Bulgarian Journal of Agricultural Science, 25 (No 3) 2019, 552–557

Micromorphometric study of the small intestines in different post-hatch periods in bronze turkey (*Meleagris meleagris gallopavo*)

David Yovchev*, Georgi Penchev, Dimitar Dimitrov, Kamelia Stamatova-Yovcheva

Trakia University, Faculty of Veterinary Medicine, Department of Veterinary Anatomy, Histology and Embryology, 6000 Stara Zagora, Bulgaria
**Corresponding author:* vetjovchev@yahoo.com

Abstract

Yovchev, D., Penchev, G., Dimitrov, D. & Stamatova-Yovcheva, K. (2019). Micromorphometric study of the small intestines in different post-hatch periods in bronze turkey (*Meleagris meleagrisk gallopavo*). *Bulgarian Journal of Agricultural Science*, *25*(3), 552–557

The normal development of the bronze turkey during post-hatch period depends on many factors. Two of the most important are: villus size and crypt size. Otherwise the structural development of the small intestinal epithelium in the birds is important for the normal gut absorption capacity and whole body growth. The aim of our investigation was to study the histological parameters – depth of the crypts and height of the villus in bronze turkeys' small intestines and the enterocytes' number in accordance to villus length. The height and width of the intestinal villi increased in age aspect. Both indices in duodenum, jejunum and ileum increased the most intensively till 90th day. The height of intestinal villi in the duodenum for the period from 1st to 90th day increased 3.4 times. The same index reported in the jejunum and ileum was higher, – 5.7 and 6.0 times respectively. The increase in the width and height of the villi corresponded with the increase of the absorption surface. The increase in the width and height of the villi corresponded with the increase of the absorption surface. The fastest growth rates were found for the ileum crypts – 5.4 times. The ratio of the height of the intestinal villi to the depth of the crypts increased from 1st day to 28th day for all segments of the small intestine.

Keywords: small intestines; bronze turkey; micromorphology

Introduction

The digestive system in birds develops intensively after the hatching period. The intensity of changes in the length, weight and diameter of intestinal structures is genetically conditioned and depends on the changes in the environment. The development of the small and large intestine in quails, broilers, pheasants, guinea fowl and geese in age aspect is the object of research by a number of authors (Mihaylov, 2006; Amerah et al., 2008; Gabriel et al., 2008; Mihaylov & Dimitrov, 2008; Mihaylov et al., 2008; Liu et al., 2010; Mihaylov et al., 2012; Mihaylov & Dimitrov, 2015).

According to Dibner et al. (2007) microscopic anatomy of the digestive system in birds is subject to certain principles. Tubular digestive organs have on their inner surface highly differentiated epithelial cells surrounded by loose fibrous connective and muscle tissue. This is the mucosal layer.

The morphological development of the glandular and muscular stomachs and the intestines in the birds associated with the increase in the secretory – absorption surface of their mucosal layer is a key factor for intensive growth in wild and domestic birds (Nitsan et al., 1991; Thompson & Applegate, 2006).

At the base of the intestinal villi are the crypts, which represent the zone of enterocyte proliferation. In the central

part of the intestinal villi is a capillary system, which transports the nutrients to the systemic circulation. The increase in number and size of the intestinal villi increases the absorption surface per unit intestinal area (Traber et al., 1991; Ferraris et al., 1992; Thomson et al., 1994).

In the post-hatching period, the volume of the intestinal villi in the duodenum, the jejunum and the ileum of the chicken broilers increases. Duodenal villi reach their final growth on the 7th day after hatching, and those in the jejunum and ileum on the 14th day after hatching (Uni et al., 1995; Uni et al., 1999).

In domestic birds after the period of hatching, the ratio crypts relative villi is 1: 1, as the number of cells is small. During the first days after hatching, the number of crypts increases while hyperplasia is observed. After 4 to 5 days, three or four villi correspond to each intestinal crypt (Uni et al., 1998; Geyra et al., 2001).

When hatching, small intestine enterocytes are round and non-prismatic. At 24 – 48 hours after hatching, these cells grow rapidly in length, acquire a pronounced polarity, their edge is defined and brush-shaped. Enterocytes are rhomboid cells located on the outer surface of the intestinal villi (Traber et al., 1991; Ferraris et al., 1992; Thomson et al., 1994; Uni et al., 2003).

