

Histological and histometric study of the small intestine in Stara Zagora white turkey in age aspect

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INTRODUCTION

ABSTRACT

Thirty clinically healthy Stara Zagora white turkeys (15 females and 15 males) were used for the study. Eighteen tissue samples (six from the middle of each segment of the small intestine) of the appropriate age group were used to prepare permanent histological specimens. The histological sections were stained by Masson - Goldner method. The small intestine of the Stara Zagora white turkey was composed of four tissue layers: tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. Tunica mucosa had lamina epithelialis, lamina propria and lamina muscularis mucosae. The layers of the mucosa formed well-defined intestinal villi - villi intestinales. Lamina epithelialis was composed of highly differentiated simple columnar epithelium. Tunica mucosa in the three segments of the small intestine had clearly distinct tubular glands - gll. Intestinales. The histometric results showed that the growth of the mucosal structures and the mucosa was more intense in the first twenty-eight days, compared to the growth of the other layers of the intestinal wall. The most intense was the increase in the height and area of the intestinal villi. The height of the intestinal villi varied most markedly during the first twenty-eight days of the duodenal examination, while the area of the villi increased most in the jejunum, followed by the ileum and duodenum. The height of the intestinal epithelium increased most intensively 2.3 times in the jejunum mucosa. The thickness of the jejunal mucosa increased the most during this period, compared to the mucosa of the duodenum ileum.

Morphometric studies of internal organs in *Anseriformes* found a variable change in the length of the tubal organsesophagus and intestine (1, 2, 3).

Objective data regarding the development of the intestine in quails, ducks, geese and guinea fowl were obtained using statistical methods (4, 5, 6, 7).

The length of the body in marine ducks and the volume of the organs that construct their digestive system are directly proportional to the food components and the type of diet (8).

All digestive organs of the duck, with the exception of the esophagus, reach peak growth levels earlier than the body weight of the birds (9).

The microscopic anatomy of the digestive system in poultry is governed by certain principles. The tubular digestive organs have highly differentiated epithelial cells on their inner surface, surrounded by loose fibrous connective and muscle tissue. This is the so-called mucosal layer (10, 11).

The basic structure of the ileum in the domestic turkey does not differ from that of the duodenum and the jejunum. The villi in the ileum are shorter and few in number, compared to the same structures in the duodenum and jejunum. The goblet cells are the most numerous (12).

On histological study is found that the small intestine of the Bronze Turkey has four layers: tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. Tunica mucosa has lamina epithelialis, lamina propria and lamina muscularis mucosae. Intestinal villi are mucous formations on the luminal surface of the intestinal wall. Lamina epithelialis is composed of a single layer of columnar epithelium. On the first day after hatching, the epithelial cells are oval in shape, which later acquire a columnar shape with pronounced morphological polarity. The height and width of the intestinal villi increase with age. These changes are observed in the three segments of the small intestine (duodenum, jejunum and ileum). The height of the intestinal villi in the jejunum and ileum is higher than in the duodenum. At the same time, the increase in the width and height of the villi corresponds to the increase in the absorption surface. The values of the area of the villi are directly proportional to the changes in their width and height. The intensity of growth of intestinal crypts in the first 90 days has similar trends in the duodenum, and the fastest growth rates have been found for crypts in the ileum. The ratio of the height of the intestinal villi to the depth of the crypts increases in the first 28 days for all segments of the small intestine (12)

The height of the intestinal villi in the duodenum and jejunum in the bronze turkey throughout the study period increases 7.8 times, while the same indicator in the ileum -6.4 times. During the period from the 1st to the 35th day the fastest growth rate of the width of the intestinal villi was found in the duodenum - 2.6 times, and with the weakest rate the same indicator was reported in the ileum - 1.9 times. The obtained data for the area of the villi are directly proportional to the changes in the width and height of the studied intestinal structures. The values concerning the depth of the intestinal crypts in the duodenum, jejunum and ileum increase more intensively in the first 90 days after hatching. The fastest growth rates are characteristic of the crypts in the ileum (12).

MATERIAL and METHODS

Thirty clinically healthy Stara Zagora white turkeys (15 females and 15 males) were used for the study.

Eighteen tissue samples (six from the middle of each segment of the small intestine) of the appropriate age group were used to prepare permanent histological specimens. The resulting tissue samples were fixed in 8% 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. Using a YD-335A rotary microtome (J. Y. M. A. Ltd., China), sections 5 to 7 μ m thick were made and stained by Masson -Goldner method. Histological samples were observed with a Leica DM 1000 light microscope.

