

The influence of various feed additives on the development of duodenal goblet cells in broilers

*Alexandra Seryakova**, *Nadezhda Cherepanova*, *Anna Semak*, *Nina Belyaeva*, and *Elena Prosekova*

Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, 127434, Moscow, Russia

Abstract. A comparative study was carried out on the effect of three drugs on the development of goblet cells in the duodenum of broilers. In the first experiment, day-old broilers Smena-8 were divided into 4 groups. The experimental groups received the phytobiotic Butitan with the main diet: E1-0.25%; E2-0.5% and E3-0.75%. In the second experiment, 3 groups were formed from broilers of the Konkurent cross; additives were added for the first 3 days. The Vetom group received a probiotic based on *Bacillus subtilis* (0.006%); Enterosgel group – Polymethylsiloxane polyhydrate sorbent (0.008%). Histological studies were carried out in the first experiment at 1, 7 and 42 days, in the second at 1, 4, 49 days. Sections were stained to identify acidic and neutral mucus. The number of goblet cells in the field of view was counted and their density over an area of 1000 μm^2 was calculated. By the end of the first experiment, the density of neutral goblet cells in the villi E1 and E2 and crypts E2 and E3 increases by 17% and 7.5%, acidic goblet cells increase in the crypts by 32.6% and 33.1%. The probiotic increased the density of acidic goblet cells in the crypts (64%; $P \leq 0.001$) without affecting the villi. Enetrosorbent reduces the density of neutral and increases the density of acidic goblet cells in the mucosa (37.3%). Due to the absence of a pathological picture in the structure of the duodenum, as well as the important role of mucus in the formation of chyme and digestion, we consider the increase in the density of goblet cells and the effect of feed additives as a positive reaction. The study of histological parameters to assess the effect of various feed additives on the body parameters of birds is important, since it shows the functional state of the organs.

1 Introduction

Intensification of production and increased technological stress negatively affect all organ systems of poultry, its health and productivity. Therefore, it is important to study fundamental adaptive mechanisms to various environmental factors, such as feeding, temperature, stocking density, etc. [1].

* Corresponding author: alseryakova@mail.ru

One of the important factors in the body's nonspecific immunity is mucus produced by goblet cells of the intestinal epithelium [2]. It protects the mucous membrane from mechanical, physical and chemical influences. Mucus can be represented by neutral and acidic glycoproteins; their ratio and distribution in different parts of the digestive system differs and changes under the influence of various factors. The MUC2 gene is responsible for the secretion of the main type of mucin in goblet cells. Many factors can influence the process of its transcription: bacteria, toxins, cytokines, hormones [3]. It was also noted that cytokines, being mediators of inflammation, increase the expression of the MUC2 gene. This leads to goblet cell hyperplasia and increased mucus secretion [4]. Experimental exposure of rats to hydrogen sulfide gas initially causes an increase in PAS-positive mucins in the lamina propria of the intestinal mucosa. This is a manifestation of adaptation mechanisms in response to irritation. With further exposure, an increase in the amount of acid sialomucins is observed, which have an enteroprotective effect and are involved in the restoration of the intestinal preepithelial barrier [5]. A similar increase in acidic and neutral glycosaminoglycans in the connective tissue of the tracheal wall in chickens was found with insufficient ventilation in production premises [6].

The amount of neutral and acidic mucins increases significantly during invasions [7], and increased goblet cell degranulation and an increase in the amount of mucus in the intestine of rats in response to intravenous administration of a high dose of endotoxins have also been noted [8]. In mice, destruction of the mucus layer leads to increased epithelial permeability to bacteria and increased cell adhesion, which leads to colitis [9]. In addition, goblet cells participate in immune defense by absorbing through endocytosis and releasing IgA into mucus [2]. Some authors note the ability of goblet cells to capture antibodies and present their antigen to presenting cells [10]. It has also been noted that an increase in the number of goblet cells has a positive effect on the body's defense against infections [11].