The small intestine of a newly hatched chicken is underdeveloped and changes significantly during the first few days after hatching. This feature is manifested by the presence of underdeveloped crypts and the lack of a clearly defined area of enterocyte proliferation. During the first 4-5 days after hatching, the interaction between intestinal crypts and villi is clearly defined by the existence of a distinct proliferative zone and constant enterocyte migration. Changes in morphological development of the small intestines are related to enterocyte differentiation, differentiation of crypts and expansion of intestinal folds in the intestinal absorption surface (Uni et al., 1998; Uni et al., 2003).

Many studies have shown that the most significant structural changes which occur with age are: increasing the length of the intestinal tract, the height and density of intestinal villi resulting in the increasing number of enterocytes, goblet cells and enteroendocrine cells (Imondi & Bird, 1966).

According to Sklan (2001), the physiological growth of the small intestine is accompanied by an increase in the size of the intestinal villi, which in turn doubles during the first 48 hours after hatching.

The duodenal villi are highest in the post-hatching period (they are two ways higher than in the other intestinal segments) (Baranylova & Holman, 1976).

At 4 days of age, the increase of the volume of the intestinal villi is inversely proportional to the relative growth of the duodenum. From 4 to 10 days of age, the height and the perimeter of the intestinal villi increased from 34 to 100% in comparison with the period immediately after hatching (Noy & Sklan, 1997).

According to Noy & Sklan (1997), the rate of increase of avian intestinal villi in the jejunum and ileum is delayed at 10 days of age.

The lack of data concerning the micromorphological changes in the small intestine in the bronze turkey, from the hatching of the birds to maturity was the motif to conduct the study.

Materials and Methods

Materials

For the study, 60 clinically healthy Bronze Turkeys (30 females and 30 males) were used. The experimental animals were purchased from state forestry Mazalat. They were divided into 10 age groups (one-day, 7-day, 14-day, 28-day, 35-day, 49-day, 56-day, 90-day, 120-day and 240-day-old). Each group consisted of six turkeys. Birds were weighed on an automatic scale with an accuracy of 10 grams. Following euthanasia, in a regulated slaughterhouse for poultry production and processing with a combined line for the slaughter of chickens, hens and turkeys, the birds were delivered to the Department of Cytology, Histology and Embryology at the Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Thracian University, Stara Zagora, Bulgaria. The requirements of the regulations (Regulation No 36 from 23/ 03/ 2006, published in Government Gazette, issue 35 from 28/ 04/ 2006) on hygiene and control at slaughter were observed. The experiments were carried out in strict compliance with the rules of the ethics committee of the Thracian University, Stara Zagora.

Methods

Processing of permanent histological preparations stained with hematoxylin (by Erlich – eosin from duodenum, jejunum and ileum.

Nine tissue samples (three from each segment of the small intestine) of the appropriate age group were used to produce permanent histological preparations. The resulting tissue samples were fixed in 10% aqueous formaldehyde solution (Merck KGaA, Darmastadt, Germany). After fixation, they were washed in running water, dehydrated in an ascending ethanol series, cleared in xylene and embedded into paraffin. Rotary microtome YD-335A (J.Y.M.A. Ltd., China) cuts with a thickness of 5 to 7 μm were made. The coloration was carried out after two times deparaffining of the cuts for 30-60 s in xylene (two cuvettes) and placed in descending

alcoholic range (absolute to 70% ethyl alcohol), every 20–30 s, then placed in water and then in hematoxylin for 2–5 min. After staining the preparations were rinsed in water, left in distilled water for 5–10 min until blue color was obtained and stained with eosin for a few seconds. After rinsing with water, the preparations were dehydrated in an ascending alcohol line and cleared in xylene. After inclusion in entelan, permanent microscopic preparations were obtained (Vitanov et al., 1995, Dimitrov, 2009).

The preparations were observed with a light microscope – VDN-200M (LUMENLAB, China), and the results were documented by a digital CMOS camera in the microscope. The obtained data were processed using the software supplied by the manufacturer for this microscope model – ScopeImage Advanced Micro-image Process Software.

The study was conducted in the Department of Cytology, Histology and Embryology, Department of Veterinary Anatomy, Histology and Embryology at the Faculty of Veterinary Medicine at Thracian University, Stara Zagora.

