

## HISTO-MORPHOMETRIC ADAPTATION IN THE SMALL INTESTINE OF BROILER CHICKEN, AFTER EMBRYONIC EXPOSURE TO – GALACTOSIDES

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### ABSTRACT

The objective of the present study was to investigate the effect of *in ovo* injection of – galactosides on the development of the intestinal villi in the small intestine of broiler chickens. In order to verify the objective 80 000 eggs were divided into two groups, the control and the experimental; 40 000 eggs in each. Solution containing – galactosides were injected *in ovo* into the air cell on the 12th d of embryonic development. At the age of 0 (DOH), 4, 7 and 14 days 10 animals of each group were randomly selected and slaughtered, and the intestine samples were collected for histological examinations. Results show that the single injection of – galactosides has a beneficial effect on morphometrical and histological parameters of intestine. In our study we observed that the each segments of small intestine and consequently the total length of the intestine was significantly higher ( $P < 0.05$ ) in the experimental group at day of hatch. Furthermore, the results showed stimulatory effect of the – galactosides on height of the villi, surface area and crypt depth in all studied segments of the intestine in the *in ovo* experimental group ( $P < 0.05$ ) at day of hatch. The observed effect of injection on studied parameters wasn't permanent and remained for 3 days after hatching.

**Keywords:** gut morphology, chicken, *in ovo*, – galactosides.

### INTRODUCTION

The small intestine, with particularly villi and crypts, which increase absorbing surface area, has an extremely important role in the final stage of nutrients digestion and assimilation (Wang and Peng, 2008). Absorption capacity depends mainly from the level of morphological and functional development of the small intestine (Akiba and Murakami, 1995; Yamauchi *et al.*, 1996). Therefore the rapid and early maturation of the intestine after hatching is crucial in the critical postnatal period. During the gut development the mucosa of the small intestine undergoes an important transformation, which determines its subsequent ability to absorb nutrients (Mitjans *et al.*, 1997; Yamauchi and Tarachai, 2000). This involves preparedness for rapid and effective transfer of *in ovo* to oral nutrition, resulting in maximum use of growth potential and complete development of the immune system (Uni *et al.*, 1998; Bar-Shira and Friedman, 2006). This period is crucial for the proper development of both digestive and immune systems as well as for the thermoregulation system in chickens. Many authors have confirmed the impact of substances with a prebiotic effect on height of villi and depth of intestinal crypts in various segments of the intestine (Xu *et al.*, 2003; Pelicano *et al.*, 2005; de los Santos *et al.*, 2007; Yang *et al.*, 2007; Baurhoo *et al.*, 2007). Furthermore, in many studies, the beneficial effect of prebiotics was routinely observed consequential by

morphological changes of intestinal mucosa which improved feed intake, weight gain and feed conversion rate (Waldroup *et al.*, 1993; Grimes *et al.*, 1997; Pelicano *et al.*, 2004; Collins *et al.*, 2009). Searching methods that stimulating the rate of development of the gastrointestinal tract, especially the small intestine, – galactosides became the subject of concern, which is ubiquitously occurring in compounds of plant seeds. The richest source of – galactosides are the seeds of legumes, among which the lupine seeds contain up to 7-15% (Villaluenga *et al.*, 2005). Substances as – galactosides are not hydrolysed in the upper parts of the gastrointestinal tract due to the lack of endogenous – galactosidase in broiler chickens. In the lower part of the gastrointestinal tract – galactosides are selectively metabolized by bifidobacteria contributing to bifidogenic effect (Crittenden, 1999). So far prebiotic substances had been applied only via feed or water, the earliest to one day old chicks. However, studies by Bednarczyk *et al.*, (2011) showed that administration of prebiotic substances in the diet of chickens can be successfully replaced by the *in ovo* method, which is a special way to deliver bioactive substances to the organism, via the direct injection into incubated eggs. Villaluenga *et al.* (2004), and Pilarski, *et al.* (2005) concluded in their studies, inter alia, that administration into the embryo –galactosides at the appropriate time causes stimulation of beneficial bacteria in two-day old chicks in comparison with other types of saccharides, which were injected. In previous studies the impact of –galactosides injected into the embryo on the