Structures from five histological sections obtained from each tissue sample were selected and measured. Micrometric measurements were processed using LAS V4.10 software for Leica DM 1000 light microscope.

The one-way ANOVA method was chosen for statistical processing of the obtained data, and for this purpose we used StatView v. 4.53 for Windows (Abacus Concepts, Inc). Statistically significant differences between the mean values of the age groups were determined at P < 0.05 (13).

The results obtained were interpreted in accordance with the histological terminology (14).

The requirements of the normative regulations were observed - Ordinance No 36 of March 23, 2006, promulgated. in SG, No 35 of 28. 04. 2006 and Ordinance No 8, promulgated, SG, iss. 4 of 16. 01. 2004, amended and add., No. 19 of 13 March 2015. The experiments were performed in strict accordance with the rules of the ethics committee at Trakia University, Stara Zagora.

RESULTS

The small intestine of the Stara Zagora white turkey was composed of four tissue layers: *tunica mucosa, tunica submucosa, tunica muscularis* and *tunica serosa. Tunica mucosa* had *lamina epithelialis, lamina propria* and *lamina muscularis mucosae*. The layers of the mucosa formed well-defined intestinal villi - villi intestinales. *Lamina epithelialis* was composed of highly differentiated simple columnar epithelium. *Tunica mucosa* in the three segments of the small intestine had clearly distinct tubular glands - *gll. intestinales* (Liberkyun glands) (Figure 1, Figure 2 and Figure 3).

Analysis of the histometric results showed that the growth of the mucosal structures and the mucosa as a whole was more



Figure 1. *Duodenum* in Stara Zagora white turkeys (age – thirtyfive days). (1) *lamina epithelialis mucosae*; (2) *lamina propria mucosae*; (3) intestinal crypt; (4) *tunica submucosa*; (5) *tunica muscularis* – internal sublayer; (6) *tunica muscularis* – external sublayer. Masson's trichrome stain. Line = 500 µm.



Figure 2. *Jejunum* in Stara Zagora white turkeys (age – thirty-five days). (1) *lamina epithelialis mucosae*; (2) *lamina propria mucosae*; (3) intestinal crypt; (4) *lamina muscularis mucosae*; (5) *tunica submucosa*; (6) *tunica muscularis*. Masson's trichrome stain. Line = 500 µm.



Figure 3. *Ileum* in Stara Zagora white turkeys (age – thirty-five days). (1) *lamina epithelialis mucosae*; (2) *lamina propria mucosae*; (3) intestinal crypt; (4) *lamina muscularis mucosae*; (5) *tunica submucosa*; (6) *tunica muscularis* – internal sublayer; (7) *tunica muscularis* – external sublayer. Masson's trichrome stain. Line = 500 μm.

intense in the first twenty-eight days, compared to the growth of the other layers of the intestinal wall. The most intense was the increase in the height and area of the intestinal villi, and the weaker was the increase in the depth of the crypts and the height of the mucosal epithelium. The height of the intestinal villi varied most markedly during the first twenty-eight days of the duodenal examination, while the area of the villi increased

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Duodenal structures	Age (days)						
	1	7	14	28	35	49	56
Height of villus	186.05 ±	285.25 ±	356.14 ±	752.36 ±	1045.25 ±	1210.25 ±	1235.89 ±
	9.04ª	11.48 ^b	10.28 ª	15.85 ª	28.36 ª	85.69ª	69.58 ª
Depth of crypts	36.89 ±	76.18 ±	82.35 ±	125.5 ±	185.65 ±	288.36 ±	304.58 ±
	5.14 ^b	6.16 ^a	10.25 ^b	12.33 ^b	9.36 ^b	12.36 ^b	26.36 °
Height of epithelium	15.17 ±	$20.58~\pm$	22.35 ±	25.36 ±	27.85 ±	32.25 ±	35.96 ±
	1.17 ^c	0.96 °	1.25 °	2.25 ª	2.96 °	2.35 °	2.48 °
Villus area	407.63 ±	679.85 ±	845.58 ±	1432.25 ±	2218.36 ±	2395.65 ±	2578.36 ±
	65.36 ª	79.36 ª	72.25 ª	125.58 °	124.85 °	112.32 °	152.28ª
Tunica mucosae	201.25 ±	365.85 ±	437.48 ±	895.62 ±	1014.35 ±	1420.25 \pm	1432.88 ±
	24.58 ^b	26.58 ^b	19.85 °	24.45 ª	85.25 ª	105.25 ª	110.25 °
Tunica submucosae	5.25 ±	8.14 ±	17.85 ±	19.25 ±	21.45 ±	23.45 ±	25.85 ±
	0.14 °	0.98 ª	1.25 ^b	2.04 ^b	0.99 ^b	1.28 ^b	1.48 ª
Tunica muscularis –	66.25 \pm	82.58 ±	97.58 ±	125.85 ±	146.28 ±	186.36 ±	209.58 \pm
internal sublayer	2.56 ª	3.25 °	2.89 ª	5.65 °	7.89 °	8.16 °	6.96 °
Tunica muscularis –	21.25 ±	28.92 ±	38.25 ±	$40.02 \pm$	52.24 ±	$63.28 \pm$	60.91 \pm
external sublayer	0.55 °	1.02ª	1.25 °	2.15 °	2.84 °	3.02 ª	3.92 ^b