Determination of the micro-morphometric parameters of the main microstructural elements of the small intestines – height and width of the intestinal villi, depth of intestinal crypts, area of villi, ratio to villus height and crypt depth.

From each histological preparation, 10 intact, strictly vertically oriented crypts and villi were selected to determine the micromorphometric parameters (270 measurements of each age group, for each intestinal segment were thirty measurements).

The height of intestinal villi was measured from the tip to the base of the villi. The width of the villi was found by measuring the distance between the outer surfaces of two adjacent epithelial edges passing through the vertical middle of the intestinal villi.

The depth of the crypts was measured, founding the depth of the invasion between the neighboring villi.

The area of the intestinal villi was calculated based on their heights and widths.

Results

The height and width of the intestinal villi increased in age aspect. These changes were observed in the three segments of the small intestine (duodenum, jejunum and ileum). Both indices in duodenum, jejunum and ileum increased the most intensively till 90th day (Table 1 and Table 2).

The height of intestinal villi in the duodenum for the period from 1st to 90th day increased 3.4 times. The same index reported in the jejunum and ileum was higher, – 5.7 and 6.0 times respectively (Table 1).

The increase in the width and height of the villi corresponded with the increase of the absorption surface.

The obtained data for the area of the villi were directly proportional to the variations of the width and height of the studied intestinal structures (Table 3).

Table 1. Height of the intestinal villi in the small intestine of the bronze turkey

Villus height (μm)			
Age (d)	Duodenum	Jejunum	Ileum
1	456.37 ± 36.24	208.56 ± 28.31	190.64 ± 18.93
7	589.12 ± 30.02	378.54 ± 50.25	307.33 ± 20.31
14	702.86 ± 31.86	475.58 ± 17.56	450.76 ± 22.56
28	1104.02 ± 29.07	561.86 ± 15.35	517.38 ± 17.76
35	1299.16 ± 30.64	668.67 ± 19.53	637.80 ± 20.34
49	1400.35 ± 31.21	720.45 ± 15.68	752.92 ± 16.34
56	1460.71 ± 21.18	976.54 ± 24.76	800.12 ± 26.63
90	1560.28 ± 25.13	1104.97 ± 31.56	925.75 ± 28.47
120	1676.99 ± 22.38	1186.13 ± 27.63	1145.83 ± 30.74
240	2348.76 ± 32.48	1528.04 ± 31.22	1620.76 ± 40.56

Table 2. Width of the intestinal villi in the small intestine of the bronze turkey

Villus width (μm)			
Age (d)	Duodenum	Jejunum	Ileum
1	65.07 ± 11.31	62.83 ± 5.78	34.02 ± 4.31
7	79.34 ± 16.91	84.58 ± 25.36	59.97 ± 6.28
14	96.91 ± 21.18	110.98 ± 14.56	75.28 ± 9.12
28	134.05 ± 22.78	130.25 ± 12.45	101.78 ± 8.64
35	174.25 ± 21.16	134.72 ± 15.28	109.49 ± 9.25
49	186.68 ± 18.69	147.28 ± 19.28	122.58 ± 10.25
56	193.48 ± 19.76	150.68 ± 14.78	137.59 ± 12.24
90	205.86 ± 24.60	195.45 ± 29.35	156.81 ± 9.42
120	218.73 ± 22.76	205.12 ± 20.59	174.73 ± 10.42
240	239.68 ± 25.86	226.86 ± 18.76	185.35 ± 12.76.

Table 3. Area of the intestinal villi in the small intestine of the bronze turkey

Villi area (x1000 μm^2)			
Age (d)	Duodenum	Jejunum	Ileum
1	30.84 ± 4.31	12.75 ± 1.96	10.42 ± 1.04
7	38.87 ± 5.63	16.72 ± 2.95	14.42 ± 2.86
14	49.67 ± 58.10	37.14 ± 6.63	25.78 ± 4.58
28	72.86 ± 5.9	50.51 ± 10.15	37.84 ± 7.17
35	81.65 ± 6.32	68.32 ± 14.25	52.62 ± 10.76
49	123.58 ± 34.25	90.32 ± 10.29	84.59 ± 12.34
56	143.28 ± 30.42	118.75 ± 19.36	98.75 ± 22.51
90	162.87 ± 27.83	140.5235 ± 21.35	112.35 ± 20.76
120	173.24 ± 21.25	250.30 ± 20.01	238.65 ± 24.42
240	200.64 ± 38.48	271.01 ± 18.73	256.78 ± 21.87