rate of resorption of the yolk sac and breeding parameters were confirmed (Brudnicki *et al.*, 2015). Moreover Pruszy ska-Oszmałek *et al.* (2015) showed in their work that *in ovo* administration of prebiotics and synbiotics increased the activity of amylase, lipase and trypsin in the pancreas. Furthermore, Madej *et al.*, (2015) studied the effect of *in ovo* administration of prebiotics and synbiotics on morphology of lymphoid organs in chickens.

Cheled-Shoval *et al.*, (2011) showed that MOS injection during embryogenesis influences the development and maturation of the intestine, including its microstructure. However, in the available literature there is no information about the effects of a single *in ovo* injection  $\alpha$ -galactosides on morphological and histological characteristics of the intestinal mucosa.

The aim of this study was to determine the effect of a single *in ovo* injection of  $\alpha$ -galactosides on the 12th day of incubation on the morphological development of the small intestine in broiler chickens in particular days of the experiment conducted under farm conditions.

## MATERIALS AND METHODS

**Birds and *in ovo* injection procedure:** The present research involved the use of hatching eggs originated from the parent stock of the Ross meat line. The experiment was performed under production conditions, on 80 000 eggs which were randomly divided into two groups: control (C) and experimental (D), 40 000 eggs each. On the 12th day of incubation the solution containing  $\alpha$ -galactosides was injected directly into the air cell according to the method proposed by Villaluenga *et al.* (2004). The applied galactosides originate from extracts of the residue after the debitering process of bitter lupin seeds galactosides (Gulewicz *et al.*, 2000). Prebiotic substances were injected in amounts of 3.43 mg / egg through an automatic injection system. After hatching, control and experimental broiler chickens were transferred in two litter chicken houses, with comparable environmental conditions, corresponding to the requirements specified in the technical manual of rearing Ross 308 broiler chickens. In the experimental period, all the birds were fed ad libitum with commercial mixtures covering the nutritional requirements of growing birds, according to the instructions given by the NRC (1994). Among the hatched chickens 10 individuals were selected randomly from each group on the day of hatching (0) and on the 4th, 7th and 14th day of the studies birds were weighed and euthanized by decapitation, after prior dislocation of the vertebrae. Subsequently, the biological material for research was collected.

**Morphological examination:** Morphometric measurements were conducted immediately after slaughter and resection of the gastrointestinal tract from

abdominal cavity of broiler chickens. Intestine was dissected entirely. Having removed the mesentery, it could be easily decomposed in a straight line without loop on the nonstick, smooth surface, then the length was measured, the intestine was divided into segments: duodenum, jejunum and colon. The length of each dissected segment was measured according to the method described by Leopold, (1953). Measurements were done with measuring tape in three replications. During the studies the total length of the intestine, the length of the duodenum, the length of jejunum and the length of the ileum, the length of the right cecum, the length of the left cecum and the length of the colon were measured. The length of duodenum was measured from the pylorus to the distal part of the duodenal loop. The length of the jejunum and ileum was measured as the distance designated from the end of the duodenum to the mouth of the caeca. The length of the colon was measured as the distance from the mouth of the caeca to the cloaca. Length of caeca was measured from the mouth of the ileum to vertex.