Table 1. Histometric parameters of some duodenal structures in Stara Zagora white turkey (mean ± SEM)

Table 2. Histometric parameters of some jejunal structures in Stara Zagora white turkey (mean ± SEM)

Jejunal structures			Age (days)				
	1	7	14	28	35	49	56
Height of villus	118.58 ±	225.58	318.48 ±	450.25 ±	478.58 ±	560.23 ±	985.25 ±
	10.25 ^a	9.85 ^b	10.23 ª	8.56 ^b	12.35 ª	48.25 ^b	96.36 ª
Depth of crypts	$20.34~\pm$	$38.58 \pm$	$63.85 \pm$	90.25 \pm	125.85 ±	156.89 \pm	185.95 \pm
	0.24 ^b	0.38 ª	0.98 ^b	1.25 ª	2.14 ^b	4.17 ^a	6.17 ^b
Height of epithelium	10.28 \pm	16.58 \pm	$20.58~\pm$	$23.65 \pm$	25.14 ±	$30.76~\pm$	32.25 ±
	0.24 ª	0.58 ^b	0.75 ª	1.12 ^b	1.52ª	2.02 ^b	1.65 ^a
Villus area	$3568.58\pm$	14587±	16854.35±	28547.58±	36958.59±	47158.25±	$50284.24 \pm$
	125.85 ^b	185.25 ª	198.58 ^b	214.74ª	617.84 ^b	624.25 ª	785.59 ^b
Tunica mucosae	131.25±	$299.65\pm$	396.58±	614.28±	728.58±	$1068.98 \pm$	1289.65±
	5.65 ^c	4.16 °	4.58 °	9.18 ^b	8.97 °	25.85 ^b	35.69 °
Tunica submucosae	8.54±	13.25±	19.56±	21.58±	25.98±	30.25±	35.98±
	0.24 ª	0.58	0.92ª	1.58 °	2.16ª	3.13 °	3.55 ª
Tunica muscularis –	56.24±	$68.58\pm$	82.56±	115.58±	143.59±	187.25±	$202.58\pm$
internal sublayer	1.24 °	2.05 ª	2.58 ^b	4.12 ^a	5.15 ^b	6.18ª	7.14 ^b
Tunica muscularis –	17.19±	24.25±	28.59±	32.59±	35.92±	58.24±	76.62±
external sublayer	0.25ª	0.28 ^b	0.65°	1.09 ^b	1.55°	4.16 ^b	5.89°

most in the jejunum, followed by the ileum and duodenum. The height of the intestinal epithelium increased most intensively 2.3 times in the jejunum mucosa. The thickness of the jejunal mucosa increased the most during this period, compared to the mucosa of the duodenum ileum. *Tunica submucosa* and the sublayers of *tunica muscularis* grew at almost uniform rates in