Studies of the distribution of goblet cells in the human intestine have shown that predominantly neutral polysaccharides are localized in the small intestine, while acidic mucins predominate in the large intestine [12]. At the same time, along the intestinal tube there are cells that contain both acidic and neutral mucins; such cells are most often observed at the base of the crypts, and in the area of the villi only neutral mucins were stained [12]. In birds, a relationship was found between the localization and activity of goblet cells and such indicators as age, sex, and breed [13]. In chickens, before hatching, only acidic mucins are found in the small intestine; after hatching, at 7 days of age, throughout the small intestine the amount of acidic and neutral mucins becomes the same. The density of goblet cells increases in the caudal direction [14]. During the growth and differentiation of the intestinal tube structures of chickens during ontogenesis, the number of goblet cells and the qualitative composition of mucus change. Their number increases in the cranial part of the duodenum [15]. At the end of incubation and in the first week after, an increase in the mitotic activity of the epithelium is observed and the intestine grows mainly due to hyperplasia [16]. In the ileum and cecum, the density and number of goblet cells containing acidic mucins increases after 21 days of ontogenesis [13, 17].

Additions of prebiotics and probiotics to feed under conditions of heat stress increase the activity of goblet cells in the duodenum and ileum, the amount of acidic mucins increases, and in the ileum also cells containing mixed mucins [18]. Yeast supplementation also causes an increase in the number of goblet cells [19]. However, some authors report no effect of probiotics and phytobiotics on the number of goblet cells and the chemical composition of mucus in the ileum and cecum of broiler chickens [13].

Since the secretion of goblet cells creates a specific environment for the intestinal microbiont, participates in digestion processes, protects against the adverse effects of external and internal factors and is a marker of inflammatory reactions, morphometric and

histochemical study of the number of goblet cells and the composition of mucus is of important diagnostic value.

2 Materials and methods

The experiments were carried out in the conditions of the experimental poultry house of the Russian State Agrarian University-Moscow Agricultural Academy named after K.A. Timiryazev. In the first experiment, broilers of the Smena-8 cross were divided into 4 groups (n=60) using the analogue pair method based on live weight. The control bird received the main diet, the experimental groups (E) received the main diet with the phytobiotic Butitan (contains sweet chestnut extract, calcium butyrate and palm oil) in dosages: E1 – 0.25%, E2 – 0.50% and E3 – 0. 75%. The duration of the experiment was 42 days.

In the second experiment, Konkurent cross broilers were used; 3 groups of 50 heads were formed from day-old chicks using analogue pair method based on live weight. Broilers in the control group received the basic diet. The experimental groups received in the starting diet: Vetom group - probiotic Vetom-1.1 (based on Bacillus subtilis) 0.006%, Enerosgel group - enterosorbent Enterosgel (based on Polymethylsiloxane polyhydrate) 0.008%. From the 4th day of life, the birds of the experimental groups received the main diet. The duration of the experiment was 49 days.

For histological studies, day-old broilers were selected before the start of feeding the diet, then, in the first experiment - at 7 days, in the second experiment - at 4 days (after completion of feeding supplements and before switching to the main diet), and at the end of the experiments - at 42 and 49 days. The duodenum was collected from the birds and fixed in 10% neutral formalin. Dehydration and paraffin embedding were carried out according to standard methods, histological sections were stained with hemoxilin-eosin, PAS (periodic acid-Schiff reagent) and AB (alcian blue) methods. The layer of villi and crypts was measured using an ocular ruler. Goblet cells in the villi and crypts were counted at a magnification of 600 times, and the density of goblet cells in a field of view with an area of 10,000 μm² was calculated. The data was processed statistically.

3 Results

On the first day after hatching, broilers showed a high content of acidic goblet cells. The presence of neutral goblet cells in the crypt layer is 8.6% greater than in the villi. There are 9.6% more acidic goblet cells in the crypt layer than in the villi (Table 1).

Significant changes in the mucosa occurred with an increase in the layer of villi and crypts in the first week of growth. In the control group, the layer of villi increased by 2.2 times, and the layer of crypts by 3.1 times. Moreover, in the control group, the number of cells with neutral and acidic mucus decreased in the crypts by 70.4% and 51.8%, respectively. In the control group, the cells in the villi layer showed the opposite pattern: the number of neutral goblet cells increased by 7.6, and acidic ones by 9.4%.

Table 1. Density of goblet cells in the duodenum when using Butitan, pcs/10000 μm².