**Histological examination:** For histological examinations, tissue samples were taken from individual segments of the small intestine. The samples were fixed in 10% buffered formalin. The fixed segments were dehydrated in graded concentrations of alcohol, cleared in xylene, and embedded in paraffin. Samples were then cut into 5- $\mu$ m thick sections and stained with periodic acid Schiff. Measurement of villus height and crypt depth was conducted using an computer image analysis system Nikon NIS-Elements (Nikon Instruments Inc., USA). The surface area of the villi was calculated according to the formula given by Sakamoto *et al.* (2000). For the measurement of intestinal villi height cross-sections of 10 villi were selected randomly. Height was measured from the top of the villi to the base at the mouth of the intestinal crypt with the exception of intestinal crypts (from the brush border membrane to the basal membrane). All the measurements taken from 10 villi were counted from 10 different preparations from each segment of the small intestine for each bird and were expressed as the average height for each bird. Finally, 10 average heights of intestinal villi from 10 birds were expressed as the average height of the villi for a group. Villus width was measured at half length. The depth of intestinal crypts was defined as the depth of invagination between adjacent intestinal villi and measured between 20 villi (Uni *et al.*, 1998). All the measurements from ten crypts were counted from 10 different samples from each segment of the small intestine of each bird. Averaged depth measurements of 10 intestinal crypts were expressed as the average depth for each bird. Finally, 10 average depths of intestinal crypts of 10 birds were expressed as the average depth of intestinal crypts of the group.

**Statistical Analysis:** The results were statistically analyzed with Student's t-test. Results were tested for normal distribution by the Shapiro Wilk test. The Mann-Whitney test was used as an alternative to a t-test when the data were not normally distributed. Differences between groups were considered as statistically significant at  $P < 0.05$ . Statistical analysis was performed using the statistical software Statistica 9.1.

## RESULTS AND DISCUSSION

**Morphological studies on intestine:** The length of each segment of the small intestine, and consequently the total length of the intestine on the day of hatch was significantly higher ( $P < 0.05$ ) in the experimental group compared to the control group. In the control group the total length of intestine was 42.04 cm, whereas in the experimental- 50.11 cm. On the 4th, the 7th and 14th day there was no statistically significant difference for this parameter. The correlation between the prebiotic supplementation and the length of the intestine was confirmed by many authors indicating the fact that it may influence an increase in its absorption capacity (Ammerman, 1988; Yusrizal and Chen, 2003). Chen *et al.*, (2005) found that the addition of prebiotics in the diet resulted in the prolongation of the intestine relatively to the control group. In the control group the duodenum length was 8.84 cm and it was significantly lower ( $P < 0.05$ ) than the length of the duodenum in broiler chickens from the experimental group, which was 9.53 cm. It should be noted, however, that this effect was not durable. Practically for 4 day of age the growth rate of the length of intestine between the control and experimental groups were aligned and this dependency lasted up to 21 days after hatching. On the day of hatching in the experimental group the total length of the jejunum and ileum was 37.37 cm and was statistically significantly higher ( $P < 0.05$ ) than the length of these sections measured in the control broiler chickens - 30.04 cm. On the 4th day of life alignment of the length of this segment of the intestine was noted, about 66 cm both in the control and the experimental group. Then, on the 7th day a high increase in the length of this part of the intestine was observed in the experimental group (85.20 cm) in comparison with the control group (76.25 cm). These differences were statistically significant ( $P < 0.05$ ). On the 14th day approximate values of this parameter were noted. For caeca and colon there was no statistically significant difference between groups in the indicated research periods. Yusrizal and Chen, (2003) suggest that the greater length of both intestine and individual segments is advantageous for absorption of nutrients, and may subsequently lead to increase of body weight. The authors also noted that this trend is present in broiler chickens that were fed a diet with prebiotics. Potentially increased intestinal absorption of nutrients as a result of

single *in ovo* injection of  $\beta$ -galactosides can be the basis for achieving better production results and finally positive economic effects.

**Histological studies of the intestine:** The structure of the intestinal mucosa can provide lots of useful information about gut health. Awad *et al.*, (2006) showed that abnormally low villi may indicate the presence of toxins in the intestine, which may lead to decrease in absorption capacity. Reduction the height of the villi accompanied by a deepening of crypts may in the end lead to poor absorption of nutrients (Xu *et al.*, 2003). However, the extension of the intestinal villi, may indicate a greater demand for energy and nutrients. Effects of a single injection of  $\beta$ -galactosides on the 12<sup>th</sup> day of incubation are shown in Table 2. In this study, statistically significant differences ( $P < 0.05$ ) were observed in broiler chickens after hatching between the control and experimental group with respect to the height, the surface area of intestinal villi and crypt depth in all studied segments of the small intestine in broiler chickens.