Ileal structures	Age (days)						
	1	7	14	28	35	49	56
Height of villus	145.85±	298.35±	458.95±	532.25±	678.59±	758.25±	835.69±
	8.56ª	7.16 ^b	12.15ª	10.85 ª	13.45 ^ь	16.78 °	20.21 ª
Depth of crypts	41.42±	78.56±	85.25±	158.95±	167.84±	178.95±	195.58±
	2.16 ^b	3.15 ª	4.58^{b}	9.50 ^b	12.58 ª	15.58 ^b	12.25 ь
Height of epithelium	12.45±	14.28±	15.23±	17.34±	22.58±	27.48±	32.58±
	0.25 ^c	0.34 ª	0.22ª	0.58 °	0.98 °	1.34 ª	1.28 °
Villus area	4560.25±	14582.36±	28352±	34568±	45895.35±	56897.23±	69854.56±
	125.87ª	156.78 ^b	168.98 °	212.58 ª	198.89ª	159.23 °	256.34ª
Tunica mucosae	235.35±	386.65±	612.22±	784.58±	854.56±	925.58±	1041.25±
	5.89 ^b	7.89 ª	12.15 ^a	19.96ª	29.59 °	28.85 ª	26.62 ^b
Tunica submucosae	8.25±	12.59±	$21.58\pm$	$28.65\pm$	45.25±	51.25±	69.58±
	0.55 °	0.69 °	0.95 °	1.12 ^b	5.65 ^b	4.28 ^b	5.16 °
Tunica muscularis –	52.28±	56.89±	86.32±	121.23±	182.56±	195.69±	$280.56 \pm$
internal sublayer	6.15 ^b	4.13 ª	5.12ª	8.16 °	14.25 ª	15.85 ª	24.58 ª
Tunica muscularis –	16.58±	18.59±	34.58±	54.25±	57.89±	62.23±	99.65±
external sublayer	1.21 ª	0.58 °	2.89 °	2.58 ª	9.16 °	5.98 °	12.19 °

Table 3. Histometric parameters of some ileal structures in Stara Zagora white turkey (mean \pm SEM)

the three segments of the small intestine, except for the outer sublayer of *tunica muscularis* of the ileum, where the thickness doubled (Table1, Table 2 and Table 3).

DISCUSSION

The data presented by us about the micrometric changes in the layers and mucous structures of the small intestine in the Stara Zagora White Turkey complement the thesis of some authors (1, 2) for the chages of the organs from the digestive tract in age aspect. In our opinion, the growth of the mucosal structures and the mucosa was more intense during the first twenty-eight days, compared to the growth of the other layers of the intestinal wall.

The results, which expressed the age-related changes in the studied structures from the histological sections, were statistically processed for completeness. Like other studies (4, 5, 6, 7), we assume that such changes are reported as objective after statistical processing.

Based on the micrometric results concerning the duodenum, jejunum and ileum, it can be concluded that the normal values of the studied parameters are a key factor for the integrity of the small intestine in Stara Zagora White turkey, and are dependent on diet conditions and animal husbandry. This thesis complements the view (8) in ducks on the relationship between intestinal mucosal integrity and the environment.

The histological structures of the small intestine in the studied birds include highly differentiated epithelial cells that participate in the formation of the mucous layer. This theory adds the published data (10, 11) on the microscopic anatomy of the digestive system in other poultry species.

The data for the histological structure of the intestinal wall in the Stara Zagora White Turkey showed that all three intestinal segments have characteristics specific to the tubular digestive organs. This thesis is confirmed by the histological findings - the presence of *tunica mucosa*, composed of epithelial cells located on layers of loose fibrous connective and muscle tissue, *tunica submucosa*, *tunica muscularis* and *tunica serosa*. The theory presented by us corresponds to the findings (12) on the histological structure of the small intestine in the Bronze turkey.

The histometric study investigated the patterns in the change in the height of the intestinal villi and the depth of the intestinal crypts in age aspect. It was found that the height of the villi intestinales in the three segments of the small intestine increased with age. These changes were more intense in the jejunum than in the duodenum and ileum. It is important to note the fact that the area of the villi changed in age more noticeably in the jejunum and ileum than in the duodenum. The results of the study convincingly showed that the changes in the depth of the crypts were more intense in the jejunum and ileum than in the duodenum. The results differ from the data (12) that this index changes most intensively in the duodenum in the Bronze turkey.

The presented results differ from the hypothesis (12) for the small intestine in the Bronze turkey, because the height of the intestinal epithelium increased most intensively in the mucosa of the jejunum, while the thickness of the jejunal mucosa increased the most during this period, compared to the mucosa of the duodenum and ileum. In addition, *tunica submucosa* and the sublayers of *tunica muscularis* grew at almost uniform rates in the three segments of the small intestine, except for the outer sublayer of *tunica muscularis* in the ileum, where the thickness doubled. Thus the theory (12) on the biological significance of these histometric indicators is complemented.

DECLARATIONS

Ethics Approval

This study was approved by the committee for animal ethics in Trakia University (No: 01/2011, No: 09/2016), Bulgaria.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contribution

All authors contributed to every step of the article.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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