The depth's values of the intestinal crypts in the duodenum, jejunum and ileum increased more intensively in the age range from the 1st to 90th day, compared to the same index, reported for the period from 90th to 240th day. The intensity of growth of intestinal crypts from the 1st to 90th day was with similar values in the duodenum – 4.7 times and jejunum – 4.8 times. The fastest growth rates were found for the ileum crypts – 5.4 times (Table 4).

Table 4. Depth of the crypts in the duodenum, jejunim and ileum of the bronze turkey

Crypt depth (μm)		
Duodenum	Jejunum	Ileum
60.37 ± 4.25	53.26 ± 5.85	58.74 ± 4.25
70.34 ± 5.65	65.34 ± 4.82	70.89 ± 5.26
80.24 ± 6.98	70.96 ± 6.45	80.25 ± 7.36
92.78 ± 8.12	80.25 ± 7.98	85.28 ± 8.14
110.90 ± 9.38	104.02 ± 11.34	110.56 ± 7.34
160.21 ± 14.03	110.75 ± 10.59	131.40 ± 9.52
170.26 ± 15.23	152.38 ± 11.12	152.38 ± 17.20
186.12 ± 17.24	165.05 ± 15.37	181.27 ± 20.35
197.21 ±13.96	190.49 ± 22.14	226.83 ± 18.26
279.67 ± 25.79	238.45 ± 22.38	311.56 ± 17.34

The ratio of the height of the intestinal villi to the depth of the crypts increased from 1st day to 28th day for all segments of the small intestine (Table 5). Then, from 49 days of age for the duodenum and 35 days of age for the jejunum and the ileum, the values of the index after a insignificantly decrease remained almost unchanged by the 240th day.

Table 5. Ratio of the height of the intestinal villi to the depth of the crypts

Villus height: crypt depth		
Duodenum	Jejunum	Ileum
7.62 ± 0.75	3.92 ± 0.26	3.35 ± 0.17
8.42 ± 0.68	5.87 ± 0.45	4.35 ± 0.24
8.73 ± 0.88	6.74 ± 0.68	5.67 ± 0.39
11.82 ± 1.31	7.01 ± 0.70	6.21 ± 0.31
11.79 ± 1.27	6.42 ± 0.58	5.87 ± 0.41
8.77 ± 0.74	6.58 ± 0.42	5.81 ± 0.26
8.68 ± 0.84	6.55 ± 0.26	5.34 ± 0.17
8.57 ± 0.79	6.61 ± 0.32	5.39 ± 0.22
8.51 ± 0.75	6.43 ± 0.42	5.41 ± 0.39
8.68 ± 0.81	6.56 ± 0.29	5.36 ± 0.25

Discussion

The results of our micromorphological investigations, demonstrating that the intensive increasing of the width, length and diameter of the turkey's small intestines was observed after hatching, was similar to the given data by some researchers for the quail, broilers, pheasants, guinea fowl and geese (Mihaylov, 2006; Amerah et al., 2008; Gabriel et al., 2008; Mihaylov & Dimitrov, 2008; Mihaylov et al., 2008; Liu et al., 2010). We assume that these parameters change and increase in age aspect and in the same time the environment has impact on their variation.

In our algorithm of study, the points were to determine the amendment in the micromorphometric parameters of the villi and crypts of the small intestines of the bronze turkey. We considered the facts that the variations of the mucosal layer's structures – villi and crypts and their micromorphometric parameters were essential for the development of the small intestines. That presents a new aspect in the morphological development of the small intestines in the bronze turkeys, according to which the intestinal growth is a subject to definitive principles, appropriate for the avian tubular digestive organs. Additionally, the development of the small intestines in the bronze turkeys was in close relation with the increasing of the height and width of the villi and crypts' depth (Nitsan et al., 1991; Thompson & Applegate, 2006; Dibner et al., 2007).

