The effect of a feed additive containing sweet chestnut extract and calcium butyrate on the histostructure of the glandular stomach of broilers.

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Abstract. The studies were carried out on broilers of the "Smena 8" cross. At one day of age, the birds were divided into 4 groups using the method of analogue pairs based on live weight. Throughout the experiment, the control group received the main diet, the experimental groups received the main diet with the feed additive "Butitan (Farmatan BCO)" O1 – 250 mg/kg, O2 – 500 mg/kg and O3 – 1000 mg/kg. Morphometry of the glandular section of the stomach at 7 days of age showed that O1 broilers in terms of the size of the submucous membrane were superior to the control group by 6,1%, and O2 and O3 were significantly inferior to the control group in terms of the size of these indicators by 16.4% and by 8,5%. Gland epithelium height of the gastric submucous membrane was higher in all experimental groups by 11.1%. At the end of the experiment at 42 days of age in O1, the mucous membrane in thickness exceeded the control group by 11.4%, the indicators of the submucous membrane did not have significant differences, O2 and O3 were inferior to the control group in the size of these membranes by 4% and by 11.6%. Gland epithelium height of the gastric submucous membrane was higher in O3 by 9.1 %.

1 Introduction

The successful development of animal husbandry farming is impossible without the production of compound feed and feed additives that replenish the need of farm animals for nutrients, minerals and biologically active substances [1].

In the domestic poultry industry, the range of non-traditional feed ingredients is growing, and plant feed additives are increasingly being used [2]. Additives of plant origin influence growth, improve feed digestibility and help increase animal productivity [3], and such additives also have antimicrobial properties [4-7]. Among other feed additives, natural plant extracts are used, which contain various biologically active components, including

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tannins, which can be divided into hydrolyzable and condensed [8]. Tannins can come from a variety of plants, the most common being sweet chestnut (*Castanea sativa* Mill.).

Tannins can serve as a substitute for feed antibiotics [9]. The positive effect of ellagitannins on animal health is explained by their ability to bind to bacterial cell membranes and remove toxins released by bacterial cells due to complex formation [10, 11]. Additives based on tannins have a powerful antibacterial effect, antidiarrheal effect, have anti-inflammatory and antioxidant effects, increase immunity, improve the digestion and absorption of nutrients from diets [12 - 17].

Researchers note the effect of tannins on the intestines. In chickens infected with C. perfringens, a tannin-based supplement suppressed the inflammatory response and strengthened the intestinal barrier [18]. A study of the effect of tannins on Cobb500 cross broilers showed their positive effect on the intestinal condition and intestinal microbiota, but growth rates and meat yield decreased [19]. The negative impact of tannins on the performance of broiler rearing is noted by Cecep Hidayat, and a direct relationship is shown between the dosage and the result [20]. Some authors suggest that a decrease in the growth of broilers when fed preparations with tannins may be caused by the fact that nutrients are used to a greater extent for intestinal growth [21]. Some studies note a decrease in the weight of lymphatic organs when using tannins [20].

In addition to plant extracts, various butyrate additives are popular in industrial poultry farming; they are recommended for use as growth stimulants and antimicrobials [22-23]. Thus, calcium and sodium butyrate contributed to an increase in the body weight of broilers at the same feed consumption, which improved feed conversion [24]. They affect the quality of meat; for example, calcium butyrate in combination with natural antioxidants or betaine has a positive effect on the quality of quail and broiler meat [25-26].

One of the complex preparations that combines sweet chestnut extract (containing hydrolyzable tannins) and calcium butyrate is «Farmatan Liquid». Its positive effect on intestinal morphology and growth performance has been described – the output of the pectoral and leg muscles has increased [27].

It is known that various biologically active substances introduced into the diet affect the digestive organs [28, 29]. Often microstructural characteristics are indicators of conditions of detention, which determines the relevance of histological research data [30-32].

To date, the number of studies on the effect of sweet chestnut ellagitannins in combination with calcium butyrate on the histological structure of the digestive organs is extremely small; the purpose of this study is to determine changes in the glandular section of the stomach when they are fed with the drug "Butitan (Farmatan BCO)".

2 Materials and methods

The study was carried out in the conditions of the experimental poultry house of the Russian State Agrarian University - Moscow Timiryazev Agricultural Academy named after K.A. Timiryazev.

Table 1. Experience scheme.

Group	n	Diet
Control (C)	60	BD
Experimental 1 (O1)	60	BD+ Butitan (Farmatan BCO) (250 mg/kg)
Experimental 2 (O2)	60	BD+ Butitan (Farmatan BCO) (500 mg/kg)
Experimental 3 (O3)	60	BD+ Butitan (Farmatan BCO) (750 mg/kg)

The experiment was carried out on broiler chickens of the "Smena 8" cross between 10.09.2019 and 11.20.2019. From day-old broilers, 4 groups (n=60) were formed using the

method of pair-analogues based on live weight, without division by sex. The experimental design is presented in Table 1.

