The influence of various feed additives on the structure of the distal cecum of broilers

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Abstract. This article concludes a study examining the effect of the probiotic Vetom-1.1 (based on B. subtilis strains) and the sorbent Enterosgel (based on polymethylsiloxane polyhydrate) on the intestines of broilers. Three groups of broilers, 50 birds each, were formed. The control group received the basic diet. Group B received the probiotic Vetom-1.1 (0.006%), and Group E received the enterosorbent Enterosgel (0.008%) during the first three days of the experiment; thereafter, the experimental groups received the basic diet. Histological studies were performed on the first day before the start of feeding, on the fourth day after the end of feeding the drugs, before the transition to the basic diet, and at the end of the experiment. The thickness of the layers of the distal cecum and the density of neutral goblet cells per an area of 10,000 μm^2 were determined. On day 4, the muscular layer in the distal part of the cecum significantly increased by 16.4% (group B) and 25.1% (group E). At the same time, the crypt layer size decreased by 8.7 ($P \leq 0.05$, group B) and 30.2% ($P \leq 0.001$, group E), the goblet cell density increased by 28.9% ($P \leq 0.01$, group B) and 72.5% ($P \leq 0.001$, group E). At the end of the experiment, the muscular layer in the experimental groups was significantly lower by 16.4% (group B) and 20.4% (group E). The crypt layer was lower by 17.5% (group B) and 9.8% (group E). There were no differences in the goblet cell density. Thus, the studied drugs inhibit the development of the wall of the distal part of the cecum throughout the experiment, which is inconsistent with the results in the small intestine.

1 Introduction

Poultry farming is an important sector of agriculture, providing the population with nutritious and wholesome food products. High productivity in poultry production is made possible by the use of intensive housing and rearing technologies aimed at fully exploiting the birds' genetic potential. High-density stocking conditions create significant stress on the birds. This increases the risk of diseases leading to reduced productivity, product quality and mortality. To prevent economic losses, it is essential to maintain the health of birds. For this purpose, and to maximize their growth potential, various feed additives are used in

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poultry feeding. These additives are aimed at maintaining health by optimizing the working conditions of the digestive and immune systems of the body. They also influence the intestinal microbiota, which plays a crucial role in maintaining health. These substances include various microorganism-based preparations, such as probiotics, and sorbents [1].

When ingested by birds with food, additives enter the digestive tract and have an initial impact effect on its sections. An important part of the intestine is the cecum, which in birds consists of two parts - proximal and distal. In the thin and short proximal part, the structure of the organ repeats the structure plan of the small intestine, containing villi. The distal part of the cecum is wider, has a thin wall, and villi are absent. According to some authors, the cecum is divided into three sections - proximal, middle, and distal, the latter being a blind-ended apex [2]. The cecum plays a decisive role in chicken digestion, as the distal portion is responsible for the decomposition of carbohydrates and nitrogenous compounds by enzymes from the microorganisms that inhabit this organ. Microbial vitamins are also produced here, and water and salts are absorbed. All this makes the cecum an important part of the digestive system for ensuring the health and productivity of the poultry. This organ is also part of the immune system, as its connective tissue contains diffuse and nodular lymphoid tissue [2, 3, 4]. The chyme of the distal portion contain abundant communities of microorganisms, some of which may be potentially dangerous, but the epithelial barrier prevents the penetration of antigens and pathogens.

Goblet cells, which form a protective layer of mucus on the surface of the epithelium, are an essential component of the cecum. Their primary function is to secrete mucin. The protein basis of mucins is rich in serine and threonine and is highly glycosylated. Mucins protect the mucosa from physical and chemical damage, microbes and their waste products, and promote wound healing. The renewal period of goblet cells ranges from 3 to 7 days. In the large intestine, acidic sulfomucins are predominantly secreted. Secretion by goblet cells of the crypts of the small and large intestines occurs upon stimulation (e.g., by acetylcholine), unlike superficial goblet cells, which secrete mucus continuously [5, 6]. At the present time, 21 genes encoding goblet cell mucins have been described. Mucins are divided into two groups: membrane-associated and secreted. Membrane-associated mucins are associated with intestinal membranes, providing protection to epithelial cells and participating in signal transduction. Secreted mucins are classified as gel-forming mucins and non-gel forming mucins. Gel-forming mucins form a mucus layer on the surface of mucous membranes, providing moisture. The function of non-gel forming mucins has not been described yet. Recently, the ability of goblet cells to absorb antigens from the lumen and transfer them to antigen-presenting cells has been studied [7].

