Original Contribution

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

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ABSTRACT
The purpose of the present study was to investigate the histologic and micrometric study of the
devontriculus 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
devontriculus 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 devontriculus 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
devontricural 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, devontriculus, 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).

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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 occur 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 tubes 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).

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).
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.

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

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 till 14th day of age (Table 2).
Table 2. Micrometric parameters of the structures in gizzard of the Bronze turkey in age aspect.

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

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
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