*In ovo* injection had an impact on the height of the villi in the duodenum, in the control group on average  $356.01 \pm 24.79 \mu\text{m}$  while in experimental group was higher ( $510.3 \pm 65.90 \mu\text{m}$ ). There was also a larger surface area of villi, in control birds was  $51,697.69 \pm 4,482.36 \mu\text{m}^2$ , while in experimental birds it was noted  $108,110.4 \pm 4,946.87 \mu\text{m}^2$ . Similar results were observed in duodenum, statistically significant differences were observed in case of crypt depth of this segment of intestine (Table 2). In the control group, crypt depth oscillated between  $32.10 \pm 1.739 \mu\text{m}$ , while in the experimental group it was  $51.94 \pm 4.466 \text{ mm}$ . Differences statistically significant ( $P < 0.05$ ) were also confirmed in height and the surface area of villi as well as crypt depth in the jejunum and ileum in day-old broiler chickens, but these differences were not as predominant as in the case of the duodenum (Table 2).

It is difficult to compare the result from this study with literature data, because research on the effects of *in ovo* injection of  $\beta$ -galactosides has not been made so far. The available literature provides only partial data on the impact of *in ovo* injection of MOS on jejunal morphology in one day-old broiler chickens. Furthermore there are no notable data on direct effects of *in ovo* injection of  $\beta$ -galactosides. The results are consistent with the results presented by Cheled - Shoval *et al.*, (2011), who studied the influence of *in ovo* injection of MOS on morphological features of jejunum in day-old broiler chickens. They observed a highly significant difference in the amount and the surface area of the villi as well as crypt depth in favor of the experimental group inoculated with MOS in comparison with the control group. The higher morphological differences of the small intestine of inoculated broiler chickens can indicate the direct or

indirect trophic effects on the intestinal mucosa before hatching.

Ruttanavut *et al.*, (2009) showed that the morphology of the intestinal villi and the epithelial cells is closely related to the function and the development level of intestines. Effective use of exogenous food is dependent on the structural and functional development of the intestine. Higher morphological parameters of the intestinal villi, which were observed in all segments of intestine in broiler chickens from the experimental group may indicate activation of the villi function (Langhout *et al.*, 1999; Yasar and Forbes, 1999). Yamauchi *et al.*, (2006) and Markovi *et al.*, (2009) in their papers showed that higher values of villi parameters can promote the growth and development of the organism. Pluske *et al.*, (1996) and Caspary, (1992) also found that increasing the length of villi influences expression of brush border enzymes and transport system of nutrients in the intestine. From morphological point of view, it can be expected that in conducted studies higher intestinal villi in the experimental group, thus more absorbent surface will bring about higher absorption of available nutrients (Caspary, 1992). Increasing the size of intestinal villi observed in these studies in the experimental group of broiler chickens may be associated with early activation of cell proliferation (Lauronen *et al.*, 1998). Xia *et al.*, (2004) and Goodlad *et al.*, (1991) showed that intestinal crypts are a region in which separating stem cells allow intestinal villi cell regeneration. Thus increased crypt depth observed in the experimental group may reflect the rapid rotation and high demand for tissue growth. Similarly, Rehman *et al.*, (2007) showed that increased crypt depth indicates increased production of epithelial cells. Many researchers agree that the depth of the crypts can be an important factor in determining the structure of the intestinal villi and maintaining an appropriate level of their height and width. Furthermore, according to de los Santos *et al.*, (2007), a larger crypt depth may be an indicator of increased numbers of mucus-producing goblet cells. It is one of the components of the intestinal barrier having a great relevance in preventing the invasion of pathogens. According to current research, the increased length of villi and crypt depth may influence the increase in the amount of epithelial cells. In consequence of this, the likely result is the increase the absorption area for nutrients. Rehman *et al.*, (2007) found that increased production of cells results in increased demand for energy. To some extent, this fact may explain the higher rate of resorption of the yolk sac observed by in previous studies in experimental broiler chickens group injected at the 12<sup>th</sup> day of embryogenesis with  $\alpha$ -galactosides (Brudnicki *et al.*, 2015).