The aim of this study was to investigate the effect of a probiotic and sorbent on the development of the distal cecum, including the possible effect on goblet cells.

2 Materials and methods

The experiment was conducted on the base of Poultry house of the Russian State Agrarian University - Moscow Timiryazev Agricultural Academy. Day-old broilers of the “Konkurent” cross were divided into 3 groups of 50 birds each using the pair-analogue method based on live weight. The birds were kept on deep litter. They were fed a main (basic) diet based on corn grain. Access to water and feed was free. The control group received the basic diet, group B received the probiotic Vetom-1.1 based on B. subtilis strains (0.006%) as supplement with the basic diet, and group E received the sorbent “Enterosgel” based on polymethylsiloxane polyhydrate (0.008%) during the first three days of life. After three days of receiving supplements, the chickens remained on the basic diet. The duration of the experiment was 49 days. Average daily weight gain was recorded throughout the experiment. For histological studies, three birds of average weight were

selected at 1 day of age (before the start of feeding), 4 days after the end of treatment and before the transition to the main diet, and at the end of the experiment. The distal cecum was removed from the broilers and fixed in 10% neutral formalin. The samples were embedded in paraffin, then histological sections were prepared on a rotary microtome, stained with hematoxylin/eosin, and subjected to the PAS reaction. The thickness of the distal cecum wall layers was determined. The number of goblet cells per field of view was counted and converted to goblet cell density per 10,000 μm^2. The data were statistically processed using Excel.

3 Results and Discussion

At the age of one day, the wall thickness of the distal part of the cecum is quite thin and amounts to 146.37 μm (Table 1). The intestinal wall is represented by four membranes – serous, muscular, submucosa and mucosa. We did not take into account the thickness of the serous membrane. The muscular membrane consists of two layers of smooth muscle tissue – an outer longitudinal one and an inner circular one. The submucosa consists of loose connective tissue and is separated from the mucosa by a muscularis mucosae one or two smooth myocytes thick, which closely underlie the crypt layer. In one bird out of three, diffuse lymphoid tissue was found, as well as two lymphatic nodules, although according to Koçak, Y. R., & Özaydın, T., lymphatic follicles appear in the cecum after 7 days of age [2]. The crypts are shallow, rounded or elongated. At the bottom of the crypts, the epithelial cells are shallower and the cytoplasm is lighter; approaching the mucosal surface, the epithelial cells become taller. We determined the average epithelial cell size. At 1 day of age, the mucosa accounts for the greatest thickness - 58.4%, the submucosa accounts for 8.2%, and the muscularis propria accounts for 33.4% (Table 2). The epithelial cell height accounts for 11.2% of the organ wall thickness.

After three days of rearing, the thickness of the cecum wall in all groups increases by approximately 100 μm, i.e., by approximately 1.61 - 1.74 times. The growth of different membranes occurs unevenly: in broilers of the control group, the thickness of the muscular membrane increases by 2.2 times, the submucosa by 1.3 times, and the mucous membrane and epithelial cells - by 1.5 times. At this time, under the influence of additives, a difference in the size of membranes appears in the distal part of the cecum between broilers of the control and experimental groups: in the experimental groups, the muscular membrane significantly increases by 16.4% (group B) and by 25.1% (group E). The relative size of the muscular membrane was also higher in the experimental groups. The submucosa at 4 days of age significantly decreased by 22.1% (group B) and 24.8% (group E). The mucosa was smaller in groups B and E by 8.2% and 29.8% ($P \leq 0.001$), respectively. The mucosa thickness decreased due to a decrease in crypt depth, with a significant difference of 8.7% (group B) and 30.2% (group E). The goblet cell density was higher in the experimental groups, with a particularly pronounced superiority in group E (72.4%).