Feeding was carried out according to a three-phase program. The birds in the control group received the basic diet. Experimental groups - the main diet with the feed additive "Butitan (Farmatan BCO)" in different proportions (Table 1). The feed additive "Butitan (Farmatan BCO)" is a microencapsulated additive that contains sweet chestnut extract, calcium butyrate and the auxiliary substance palm oil as a shell. The additive was consumed by broilers throughout the experiment along with feed.

The birds were kept in battery cages throughout the study. Microclimate parameters were maintained within normal limits.

The duration of the study was 42 days. On days 7 and 42, for the purpose of conducting morphological studies, control slaughter of 3 heads of chickens from each group (average by weight) was carried out. After slaughtering the chickens, they were anatomically dissected and the mass of internal organs was determined.

Samples of the glandular stomach were fixed in 10% neutral formalin. Wiring, compaction, and preparation of histological sections were carried out according to Suvarna [33]. Histological sections were stained with hematoxylin-eosin according to Romeis. Using survey preparations, the thickness of the layers and membranes in the organs under study was determined using an ocular ruler; the conversion factor into micrometers was determined using an object-micrometer.

The results obtained were processed using mathematical statistics methods in the Microsoft Excel spreadsheet processor using the capabilities of the "data analysis package".

3 Results and Discussion

Data on the weight of the digestive organs are presented in Table 2.

Table 2. Absolute and relative masses of the digestive organs of broilers.

Group	Indicator					
	absolute weight of the stomach, g	relative mass stomach, %	absolute weight of the glandular stomach, g	relative mass of the glandular stomach, %	absolute mass of the muscular stomach, g	relative mass of the muscular stomach, %
7 days of age						
C	7.01 ± 0.3	5.81 ± 0.29	1.36 ± 0.12	1.13 ± 0.11	5.65 ± 1.73	4.69 ± 0.19
O1	7.28 ± 0.27	5.56 ± 0.36	1.31 ± 0.07	1 ± 0.05	5.97 ± 0.44	4.56 ± 0.33
O2	7.63 ± 0.39	5.4 ± 0.24	1.39 ± 0.13	0.98 ± 0.08	6.24 ± 0.32	4.42 ± 0.2
O3	8.11±0.66	6.08 ± 0.42	1.51 ± 0.23	1.13 ± 0.15	6.60 ± 0.47	4.95 ± 0.3
42 days of age						
C	38.85 ± 4.06	2.18 ± 0.21	6.79 ± 0.74	0.38 ± 0.04	32.06 ± 3.58	1.8 ± 0.18
O1	31.22 ± 4.88	1.67 ± 0.24	6.53 ± 0.49	0.35 ± 0.03	24.69 ± 4.86	1.32 ± 0.24
O2	29.71 ± 1.11	1.60* ± 0.06	6.29 ± 0.28	0.34 ± 0.01	23.42 ± 1.34	1.26* ± 0.08
O3	30.67 ± 6.18	1.58 ± 0.33	6.3 ± 0.63	0.32 ± 0.03	24.37 ± 5.84	1.25 ± 0.31

The differences with control treatment were significant at: *p < 0.05; **p < 0.01; ***p < 0.001

Feeding the drug "Butitan (Farmatan BCO)" during the first week of life stimulates the growth of the absolute mass of the glandular stomach in the O2 and O3 groups by 2.2% – 11%, while the relative weight in the O2 group decreased. The absolute mass of the muscular stomach increased in broilers in the experimental groups by 5.7% in O1, by 10.4% in O2 and by 16.8% in O3. An increase in relative weight of 0.26% was observed only in the O3 group. Birds in this group are distinguished by higher rates of intestinal tract development (Table 2).

By the end of the experiment, it was noted that the absolute mass of organs in the experimental groups was lower by 19.6 – 23.5% for the two chambers of the stomach, by 3.8 – 7.4% for the glandular stomach and by 22.9 – 26.9% for the muscular stomach, but the difference between the results is unreliable. The relative weight of the digestive organs also decreased relative to the control group (Table 2).

The main effect of "Butitan (Farmatan BCO)" on the mass of the stomach chambers was manifested in the first 7 days of postnatal ontogenesis, which laid the foundation for increasing the more intensive growth of broilers of the experimental groups.

Morphometry of the glandular section of the stomach at 7 days of age showed that broilers in the experimental groups were significantly inferior to the control group in the size of mucosal folds by an average of 13.8%, the thickness of the muscular plate of the mucosa by 20.6% and the thickness of the mucous membrane by 15.3%. The value of the submucous membrane in the O1 group was significantly higher than in the control group by 6.1% (P≤0.01). In groups O2 and O3, the values of submucous, muscular membrane and organ wall were lower, the difference with the control was significant (Table 3). In terms of the height of glandular epithelial cells, the experimental groups significantly exceeded the control by 11.1%.

Table 3. Morphometry of the glandular stomach of broiler, microns.