Group	Neutral cells		Acidic cells		Layer thickness, μm	
	Villi	Crypt	Villi	Crypt	Villi	Crypt
1 day						

Beginning of experiment	11,20 ± 0,05	12.26 ± 0.19	17,58 ± 0,11	19,45 ± 0,33	599 ± 9,1	64 ± 1,8
7 day						
Control	12.06 ± 0.07	3.63 ± 0.09	19.25 ± 0.12	9.38 ± 0,07	1306 ± 13.4	199 ± 5.9
E1	12.49*** ± 0,08	4.33*** ± 0.06	19.05 ± 0.10	11.03*** ± 0.09	1297 ± 26.6	187 ± 7.9
E2	12.44** ± 0,09	3.79 ± 0.05	19.14 ± 0.08	9.35 ± 0.15	1271 ± 47.5	214 ± 12.9
E3	12.42*** ± 0.07	4.35*** ± 0.04	19.01 ± 0.08	9.50 ± 0.10	1214*** ± 25.0	207 ± 10.3
42 day						
Control	18.50 ± 0.45	7.11 ± 0.22	23.76 ± 0.53	10.07±0,23	1806 ± 28,5	183 ± 4,4
E1	21.64*** ± 0.24	7.15 ± 0.20	23,10 ± 0.42	13.35*** ± 0,13	1643*** ± 17.7	197 ± 21.4
E2	19.88** ± 0.23	8.48*** ± 0.19	23.91 ± 0.16	13.40*** ± 0.16	1665*** ± 14.9	163*** ± 3.7
E3	19.20 ± 0.28	7.89* ± 0.27	24.71 ± 0.12	13.41*** ± 0.08	1666*** ± 12.8	160*** ± 3.8

Here and further in the tables: ** - the difference with the control group is significant at $P\leq0.01$; *** - the difference with the control group is significant at $P\leq0.001$

In the first week of growth, the Butitan additive had an effect on the depth of the crypt and the height of the villi. In E3, a significant decrease in villi thickness by 7% was noted. In E1, there is an increase in neutral cells in the villi by 3.6% ($P\leq0.001$), in the crypts by 19.3% ($P\leq0.001$), the number of acidic goblet cells increased in the crypt layer by 17.6% ($P\leq0.001$). In the E2 group, the number of neutral cells also increased in the villi by 3.2% ($P\leq0.01$), and in the crypts by 4.4%. In E3, the number of neutral cells increased in the villi by 3% ($P\leq0.001$), and in the crypts by 19.8% ($P\leq0.001$) (Table 1).

By the end of the experiment, the layer of villi in the control group decreased 1.4 times compared to the first week of growing, the thickness of the layer decreases slightly. At the same time, the number of goblet cells in the control group increases compared to day 7; when comparing the number of goblet cells in the crypt layer at the beginning of the experiment and at the end of the experiment, we can conclude that the number of neutral cells decreased by 42%, and acidic goblet cells decreased by 48.2%.

In E1 at 42 days of age, there is an increase in neutral goblet cells in the villi by 17% ($P\leq0.001$); in the crypt layer the number of acidic goblet cells increased by 32.6% ($P\leq0.001$). In E2, the number of neutral cells increases in the villi by 7.5% ($P\leq0.01$), in the crypts by 18.8% ($P\leq0.001$), the number of acidic cells increases in the crypt layer by 33.1% ($P\leq0.001$). In E3, the number of neutral cells in the crypts increases by 10.9% ($P\leq0.05$) and acidic cells by 33.2% ($P\leq0.001$) (Table 1).

It can be concluded that by the end of the experiment, as the thickness of the villi layer decreases, the density of neutral cells increases, and as the thickness of the crypt layer decreases, the density of acidic and neutral cells also increases (Table 1).

The results of the second experiment are shown in Table 2. At one day of age in broilers, a higher density of goblet cells was noted in the crypts compared to the villi, while the density of acidic goblet cells was greater compared to neutral cells.