Publications of many authors prove that the gastrointestinal tract of broiler chickens before hatching is devoid of intestinal flora (Klasing 1998, Bort *et al.*, 2011, Cheled-Shoval *et al.*, 2011). Therefore, to clarify

the effect of oligosaccharides on the morphological characteristics of intestine in day-old broiler chickens injected *in ovo* with prebiotic, Cheled-Shoval *et al.*, (2011) proposed a mechanism for direct interaction of oligosaccharides with the carbohydrate receptors of intestinal epithelial cells and a partial absorption of oligosaccharides without the intestinal microflora. The above mentioned mechanism is described by Seifert and Watzl, (2007) on the basis of several studies conducted both in animals and humans. On the basis of this paper it can be assumed that the described mechanism implies the morphological characteristics of the small intestine in broiler chickens injected *in ovo* with  $\alpha$ -galactosides observed on the day of hatching in the present study.

On the fourth day more rapid growth of the duodenal villi in the experimental group of broiler chickens was still observed ( $835.42 \pm 62.90 \mu\text{m}$ ) compared with the control group ( $584.70 \pm 56.39\mu\text{m}$ ). The differences statistically significant ( $P < 0.05$ ) were also observed in the case of ileum. In the ileum villus height in the control group was  $391.06 \pm 19.72 \mu\text{m}$ , while in the experimental group  $456.78 \pm 27.18 \mu\text{m}$ . There was statistically significant increase of crypt depths in the duodenum ( $p < 0.05$ ) in the experimental group ( $64.32 \pm 5.94 \mu\text{m}$ ) as compared to the control group ( $53.82 \pm 5.31 \mu\text{m}$ ). In 4-day broiler chickens, due to the differences in the villus height in the duodenum and ileum, in these parts of the intestine also significantly higher ( $P < 0.05$ ) surface area of the villi was observed in experimental group compared with the control group. The growth rate of the villi in the duodenum could be associated with the effect of a good start, which was potentially provided by *in ovo* injection of  $\alpha$ -galactosides. It is confirmed by the height of the villi in the duodenum in the experimental group. Noy and Sklan, (1997) demonstrated that duodenal villi highest increase takes place up to 4 days or on the 4th day, and then the rate of growth is reduced. Smith *et al.*, (1990) suggested that a significant increase in the surface villi observed on 4th day in the experimental group allows growing birds to optimize absorption and assimilation processes in this important period. In our study, it was found that *in ovo* injection of  $\alpha$ -galactosides on the 12<sup>th</sup> day of breeding significantly affects the increase in the villi surface area in all segments of the intestine up to 4th days and duodenum up to 7 th days, which could potentially lead to an increased growth and productivity of birds. The faster rate of maturation of the duodenal mucosa in the first few days after hatching may be associated with the presence of bigger amount of nutrient substrates that can stimulate its functions. According to Bort *et al.*, (2011), during the first 2 - 4 days after hatching microbes colonize the cecum and the small intestine. In studies of Villaluenga *et al.*, (2004) and Pilarski *et al.*, (2005) it was showed that administration of oligosaccharides of raffinose family

Table 1. Intestinal morphometric parameters in broilers.

