrov, R., Comparative weight and metric traits of intestines in ducks, quails and broiler chickens. *Journal of Animal Science*, 47: 31-38, 2010.
3. Mihaylov, R. and Dimitrov, R., Comparative weight and metric traits of intestines in Japanese quails (*Coturnix coturnix Japonica*), common quails (*Coturnix coturnix, Lineus*, 1758) and their hybrids. *International Journal in Physical & Applied Sciences*, 2: 33-38, 2015.
4. Qureshi, AS., Faisal, T., Saleemi, K., and Ali, M. Z., Histological and histometric alterations in the digestive tract and accessory glands of duck (*Anas platyrhynchos*) with sex and progressive age. *J Anim Plant Sci* 27: 1528-33, 2017.
5. Kausar, R., Raza, S., Hussain, M. and Bahadur, S. U. K., Histometerical and morphological studies of digestive tract and associated glands in domestic pigeon (*Columba livia*) with regard to age. *Pak Vet J*, 39: 573-577, 2019.
6. Wasilewski, R., Kokoszyński, D., Mieczkowska, A., Bernacki, Z. and Górska, A., Structure of the digestive system of ducks depending on sex and genetic background. *Acta Vet Brno*, 84: 153-158, 2015.
7. Dong, X. Y., Wang, Y. M., Dai, L., Azzam, M. M. M, Wang, C. and Zou, T. C., Posthatch development of intestinal morphology and digestive enzyme activities in domestic pigeons (*Columba livia*). *Poultry Science*, 91:1886–1892, 2012.
8. Klasing, KC. Comparative avian nutrition. *CAB International*, Wallingford UK, 1998.
9. Mabelebele, M., Norris, D., Brown, D., Ginindza, M. and Ngambi, J. Breed and sex differences in the gross anatomy, digesta ph and histomorphology of the gastrointestinal tract of *Gallus gallus domesticus*. *Brazilian Journal of Poultry Science*, 19: 339 – 346, 2017.
10. Klasing, K. C. Avian gastrointestinal anatomy and physiology. *Semin Avian Exotic Pet Med*, 8: 42-50, 1999.
11. Hassouna, E. M. A., Some anatomical and morphometrical studies on the esophagus and stomcah in goose, turkey, sparrow, kestrel, hoopoe, owl and darter. *Assiut Vet Med J*, 44: 21-46, 2001
12. Dyce, K. M. and Sack, W. O., Text book of Veterinary anatomy. fourth edition, W.B. Saunders Company, Philadelphia, London and Toronto. 2010.
13. Abumandour, M. M., Morphological studies of the stomach of falcon. *Scientific Journal of Veterinary Advances*, 2: 30-40, 2013.
14. Hristov, H., Vladova, D., Kostov, D., Dimitrov, R., Gross anatomy of some digestive organs of the domestic canary (*Serinus canaria*). *Trakia Journal of Sciences*, 15: 106 – 112, 2017.
15. Hristov, H., Vladova, D., Kostov, D., Dimitrov, R., Chaprazov, C. and Goranov, N., Proventricular dilatation disease: Anatomical aspects of the diagnostics of the domestic canary (*Serinus canaria*). *Bulgarian Journal of Veterinary Medicine*, 20: 37 – 44, 2017.
16. Age morphological and imaging anatomical study of the glandular part of the stomach in domestic canary bird (*Serinus canaria*). Dissertation, Trakia University, Stara Zagora, Bulgaria, 2020.
17. Hristov, H., Avian stomach anatomy – a mini review. *Bulgarian Journal of Veterinary Medicine*, 24: 461 - 468, 2020.
18. Denbow, D. M., Gastrointestinal anatomy and physiology. In: Whittow G.C. (ed), *Sturkie's Avian Physiology*, Academic, San Diego, CA, pp. 299- 325. 2000.

19. Gartrell, B. D., The nutritional, morphologic, and physiologic bases of nectarivory in Australian birds. *J Avian Med Surg*, 14: 85-94, 2000.
20. Taylor, M., Anatomy and physiology of the gastrointestinal tract for the avian practitioner. In: Birds. *Post Grad* Found in Vet. Sci. Univ. of Sydney, Australia, pp. 107- 113, 2000.
21. Ahmed, Y., Kamel, G. and Ahmad, A., Histomorphological studies on the stomach of the Japanese quail. *Asian Journal of Poultry Science*, 5: 56 – 67, 2011.
22. Yovchev, D., 2020. Histological, histometric and histochemical investigation on the small intesines of the Bronze turkey (*Meleagris meleagris gallopavo*). Dissertation, Trakia University, Stara Zagora, Bulgaria, 2020.
23. Nomina Histologica Veterinaria, 2017. http://www.wava-amav.org/downloads/NHV_2017.pdf (10 February 2020 date last accessed).