Table 2. Density of goblet cells in the duodenum when using Vetom and Enterosgel, pcs/10,000 μm²

Group	Neutral cells		Acidic cells		Layer thickness, μm	
	Villi	Crypt	Villi	Crypt	Villi	Crypt
1 day						
Beginning of experiment	6.43 ± 0.27	9.29 ± 0.74	8.81 ± 0.35	21.40 ± 0.62	568 ± 8.3	61 ± 0.4
7 day						
Control	6.13 ± 0.27	14.67 ± 0.85	6.74 ± 0.42	14,30 ± 1,16	856 ± 10.4	105 ± 2.42
Vetom	7.49** ± 0.36	14.02 ± 0.55	6.21 ± 0,48	13.63 ± 1.46	841*** ± 7.8	96 ± 2.0
Enterosgel	5,68 ± 0.31	7.95*** ± 0.63	6.86 ± 0.37	10,97*** ± 0.50	953*** ± 6.3	103 ± 2.1
42 day						
Control	8.08 ± 0.35	13.82 ± 0,59	7.21 ± 0,43	7.68 ± 0.42	1388 ± 18.0	169 ± 3.5
Vetom	9.29 ± 0,51	12.81 ± 0,51	7.44 ± 0.29	12.60*** ± 0,75	1573*** ± 24.1	181*** ± 3,1
Enterosgel	6.47*** ± 0.29	7.27*** ± 0.33	9.26*** ± 0.34	11.96*** ± 0.66	1563*** ± 18.19	197*** ± 6.0

By 4 days of age, the layers of the mucous membrane increase 1.5-1.7 times. In broilers of the control group, by 4 days of age, compared to one day, the density of neutral goblet cells in the crypts increases, and from acidic ones, it decreases in the villi and crypts. In the Vetom group, there was a significant decrease in the villi layer (1.7%), but the density of neutral goblet cells in them increased by 22.2% (P≤0.01). There was no difference in the density of acidic goblet cells at this age. In the Enterosgel group, the height of the villi significantly increases (11.3%) with a constant density of goblet cells, the size of the crypt layer does not change, and the density of goblet cells in them decreases, regardless of the secretion (Table 2).

By 49 days of age, compared with 4 days in the control group, the size of the layer of villi and crypts increases by 1.6 times, in the crypts the density of goblet acidic cells decreases by 1.9 times. The Vetom group is superior to the control in terms of the size of mucosal layers and the density of acidic goblet cells in the crypts (64%; P≤0.001). The Enterosgel group was significantly superior to the control group in terms of the size of the layers of the mucous membrane (12.6% - the villi layer and 16.6% - the crypt layer), and the density of acidic goblet cells both in the villi and in the crypts of the duodenum. The number of neutral goblet cells in the crypts was significantly lower by 47.4% (P≤0.001), in the villi by 19.9% (P≤0.001) (Table 2).

4 Discussion

Broilers of the Smena-8 cross are characterized by an increase in the density of neutral and acidic goblet cells in the villi throughout the experiment. In Konkurent cross broilers, there is no significant increase in the density of goblet cells with any secretion in the villi. This feature is independent of the additives used and is probably a characteristic feature of the crosses. In two experiments, the maximum density of acidic goblet cells was observed in the crypts of a day-old chick; subsequently, their density decreased. This pattern for neutral

goblet cells was observed only in the first experiment. A decrease in the density of acidic goblet cells in the crypts may be caused by the transition to external nutrition. Comparing the results of two experiments, it can be seen that, regardless of the use of feed additives, in experiment 1 the density of goblet cells with any secretion was higher in the villi, and in the second - in the crypts. These differences are probably also the distinguishing features of the crosses. The literature describes breed differences in the localization of goblet cells [13]. Age-related changes in the number of goblet cells in the intestine are natural and noted by researchers [13, 17], and the qualitative composition of mucus also changes [15].

The additives used had an effect on the duodenal mucosa, which was manifested in changes in the height of the villi layer and the depth of the crypts, and an effect on the density of goblet cells was also observed. In the first experiment, regardless of the dosage of Butitan, the density of neutral goblet cells in the mucosal epithelium slightly increased, and the density of acidic ones, with the exception of E1 crypts, remained at the control level. By the end of the experiment (by the age of 42 days), the density of neutral goblet cells in the villi E1 and E2 and crypts E2 and E3 continued to increase; the density of acidic goblet cells significantly increased in the crypts of all experimental groups. There is a relationship between an increase in goblet cell density in the experimental groups and a decrease in villi height. In our case, we believe that the increase in goblet cell density compensates for the decrease in villi height and crypt depth that occurred under the influence of the feed additives. Probably, the duodenum tends to produce a constant amount of mucus, regardless of fluctuations in the dimensional characteristics of the mucous membrane. In some cases, phytobiotics do not affect the quantitative parameters of goblet cells [13].