Day of life	Group	Intestine length [cm]	Duodenum length [cm]	Jejunum and ileum length [cm]	Right cecum length [cm]	Left cecum length [cm]	Colon length [cm]
Day of Hatch	C control	42,04 ± 3,33 <sup>a</sup>	8,84 ± 0,49 <sup>a</sup>	30,04 ± 2,96 <sup>a</sup>	4,05 ± 0,18	3,86 ± 0,15	3,16 ± 0,57
	E <i>in ovo</i>	50,11 ± 3,90 <sup>b</sup>	9,53 ± 0,63 <sup>b</sup>	37,37 ± 3,40 <sup>b</sup>	4,33 ± 0,51	3,92 ± 0,54	3,21 ± 0,36
Day – 4	C control	88,28 ± 5,21	17,45 ± 0,80	66,04 ± 5,73	6,78 ± 0,80	6,41 ± 0,78	4,79 ± 0,47
	E <i>in ovo</i>	88,34 ± 9,62	17,23 ± 2,31	66,22 ± 7,73	7,17 ± 0,66	6,79 ± 0,73	4,89 ± 0,63
Day – 7	C control	100,11 ± 6,63	19,10 ± 1,67	76,25 ± 6,84 <sup>a</sup>	8,34 ± 0,99	7,81 ± 0,79	4,76 ± 0,43
	E <i>in ovo</i>	109,75 ± 8,81	19,25 ± 1,42	85,20 ± 7,42 <sup>b</sup>	8,88 ± 1,41	8,04 ± 1,89	5,30 ± 1,06
Day – 14	C control	131,50 ± 11,30	25,22 ± 1,51	100,58 ± 11,25	10,86 ± 0,86	9,99 ± 0,69	5,70 ± 0,73
	E <i>in ovo</i>	134,10 ± 13,33	24,92 ± 2,87	102,54 ± 11,96	11,47 ± 1,66	10,55 ± 1,30	6,64 ± 0,66

Mean ± standard deviation

<sup>a, b</sup> - different superscripts indicate significant  $P < 0.05$  differences between groups in different days

Table 2. Histological parameters of the different small intestine segments in broiler chickens.

Day of life	Group	Villus height (µm)		Villus surface area (µm <sup>2</sup> )		Crypt depth (µm)	
		C control	E <i>in ovo</i>	C control	E <i>in ovo</i>	C control	E <i>in ovo</i>
Day of Hatch	Duodenum	356.01±24.79 a	510.3±55.90b	51,697.69±4,482.36a	108,110.4±4,946.87b	32.10±1.739a	51.94±4.466b
	Jejunum	280.30±33.23a	437.89±37.85b	41,363.61±5,734.97 a	61,359.03±8,712.119b	41.12±1.97a	46.89±3.02b
	Ileum	266.90±14.58a	370.81±49.62 b	44,963.93±5,476.938a	48,293.72±9,438.32b	33.49±2.88a	40.95±2.70b
Day – 4	Duodenum	584.70±56.39a	835.42±62.90b	132,378.54±18,446.09a	219,016.3±32,289.43b	53.82±5.31a	66.32±5.94b
	Jejunum	505.70±27.41	502.40±32.45	112,359.03±7,918.14	126,771.7±22,572.27	58.93±4.83	64.29±6.19
	Ileum	391.06±19.72a	456.78±27.18b	90,365.20±12,908.43a	124,335.6±8,960.007b	49.32±3.51	53.18±3.40
Day – 7	Duodenum	956.70±42.31a	1045.93±48b	261,532.38±18,310.83 a	281,568.4± 26,734.91 b	58.23±6.90 a	110.21±5.21 b
	Jejunum	607.85±49.53	631.30±32.01	168,473.0±21,961.32	169,551.72±18,709.50	57.77±9.33a	98.97±5.88b
	Ileum	528.01±44.06,	562.01±19.40	135,232.44±14,416.78	146,128.18±17,063.08	52.32±6.55	78.84±3.83
Day – 14	Duodenum	1099.03±67.58	1121.34±60.93	272,813.85±24,042.30	304,796.27±28,839.99	120.02±9.06a	169.05±8.90b
	Jejunum	922.70±32.82	933.53±92.19	199,643.02±12,473.22a	269,239.46±31,104.32b	116.58±8.84a	142.28±15.92b
	Ileum	707.11±40.11	729.20±70.44	171,847.28±84,98.71	183,886.14±22,928.86	92.95±2.45a	107.93±10.32b

Mean ± standard deviation

<sup>a, b</sup> - different superscripts indicate significant  $P < 0.05$  differences between groups in different days

during embryogenesis stimulates the formation of beneficial bacterial profile in two-day old broiler chickens. Therefore, it can be expected that the mentioned mechanism of direct interaction of the oligosaccharides on the carbohydrate receptor is less significant in comparison with the selective stimulation of the growth and activity of the selected strains of bacteria by oligosaccharides. The consequence of this is the production of SCFA by intestinal microorganisms preferably affecting several physiological processes in the host organism.