The increase in the number and size of the intestinal villi in the bronze turkey was related to villi area. The area of the intestinal villi increased after hatching. The variations of this parameter were important for the morphological development of the small intestines. Therefore, we support the thesis of some authors (Traber et al., 1991; Ferraris et al., 1992; Thomson et al., 1994) that the increase of the density and size of the villi and respectively villi area after hatching corresponds to increasing of the intestinal absorption surface.

Our results which concern that the most intensive changes of the height and width of the villi in the bronze turkeys' small intestines were till 90th day for the three segments – duodenum, jejunum and ileum contradicted the attitude of Uni et al (1995) and Uni et al. (1999) for the most intensive growth of the broilers' intestines till 7th day after hatching. Additionally, we found that these alterations have been less intensive in the duodenum (3.4 times), compared to the same index in the jejunum and ileum (5.7 and 6.0 times), which was a marker for faster growth of both segments.

Our algorithm of investigation was focused only to specify the width and height of the villi, villi area and crypt depth and the ratio between villus heights to crypt depth in age aspect. Contrary to the published data (Uni et al., 1998; Geyra et al., 2001), we confirm that these parameters are adequate to investigate the normal development of the small intestines of the bronze turkeys in age aspect.

The identified by us specifics of the enterocytes' shape in the bronze turkey's small intestines from hatching to 240th days of age (at the first day after hatching – round and in the following periods – prismatic with polarity) corresponded to the results for the same cells in other avian species (Traber et al., 1991; Ferraris et al., 1992; Thomson et al., 1994; Uni et al., 2003).

We claim that the increase in the width and height of the villi corresponded with the increase of the absorption surface; the increasing of the villi area was directly proportional to the variations of the width and height of the studied intestinal structures. Furthermore, we assert that the intensity of crypts' growth in the duodenum, jejunum and ileum increased more rapidly in the period of intensive growth of the turkeys as the fastest changes were found in the ileum. Our hypothesis is that the variations of these micromorphometric parameters are age related and that they are key factor for the development of the small intestines. We confirm that only these parameters are important to follow the growth of the studied organs without comparing them with the density of other cellular elements in the small intestines, contrary to the thesis of Imondi & Bird (1966).

According to us the morphological growth of the turkeys' small intestine is accompanied by an increase in the size of the intestinal villi, during the first week after hatching. Our thesis supports the published theory of Sklan (2001), for the small intestines in broilers.

Our results for the duodenal villi height gradual increasing after hatching and the greatest values of this parameter in duodenum, contrary to the same index, studied in jejunum and ileum add the data of some authors (Baranylova & Holman, 1976).

Collected results for the intensity of the growth of duodenal villi of the bronze turkey are a definitive reason to conclude that this parameter is slower than the same in the broilers (Noy & Sklan, 1997).

Conclusions

• The height and width of the intestinal villi increased in age aspect. These changes were observed in the three segments of the small intestine (duodenum, jejunum and ileum).

• The intensity of growth of intestinal crypts from 1st to 90th day was with similar values in the duodenum – 4.7 times and jejunum – 4.8 times. The fastest growth rates were found for the ileum crypts – 5.4 times.

• The ratio of the height of the intestinal villi to the depth of the crypts increased from 1st day to 28th day for all segments of the small intestine. Then, from 49 days of age for the duodenum and 35 days of age for the jejunum and the ileum, the values of the index after an insignificantly decrease remained almost unchanged by 240th day.

• The results will be important as a base for standard values of the small intestinal micromorphometric parameters.

References

Amerah, A. M., Ravindran, V., Lentle, R. G., & Thomas, D. G. (2008). Influence of feed particle size on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters fed wheat-and corn-based diets. *Poultry Science*, *87*(11), 2320-2328.

Baranyiova, E., & Holman, J. (1976). Morphological changes in intestinal wall in fed and fasted chickens in the 1st week after hatching. *Acta Veterinaria Brno*, *45*(3), 151-158.

Dibner, J. J., Richards, J. D., Kitchell, M. L., & Quiroz, M. A. (2007). Metabolic challenges and early bone development. *Journal of Applied Poultry Research*, *16*(1), 126-137.

Dimitrov, R. (2009). Morphofunctional and imaging features of the male accessories glands and pelvic part of the urethra in the tomcat. Dissertation, Trakia University, Stara Zagora, Bulgaria.