In the second experiment, at the initial stage of growing, the Vetom probiotic caused some inhibition of the villi layer while simultaneously increasing the density of neutral goblet cells in them. When using the sorbent, there was an increase in the layer of villi and a decrease in the density of all goblet cells in the crypts. During this period, changes in the size of the villi are accompanied by a compensatory change in the density of the crypts, and probably leads to a constant number of goblet cells in the organ area. At the end of the second experiment, after discontinuation of the probiotic and sorbent, their prolonged positive aftereffect on the height of the villi layer and the depth of the crypt layer is visible. Also in both cases, the density of acidic goblet cells in the crypts increases. This effect of probiotics has been noted in other studies [18]. In the Enterosgel group, with a decrease in the density of neutral goblet cells, the density of acidic goblet cells increases; probably, the use of Enterosgel at the initial stage caused the need to increase the production of secretion with a low pH value. In both experiments, an increase in the density of acidic goblet cells is noted; in the first experiment, this may compensate for the decrease in the villi layer; in the second, it requires additional research with the involvement of microbiologists, geneticists and other specialists.

The mucus of goblet cells performs important functions in the intestine: they participate in immune reactions, protect against mechanical, physical and chemical damage [2, 10, 11]. In addition to the well-known functions of goblet cell mucus, some researchers believe that it plays an important role in the digestive processes: it participates in the formation of a dense endogenous fraction of chyme and structures the chyme to ensure that the substrate meets the enzyme. The secretion of goblet cells, forming a dense endogenous fraction of chyme, promotes cavity hydrolysis and the movement of nutrients to the surface of the epithelium [20, 21]. Changes in the number of goblet cells and the chemical properties of their secretions can be interpreted ambiguously. In some cases, increased formation of goblet cells is the body's response to negative influences. For example, an increase in the number of goblet cells and glycosaminoglycans in the lamina propria of the mucous membrane is interpreted as an adaptation mechanism in response to damaging influences;

in this regard, an increase in the number of goblet cells may indicate negative processes in the intestine [5]. Similar processes occur in the case of invasions [7]. In the respiratory organs, the interpretation of an increase in goblet cells is clearly negative; it is a marker of poor organ condition, and occurs in response to insufficient ventilation of poultry house premises [6]. In our case, the general histological picture of the sections was within normal limits without signs of pathological processes. We interpret the increase in the number of goblet cells as a positive factor, since in birds the only source of the dense endogenous fraction of chyme is the secretion of goblet cells; their increased number contributes to the creation of such a fraction, which ensures the creation of optimal conditions for cavity digestion.

5 Conclusion

In the first experiment, under the influence of a phytobiotic, an increase in neutral goblet cells in the villi of the duodenum was noted; this process begins at 7 days and intensifies by the 42nd day. The neutral goblet cells of the crypts increase to a lesser extent. Goblet cells with acidic secretion also tend to enlarge, but this occurs by day 42 in the crypts. The probiotic Vetom-1.1 at 4 days of age causes an increase in neutral goblet cells in the villi while reducing their density in the crypts. At the end of the experiment, it has no effect on neutral goblet cells. Acidic goblet cells increased at the end of the experiment only in the crypts. Enterosgel sorbent reduces the number of neutral goblet cells at 3 and 49 days, but increases acid cells at the end of the experiment. It should be noted that crypt acidic goblet cells increase by the end of the experiment, regardless of the additive. Due to the absence of a pathological picture in the structure of the duodenum, as well as the role of mucus in the formation of chyme and digestion, we consider an increase in the density of goblet cells as a positive reaction. The study of the histological picture of the duodenum allows us to draw a conclusion about the effect of feed additives on the functional state of the organ, which makes such studies important for assessing the effect of various drugs on the health of birds.

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