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EFFECT OF IMMUNOMODULATOR IMMUNOBETA ON HISTOLOGICAL FEATURES OF INTESTINAL VILLI AND CRYPTS IN BROILER CHICKENS

N. Bozakova[1], D. Dimitrov[1], L. Sotirov[1*], P. Petrov, V. Gerzilov, Ts. Koynarski[1]

[1]Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria.

[2]Department of Animal Science, Faculty of Agronomy, Agricultural University, 4000, Plovdiv, Bulgaria,

***Corresponding author:** Lilyan Sotirov

Student's town, Department of Animal Genetics, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria.

E-mail: sotirov54@gmail.com

ABSTRACT

The present histometric study was performed on clinically healthy male broiler chickens, 40 days of age. After decapitation preceded by euthanasia specimens for analysis were collected from the jejunum of all birds. The biopsies were fixed in 10% aqueous solution of neutral formalin. The fixed specimens were processed using the classical histological techniques. The number of intestinal villi per microscopic field was the highest in broiler chickens treated at 3 g/kg Immunobeta but the differences were not statistically significant. According to measurements, the height of intestinal villi was the highest in broiler chickens treated at 4 g/kg ($P<0.001$). The experiments have shown that control birds had most numerous glandular crypts per microscopic field. The outer diameter of glandular crypts was the largest in small intestine of broiler chickens that received 3 g/kg of the preparation in their diet ($P<0.001$). The analysis of data suggested that the intestinal villi adjacent to glandular crypts were the tallest in birds treated with 2 g/kg immunomodulator ($P<0.001$).

KEYWORDS: chickens, villi, crypts, immunomodulator Immunobeta.

INTRODUCTION

According to data from specialised educational and scientific literature (Eurell et al., 2006; Georgescu et al., 2007; Samuelson, 2007; Dimitrov, 2014), the mucosa of small intestine in gallinaceous birds is outlined with structural features, ensuring a 1000-fold increase of intestinal mucosal surface – longitudinal mucosal folds, projections of various density and height protruding to the intestinal lumen, known as intestinal villi. Every enterocyte from the absorptive type possesses on its apical surface up to 2000 finger-like formations of the cellular plasmolemma, known as microvilli, which are actively involved in the bidirectional cellular transport. The epithelium lining on the villous surface in intervillous spaces protrudes into the mucosal propria, forming intestinal glands of various shape, size, density and secretion type – the intestinal crypts. The intestinal propria adjacent to intestinal glands which penetrates inside the intestinal villi has microstructural elements responsible for the continuous morphological and functional contact both with intestinal villi and glandular crypts.

Even this brief description of the microarchitectonics of small intestinal mucosa in gallinaceous birds suggests why it has always attracted the interest of researchers, and nowadays, with the increasing economic significance of gallinaceous birds reared under free-range or intensive production systems, it is a particularly important topic of research.

Without going back to the distant past, we should note the already-classic studies on microstructural features of small intestinal mucosa of different gallinaceous bird species before and after hatching (Uni et al., 1996; Mitjans et al., 1997). Their description was possible by virtue of studies on lymphocyte population in small intestinal mucosa of chickens after the hatch (Lillehoj et al., 1992; Vervelde et al., 1993). Later studies on proliferative processes in the epithelium lining the villi and glandular crypts in chicken small intestine (Uni et al., 1998), as well as the association of post hatch structural and functional changes in small intestine of chickens (Uni et al., 1999), have focused the attention of scientists on the immunological competence of avian intestines.

Initially, the dynamics of structural changes in enterocytes were associated with the development of small intestine mucosa of chickens prior to hatching (Kajiwara et al., 2003) as well as after the hatch (Geyra et al., 2001). Then followed many reports of individual researchers and research teams on immune competence of intestinal tract of chickens (Bar-Shira et al., 2003, 2005; Friedman et al., 2003; Olah et al., 2003). Two reviews (Dibner & Richards, 2004; Bar-Shira & Friedman, 2005) present the most important achievements in this field. Later Islam et al. (2008) compared the intestinal mucosa and immunoglobulin-containing plasma cells in native and broiler chickens reared in Bangladesh. One of the first reports that prebiotics could modulate the immune response in gut-associated lymphoid tissue of chickens is that of Janardhana et al. (2009). Today, there are literature reports on experimental studies of various immunomodulators on gut-associated lymphoid tissue in other animal species. This was the incentive to perform a histomorphometric investigation on the effect of immunomodulator Immunobeta on primary microstructural elements of small intestine of chickens, whose ration was supplemented with different doses of the preparation.