Ferraris, R. P., Villenas, S. A., & Diamond, J. (1992). Regulation of brush-border enzyme activities and enterocyte migration rates in mouse small intestine. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *262*(6), 1047-1059.

Gabriel, I., Mallet, S., Leconte, M., Travel, A., & Lalles, J. P. (2008). Effects of whole wheat feeding on the development of the digestive tract of broiler chickens. *Animal Feed Science and Technology*, *142*(1-2), 144-162.

Geyra, A., Uni, Z., & Sklan, D. (2001). Enterocyte dynamics and mucosal development in the posthatch chick. *Poultry Science*, *80*(6), 776-782.

Imondi, A. R., & Bird, F. H. (1966). The turnover of intestinal epithelium in the chick. *Poultry Science*, *45*(1), 142-147.

Liu, B. Y., Wang, Z. Y., Yang, H. M., Wang, X. B., Hu, P., & Lu, J. (2010). Developmental morphology of the small intestine in Yangzhou goslings. *African Journal of Biotechnology*, *9*(43), 7392-7400.

Mihaylov, R. (2006). 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.

Mihaylov, R., & Dimitrov, R. (2010). Comparative weight and metric traits of intestines in ducks, quails and broiler chickens. *Journal of Animal Science*, *47*(6), 31-38.

Mihaylov, R., & Dimitrov, R. (2015). 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*(5), 33-38.

Mihaylov, R., Dimitrov, R., & Yordanova, V. (2012). Comparative electronmicroscopical study of the enterocytes of the duodenum of the Japanese quail (*Coturnix japonica*) and the wild type (*Coturnix coturnix*). *Agricultural Science & Technology*, *4*(3), 328-331.

Mihaylov, R., Genchev, A., & Kabakchiev, M. (2008). Metric and

weight development of some organs from the digestive tract of Japanese quails (*Coturnix japonica*) from the hatching to maturity. *Journal of Animal Science*, *45*(1), 63-71.

Nitsan, Z., Dunnington, E. A., & Siegel, P. B. (1991). Organ growth and digestive enzyme levels to fifteen days of age in lines of chickens differing in body weight. *Poultry Science*, *70*(10), 2040-2048.

Noy, Y., & Sklan, D. (1997). Posthatch development in poultry. *Journal of Applied Poultry Research*, *6*(3), 344-354.

Sklan, D. (2001). Development of the digestive tract of poultry. *World's Poultry Science Journal*, *57*(4), 415-428.

Thomson, A. B. R., Cheeseman, C. I., Keelan, M., Fedorak, R., & Clandinin, M. T. (1994). Crypt cell production rate, enterocyte turnover time and appearance of transport along the jejunal villus of the rat. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1191*(1), 197-204.

Thompson, K. L., & Applegate, T. J. (2006). Feed withdrawal alters small-intestinal morphology and mucus of broilers. *Poultry Science*, *85*(9), 1535-1540.

Traber, P. G., Gumucio, D. L., & Wang, W. (1991). Isolation of intestinal epithelial cells for the study of differential gene expression along the crypt-villus axis. *American Journal of Physiology – Gastrointestinal and Liver Physiology*, *260*(6), 895-903.

Uni, Z., Ganot, S., & Sklan, D. (1998). Posthatch development of mucosal function in the broiler small intestine. *Poultry Science*, *77*(1), 75-82.

Uni, Z., Noy, Y., & Sklan, D. (1995). Posthatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. *Poultry Science*, *74*(10), 1622-1629.

Uni, Z., Noy, Y. & Sklan, D. (1999). Posthatch development of small intestinal function in the poult. *Poultry Science*, *78*(2), 215-222.

Uni, Z., Smirnov, A., & Sklan, D. (2003). Pre- and posthatch development of goblet cells in the broiler small intestine: effect of delayed access to feed. *Poultry Science*, *82*(2), 320-327.

Vitanov, S., Dimitrov, D., & Bochukov, A. (1995). Manual of cytology and histology with histological techniques. In: M. Popova (Ed.) *Methods for investigation in Cytology and Histology*. Zemizdat, Sofia, Bulgaria, 3-22.

Received: October, 29, 2018; *Accepted:* November, 7, 2019; *Published:* June, 30, 2019