On the 7th day villi were significantly higher ( $P < 0.05$ ) and larger surface area was observed in the duodenum of broiler chickens from the experimental group ( $1045.93 \pm 48$ ) compared with the control group ( $956.70 \pm 42.31$ ). On the 7th day of age equalization of the height of the villi in the jejunum and in the ileum was observed in control and experimental group.

In the duodenum, despite statistically significant differences, decrease in the growth rate of the villi in the experimental group as compared to the previous period can be clearly observed. This is probably the effect of *in ovo* injection of a single dose of  $\alpha$ -galactosides. To keep the beneficial effects of the added substance on the microstructure of the small intestine, and thus the functionality of the intestine, it may be necessary to repeat the application of a prebiotic supplement or modify diet. In another case, the effect may be short-lasting, and the support for the rate of the morphological development of the intestine can be limited up to 7 days after hatching. The results are consistent with the results of Cheled-Shoval *et al.*, (2011), that injection of a single dose of prebiotic does not give long-lasting effects. The effect of injection on studied parameters remained for only 3 days after hatching. In our studies on the 14th day, there were no significant differences in height and the surface area of the villi, except for the surface of the villi of the jejunum, where there was a statistically significant difference ( $P < 0.05$ ) in experimental group ( $269,239.46 \pm 31,104.32$ ) compared to the control group ( $199,643.02 \pm 12,473.22$ ). On the 14th day after hatching statistically significant differences were observed ( $P < 0.05$ ) in crypt depth in all segments of the intestine (Table 2). Research by van Leeuwen *et al.*, (2004) showed that between 7th and 28th day after hatching villi are continuously thickening, particularly in the middle and the final section of the small intestine, probably by fusing previously separated villi. This may be the explanation why in current studies was observed that despite the relatively similar height of the villi the surface area increased. Geyre *et al.*, (2001) reported that increase of the depth of intestinal crypts can compensate a reduction in the level of proliferation in the villi. Awad *et al.*, (2009) and Fan *et al.*, (1997) suggested that an increase in crypt depth may result in increased production of regenerative cells contributing to faster growth. The positive effect of

prebiotics on the development and structure of the intestines in broiler chickens, including a significant increase in villi height in different segments of intestine is well documented (Sonmez and Eren 1999, Iji *et al.*, 2001, Xu *et al.*, 2003). Similarly, in our study effects of administered oligosaccharides on the structure of the intestine was observed.

**Conclusion:** In our study we demonstrated that a single *in ovo* injection of  $\alpha$ -galactosides can assist the process of early development of morphological and functional gastrointestinal tract, thereby potentially minimize the negative effects of the lack of or delayed access to feed. Conducted research shows that *in ovo* injection of  $\alpha$ -galactosides supports morphological and functional development of gastrointestinal tract, suggesting that  $\alpha$ -galactosides may be a substance used as a growth promoter especially in the early stages of development. Furthermore, it is a natural product and thus can be used as a support of organic poultry farming. The main challenge for the poultry industry nowadays is the identification of various factors influencing the rate of maturation of the gastrointestinal tract shortly after hatching, which is the key to improve the production rates and reduce the costs of poultry production. Many researchers draw attention to the fact that there is a considerable shortening of broiler chicken rearing and that the period of embryogenesis with a critical postnatal period is a significant part of bird's life. Therefore, it seems appropriate to interest in this metabolically complicated period in the life of the birds and the potential benefits of the interference at this early stage can be enormous.

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