Group	Layers						
	mucosal fold	**muscular lamina of the mucosa**	**mucous**	**submucous**	**gland epithelium height**	**muscular**	**Wall**
7 days of age							
C	867 ± 14.8	217 ± 5.7	1086 ± 15.5	2984 ± 54.9	8 ± 0.2	277 ± 6.1	4347 ±59.0
O1	754*** ±5.2	189*** ± 3.6	943*** ± 6.8	3166** ± 37.7	9** ± 0.3	271 ± 3.9	4380 ±38.6
O2	761*** ±10.9	170*** ± 4.3	931*** ± 11.6	2657*** ± 10.5	9*** ± 0.2	212*** ± 4.1	3800*** ±16.1
O3	726*** ±13.1	158*** ± 3.3	884*** ± 14.3	2803** ± 32.5	9*** ± 0.2	203*** ± 3.2	3890*** ±34.9
42 days of age							
C	780 ± 16.6	200 ± 6.2	989 ± 17.6	3051 ± 73.9	11 ± 0.4	295 ± 8.1	4537 ± 64.9
O1	877*** ±20.5	225* ± 7.5	1102*** ±20.5	2966 ± 58.2	11 ± 0.3	331** ± 7.7	4399 ± 58.9
O2	728* ± 13.5	208 ± 9.1	936* ± 18.2	2637*** ±59.0	11 ± 0.3	267* ± 8.2	3840*** ±54.9
O3	742 ± 12.6	220 ± 8.8	962 ± 17.1	2760** ±50.3	12* ± 0.3	274 ± 6.6	3996*** ±54.5

The submucous membrane contains complex glands that produce enzymes and hydrochloric acid, so the development of this membrane in O1 broilers seems to be a positive fact. In groups O2 and O3, there was a decrease in all layers of the stomach, which is probably compensated by the greater mass of the organ and, therefore, better dimensional characteristics, as well as the greater height of the glandular cells of the submucous membrane.

At the end of the experiment at 42 days of age in group O1 there were higher: thickness of the layer of mucosal folds (12.4% (P≤0.,001)), thickness of the muscular lamina of the mucosa (12.5% (P≤0.05)), thickness of the mucosa tunica (11.4% (P≤0.001)) and muscularis propria thickness (12.2% (P≤0.01)). The opposite pattern is typical for O2 and O3. In the O2 group, there was a tendency to reduce the size of the folds of the mucous membrane (by 6.7%), mucous membrane (by 5.4%), submucous membrane (by 13.6%), muscular membrane (by 9.5%) and organ walls in overall (by 15.4%), the difference with the control is significant. In O3, the thickness of the submucous membrane (by 9.5%) and the organ wall (11.9%) also decreased; the difference with the control was significant. In gland epithelium height of the stomach, only the O3 group was superior to the control 9.1% (P≤0.05).

That is, the effect of "Butitan (Farmatan BCO)" at a dosage of 250 mg/kg of feed has a positive effect on the mucous membrane and muscle elements of the glandular section of the stomach (Table 3), without affecting the submucous. The effect of high concentrations of the drug on the glandular section of the stomach can be considered negative. This is also confirmed by the decrease in organ mass at the end of the experiment. Our previous studies noted an increase in the layer of villi in the jejunum when feeding "Butitan (Farmatan BCO)" [34]. Such opposite results of the influence of the active components of the drug in different parts of the gastrointestinal tract may be associated with the peculiarities of the action of the palm oil capsule in which they are enclosed. Medium-Chain Fatty Acids, which form a capsule, have been shown to dissolve in the jejunum [23], which confirms our results where the beneficial effect occurs in the jejunum. It has been noted that Medium-Chain Fatty Acids have a positive effect on the intestinal epithelium, improving the formation of tight junctions between cells [35], but their effect on the wall of the glandular stomach has not been described in the literature. The positive zootechnical results of cultivation achieved in this experiment are achieved due to the stimulating effect of the drug components on the intestines [34].

When feeding tannins, researchers note a decrease in the mass of lymph-forming organs [20]. In our case, there was good development of lymphoid tissue in the mucous membrane of the stomach in O3 group broilers. Since we did not study the mass of the bursa of Fabricius and the thymus, we cannot exclude a decrease in their mass; in our case, its compensatory development can be assumed in the gastric mucosa.

4 Conclusion

According to previous experiments, broilers in experimental groups at 42 days of age reached a weight that significantly exceeds the weight of birds from the control group [34]. We hypothesize that this was due to the fact that the supplementation of sweet chestnut ellagitannins promoted the development of the digestive organs, which was reflected in the growth of the stomach in the first week of life.

The supplement "Butitan (Farmatan BCO)" had different effects on the glandular section of the stomach, depending on the dosage. The low dosage (250 mg/kg) had no effect on the thickness of the submucous membrane, but there was an increase in the size of glandular cells. At the same time, the mucous and muscular membranes increased. Dosages of 500 and 750 mg/kg had a negative impact on the weight of the organ and the thickness of its wall. A dosage of 750 mg/kg caused an increase in gland epithelium height, which suggests greater production of gastric juice, and may partially compensate for the negative effect on the microstructure of the organ wall. An increase in lymphoid tissue in the mucous membrane of the organ was noted when using the supplement at a dose of 750 mg/kg, which we consider to be an increase in immune properties.

Currently, there is a need for further research to establish the effect of various doses of "Butitan (Farmatan BCO)" on the anatomical, hematological and microbiological parameters of the digestive system of birds.

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