MATERIAL AND METHODS

The present histometric study was performed on clinically healthy male broiler chickens, 40 days of age, divided in four groups (n=6), owned by the Experimental base of the University of Agriculture, Plovdiv. The broilers from the first group were used as controls. The food of broilers from second group was supplemented with 2 g/kg Immunobeta in the food, the

broilers from the third group received 3 g/kg Immunobeta and the broilers from the fourth group - 4 g/kg Immunobeta from the 1st day of age to the end of the experiment - 42th day of age. After decapitation preceded by euthanasia in full compliance with Bulgarian legislation, specimens for analysis were collected from the jejunum of all control and treated birds. The bioptates were fixed in 10% aqueous solution of neutral formalin. The fixed specimens were processed in the laboratory of the Cytology, Histology and Embryology Unit, Faculty of Veterinary Medicine – Stara Zagora using the classical histological techniques (Vitanov et al., 1995) – removal of fixative from tissues on a water bath, dehydration in ascending ethanol series, clearing in two consecutive xylene baths, embedding of biospecimens in melted paraffin. From the paraffin blocks, numerous single and serial histological sections were cut on paraffin microtome (Reichert – Jung, Austria). The sections were stained with haematoxylin (Erlich) – eosin (Kiernan, 2008) to obtain permanent histological preparation. During all stages of histological processing, high-quality analytical grade chemicals (Fluka, Merck) were used. Histomorphometric studies were conducted on a sufficient number of histological preparations from the intestinal tract of all control and experimental birds by light microscope "Ergaval" (Karl Zeiss – Jena, Germany), equipped with eyepiece micrometer and stage micrometer using the methods of Avtandilov (1990).
Data from the morphometric study were statistically analysed (StatMost for Windows®, USA, 1994).

RESULTS AND DISCUSSION

The results from the histometric study are presented in Table 1. They demonstrate that the number of intestinal villi per microscopic field was the highest in broiler chickens treated at 3 g/kg Immunobeta (Fig. 3). The differences between control and treated chickens were not statistically significant but a steady tendency could be outlined (Fig.1, 1a).

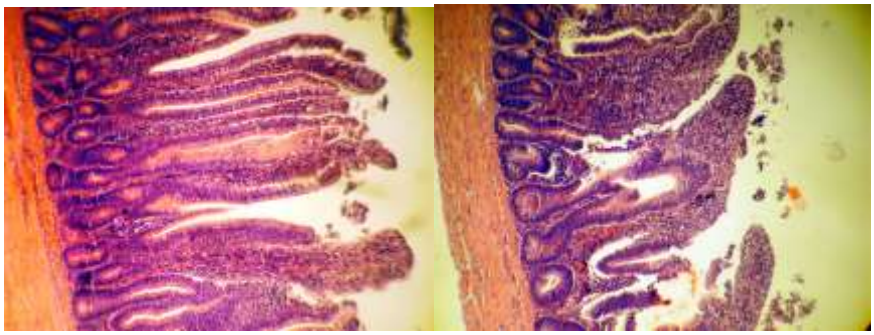

Fig.1, 1a. Number of intestinal villi per microscopic field in control broiler chickens.

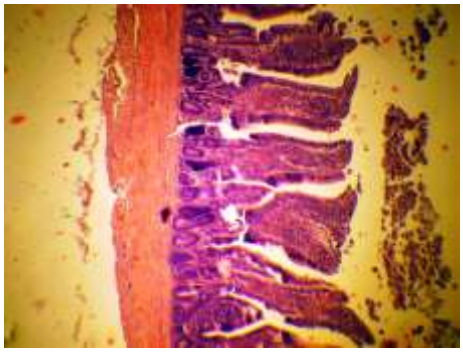

Fig. 3. Number of intestinal villi per microscopic field in broiler chickens chickens treated at 3 g/kg Immunobeta.

According to measurements, the height of intestinal villi was the highest in broiler chickens treated at 4 g/kg ($P<0.001$) (Fig. 4).

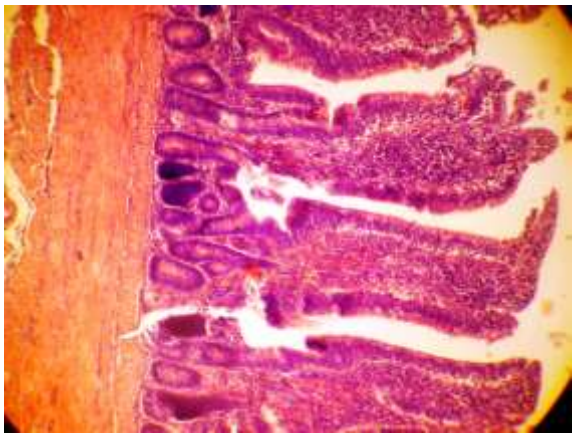

Fig. 4. The height of intestinal villi in broiler chickens treated at 4 g/kg.

The experiments have shown that control birds had most numerous glandular crypts per microscopic field. The outer diameter of glandular crypts was the largest in small intestine of broiler chickens that received 3 g/kg of the preparation in their diet ($P<0.001$) (Fig. 3). The analysis of data suggested that the intestinal villi adjacent to glandular crypts were the tallest in birds treated with 2 g/kg Immunobeta ($P<0.001$) (Fig. 2).