In our previous studies, we found that the mucosa height also decreased in the proximal cecum, but the crypt layer remained unchanged, with a shortening of the villi. The crypt layer also was significantly thinner in the duodenum. Thus, at the beginning of the experiment, the use of probiotic based on Bacillus subtilis and a sorbent had a suppressive effect on the development of the intestinal mucosa. The mucosal epithelium was intact in poultry of all groups. Lymphoid tissue is equally developed in all groups and occurs in the form of diffuse clusters and lymphatic nodules.

By the end of the experiment, the distal cecum wall increased by 2.1-2.6 times compared to 4 days of age. The muscular membrane thickness in the control group increased by 3 times, while in the experimental group it increased only by 2.2 and 1.9 times, resulting in a reliable lag in the muscular coat size of 16.4-20.0% for groups B and

E, respectively. There were no reliable differences in the size of the submucosa and muscular plate. The crypt depth in broilers of the experimental groups, as well as at 4 days of age, was less than in the control group; the reliable difference in this indicator was 17.5% (group B) and 9.8% (group E). At the end of the experiment, the epithelial height decreased compared to 4 days of age in absolute (by 5.4–8.7 μm) and relative (from 10.8% to 2.8–3.7%) terms. Moreover, the birds of group E exceeded the control birds in this parameter by 8.7% ($P \leq 0.05$). In group B, where the crypts were smallest, the highest goblet cell density was noted, although the difference was not significant. It is likely that the increase in epithelial cell height and goblet cell density in birds of the experimental groups can be regarded as a mechanism aimed at compensating for the decrease in crypt depth. Our previous studies have shown that in the proximal cecum at the end of the experiment, crypt depth was also lower in the experimental groups; however, in terms of villi height and mucosal thickness, groups B and E exceeded the control. In the small intestine - the duodenum and jejunum - all components of the mucosa - the villi and crypts - were better developed in groups B and E. This means that in the distal cecum, we obtained opposite results compared to the small intestine and proximal cecum studied earlier. At the end of the experiment, the mucosal epithelium in the distal cecum did not always maintain its integrity; the experimental groups performed a little better in this respect. Lymphoid tissue was more prominent than at 4 days of age, appearing diffuse and grouped into follicles. It was slightly better developed in the birds from the experimental groups.

In terms of live weight at the end of the experiment, Group B exceeded the control group by 2.2%; Group E – by 1.2%. The average daily live weight gain in the control group was 49.21 g; in Group B – 50.23 g, and in Group E – 79.73 g. Feed costs per 1 kg of gain were lower by 0.19 kg in the experimental groups.

The distal portion of the cecum differs significantly in morphology from the small and large intestines, as well as from its proximal portion. Villi do not develop here; a crypt layer is present, and folds form, particularly expressed in the middle portion. The narrow proximal portion of the cecum apparently selectively allows substances to pass into the distal portion; at least, the microbial community in the distal portion differs significantly from the rest of the intestine and from feces [8]. In the cecum, the microbiome breaks down fiber into volatile fatty acids, making it an important part of the intestine to develop productive qualities of poultry. It is suggested that poultry productivity can be improved by manipulating the cecal microbiome [3].

Probiotics are popular additives that influence the intestinal microbial community, with spore-forming microorganisms better able to overcome the acidic environment of the stomach for further intestinal colonization. In addition to influencing the species composition of the microbiome, probiotics affect the intestinal mucosa. For example, when taking additives based on B. subtilis, an increase in the layer of villi in the jejunum and ileum was noted, which creates conditions for intensive parietal digestion and absorption of dietary nutrients [9, 10, 11]. In addition to an increase in the layer of villi, they also improve contacts between epithelial cells, which creates a strong intestinal barrier. Some strains also reduce the production of IL-8 and lower inflammatory reactions [12]. The complex effect on the intestine from feeding addititives based on B. subtilis leads to improved zootechnical results, such as an increase in average daily gains of up to 5.7% and improved feed conversion [16, 18]. In our studies, the effect observed in the distal cecum was not positive, unlike in other intestinal regions. We believe that this condition of the distal cecum explains the slight superiority of Group B broilers over the control group in terms of growth parameters. most likely that this condition of the distal part of the cecum explains the insignificant difference of group B broilers over the control ones in terms of growth indicators.