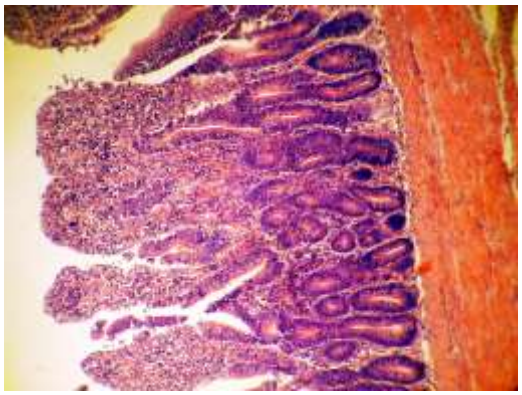

Fig. 2. Glandular crypts in intestinal villi in birds treated with 2 g/kg Immunobeta.

On the basis of reports from the specialised literature and our long-term experience in histomorphometric studies on various systems in gallinaceous birds, it could be affirmed that the number of intestinal villi and glandular crypts is relatively constant. These microstructures appear during the embryogenesis after segmentation of longitudinal rugae in the primary intestinal loop and the start of epithelium differentiation. Regardless of the exceptionally short genetically programmed period for development of organ systems, newly hatched broiler chickens have the set of microstructural elements of the intestinal tract at hatch. After hatching, these organs grow and undergo structural and functional differentiation until the

birds attain body and commercial maturity. That is why we assume that the effects of the tested immunomodulator could be consistently monitored through values characterising the micrometric indices of intestinal villi, glandular crypts and the lining epithelium.

According to Hermiston and Gordon (1995) the villus/crypt unit have zonal organization and enterocytes of many different types are found at distinct locations within the villus or crypt. The same authors also say that enterocytes derived from each crypt are responsible for feeding enterocytes into four villi, and each villus was formed from cells derived from four crypts. Cook and Bird (1973), Smith and Peacock (1989), Uni et al. (1995, 2000) in their ivestigations found that the enterocytes derived from the crypts migrate along the villus structure in a spiral path and survive for between 48 and 96 h. Davidson et al. (2008) let us know that the gut-associated lymphoid tissues (GALT) form series of structures that comprise part of the more extensive mucosa-associated lymphoid tissues (MALT; described in Chapter 2) which also includes nasal, lung and reproductive tract tissues (see Chapter 14). According the same authors the homing of immune cells is governed by interactions between molecular addressins on tissue vessel walls (e.g. high endothelial venules) with immune cell-expressed integrins and adhesins as well as the ability of the immune cells to respond to particular chemokines and other chemoattractant molecules (described in Chapter 10). The nature of these interactions links different areas of the mucosal immune system, and cells stimulated in one mucosal site migrate through other mucosal sites.

Our unpublished data (Bozakova et al., 2016) show that Immunobeta has strong dose dependent effect on serum lysozyme concentrations, alternative pathway of complement activity, beta lysins (humoral factors of natural immunity), IgG and IgM (humoral factors of acquired immunity) and these results hardly support the opinion of Davidson et al. (2008) described above.

Karakolev et al. (2013, 2015) reported that other immunomodulator “Helpankar” increases serum lysozyme, alfa and gamma interferon concentrations and complement activity in layer hens and chicken broilers. Similar results obtained Oblakova et al. (2015) and Lalev et al. (2015) also in broiler chickens and layer hens treated by immunomodulator “Natstim”. Both immunomodulators are based on thermally killed bacteria as a source of lipopolysaccharides. These investigations are very important because they are in high correlation with described above microstructural changes in small bird’s guts and explain “interactions between molecular addressins on tissue vessel walls (e.g. high endothelial venules) with immune cell-expressed integrins and adhesins as well as the ability of the immune cells to respond to particular chemokines and other chemoattractant molecules” (Davidson et al., 2008).

CONCLUSIONS

In the light of above mentioned and on the basis of numerical results from the histometry, we suggest that the tested immunomodulator Immunobeta, applied as dietary supplement at doses of 3 and 4 g/kg feed, stimulated the intestinal villi height and outer diameter of glandular crypts in the small intestine of broiler chickens. The application of the preparation at doses of 2 and 4 g/kg had beneficial effects on the growth of epithelium lining the glandular crypts and adjacent intestinal villi.

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Table 1. Effect of immunomodulator Immunobeta on microstructural parameters of small intestinal wall in broiler chickens.

Immunobeta g/kg feed	Number of intestinal villi per microscopic field (10x lens)	Height of intestinal villi (µm, 10x lens)	Number of glandular crypts per microscopic field (10x lens)	Outer diameter of glandular crypts (µm, 20x lens)	Height of glandular crypts epithelium (µm, 20x lens)	Height of epithelium lining the adjacent intestinal villi (µm, 20x lens)
Controls	3.55±0.3	392.8±1.9	19.4±0.8*	71.2±0.2	33.5±0.1***	23.9±0.8
2	4.05±0.3	354.6±0.2	17.7±0.8	73.1±0.3	29.2±0.2	31.4±0.2***
3	4.25±0.4	403.4±0.15	17.2±0.6	76.6±0.3***	27.2±0.3	27.6±0.6
4	3.8±0.4	417.9±0.15***	17.9±0.8	72.7±0.19	31.5±0.17	30.8±0.1

* - P < 0.05; *** - P < 0.001