Sorbents can also influence the intestinal microbiota, and through it, the health and performance of birds. The introduction of sorbents into the diet has a positive effect on broiler growth performance, but when feeding a additives based on biochar, bird growth was slower at the initial stage (0-21 days) [13, 14]. In our case, by contrast, the positive effect of the sorbent was observed early in the experiment and was confined to its effect on the muscular membrane. Thus, the effect of sorbents on the intestine is poorly understood and requires further investigation.

Table 1. Morphometry of the distal cecum.

	Tunica, microns						**Total woll**	**Goblet cells, pcs/10000 microns ^{2}**
	Muscularis	**Submucosa**	**Mucosa**					
			Lamina muscularis	**Crypts**	**Epithelium**	**Total**		
1 day								
B	48.88 ± 1.66	11.98 ± 0.80	3.44 ± 0.24	82.07 ± 3.97	17.43 ± 1.14	85.51	146.37 ± 5.66	8.00 ± 1.00
4 days								
C	108.39 ± 3.15	16.01 ± 1.28	4.55 ± 0.22	122.18 ± 2.80	27.08 ± 0.89	126.73 ± 5.34	251.13 ± 5.34	8.07 ± 0.77
V	126.21** ±5.10	12.47* ± 1.04	4.79 ± 0.71	111.49* ± 4.07	27.64 ± 0.91	116.28 ± 7.60	254.96 ± 7.60	10.40** ± 0.64
E	135.63*** ± 4.36	12.04* ± 1.05	3.69** ± 0.18	85.23*** ± 2.71	25.35 ± 1.32	88.92*** ± 5.42	236.59 ± 5.42	13.92*** ± 1.10
49 days								
C	327.41 ± 12.68	21.58 ± 2.30	14.06 ± 0.73	286.09 ± 7.53	18.33 ± 0.53	300.15 ± 13.55	649.14 ± 14.83	5.22 ± 0.43
V	273.79*** ± 8.45	18.02 ± 1.15	13.99 ± 0.56	236.09*** ± 4.76	19.83 ± 0.55	250.18*** ± 7.49	541.99*** ± 9.63	6.26 ± 0.54
E	261.85*** ± 9.75	16.26 ± 1.50	15.49 ± 1.02	258.10** ± 9.38	19.92* ± 0.52	273.59 ± 8.31	551.70*** ± 16.11	5.11 ± 0.29

B - the beginning of the experiment;
* - the difference compared to the control group is significant: * - at P≤0.05; ** - at - P≤0.01; *** - at P≤0.001

Table 2. Relative sizes of the membranes of the distal part of the cecum, %.

	Tunica					
	Muscularis	Submucosa	Mucosa			
			Lamina muscularis	Crypts	Epithelium	Total
1 day						
B	33.4	8.2	2.35	56.1	11.9	58.4
4 days						
C	43.2	6.4	1.8	48.6	10.8	50.5
V	49.5	4.9	1.9	43.7	10.8	45.6
E	57.3	5.1	1.6	36.0	10.7	37.6?
49 days						
C	50.43	3.3	2.2	44.1	2.8	46.2
V	50.51	3.3	2.6	43.6	3.7	46.2
E	47.5	2.9	2.8	46.8	3.6	49.6

4 Conclusion

Our data on the effect of a supplement containing a B. subtilis-based probiotic and a polymethylsiloxane polyhydrate-based sorbent on the distal cecum show that, at the beginning of use, both preparations had a positive effect on the development of the muscular layer, which potentially improves the motor function of the organ. However, the effect on connective tissue and epithelial structures - the submucosa and the crypt layer of the mucosa - was suppressive. At the end of the experiment, the experimental groups were smaller than the control groups in the size of all layers of the distal cecum. This contradicts our earlier results obtained in the same experiment on small intestines, where we observed better development of mucosal elements - the villi of the duodenum and jejunum. Thus, the preparations we studied exerted opposing effects in different parts of the intestinal tract. The zootechnical results obtained in this experiment showed minor increases compared to the control group, which is likely due to the poor development of the mucosa in the distal cecum. We hypothesize that the different effects of the drugs on the small intestine and the distal cecum may be due to differences in the microbiome of these intestinal regions or to functional differences. This may also be related to dosage selection. In any case, this topic requires further research.

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