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Influence of sapropel on the activity of intestinal peptidases of broiler chickens

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ABSTRAK

Pada awal percobaan, bobot mingguan ayam jantan persilangan putih telur Hisex (n = 90) adalah 64,6 ± 0,47 g. Pada umur satu minggu dibentuk 3 kelompok ayam (30 ekor tiap kelompok). Ayam percobaan menerima pakan lengkap. Kelompok pertama berfungsi sebagai kontrol. Ayam kelompok kedua dan ketiga mendapatkan pakan gabungan, masing-masing 3% dan 5% dari bobot pakan diganti dengan sapropel kering. Sapropel kering (3% dari berat pakan harian) meningkatkan aktivitas peptidase kasein-litik dan hemoglobin-litik di chyme sebesar 1,5 (P <0,05), di mukosa usus besar sebesar 1,3 (P <0,05) dan di usus halus mukosa 1,7 kali (P <0,01) dibandingkan dengan kontrol. Peningkatan konsentrasi sapropel dalam pakan hingga 5% tidak menyebabkan peningkatan aktivitas peptidase lebih lanjut. Aktivitas peptidase minimal pada pH 5 dan pH 12 dan maksimal pada pH 7–8. Dosis optimal sapropel kering yang dimasukkan ke dalam pakan ayam untuk merangsang proses pencernaan adalah 3% dari massa pakan.

Kata kunci : ayam pedaging, sapropel, intesti, aktivitas peptidase, dependensi pH

ABSTRACT

At the beginning of the experiment, the weight of weekly cockerels of the Hisex White egg cross (n = 90) was 64.6 ± 0.47 g. At the age of one week old, 3 groups (30 birds in each group) were formed. They received complete feed. The first group served as a control. The chickens of the second and third groups received combined fodder, in which 3% and 5% of the feed weight was replaced with dry sapropel. The dry sapropel (3% of the daily feed weight) increased the activity of casein-lytic and hemoglobin-lytic peptidases in the chyme by 1.5 (P<0.05), in large intestine mucosa by 1.3 (P<0.05) and in small intestine mucosa by 1.7 times (P<0.01) compared with the control. The increase of sapropel concentration in feed up to 5% did not lead to a further increase in the activity of peptidases. The activity of peptidases were minimal at pH 5 and pH 12 and reached maximal at pH 7–8. The optimal dose of dry sapropel introduced into the diet of chickens ~~to~~ that stimulated the digestive processes is 3% of the mass of feed.

Keywords: broiler chickens, sapropel, intestines, peptidase activity, pH dependence

INTRODUCTION

As it is known, poultry farming is one of the leading branches of agricultural production. Currently, the main research trend in the field of poultry feed production is the search of feed additives which can reduce the proportion of grains and legumes in the diet. The effect of enzyme supplements on the condition of broiler chickens and body weight increase was studied in several studies. So, it is shown that the introduction of amylase in the diet, as well as amylase and protease together during the first 2 weeks do not influence on characteristics of broilers. However, the addition of a non-starch polysaccharide enzyme in feed increases body weight gain and feed conversion rate (Kaczmarek *et al*., 2014). In the study of broiler chickens (Ross-308) aged from 1 to 42 days old which were fed with non-starch polysaccharide enzyme and proteases, the activity of pancreatic enzymes and the expression of mRNA of trypsin, lipase and amylase in the experimental group significantly increased compared with the control group (Yuan *et al*., 2017). In addition, it is shown that the introduction of proteases (0.2 g / kg) into broiler chicken (Cobb) feed increases the phylogenetic diversity and abundance of the microbiota (Jeferson *et al*., 2020), and also improves the condition of the intestinal mucosa (Cardinal *et al*., 2019). It is also found that enzyme preparations have the positive effect on the immune system of chickens and broiler productivity (Sugiharto and Ranjitka, 2019).

In order to improve the productivity of the poultry industry, affordable but effective feed additives are required. One of the most promising feed additives is sapropel (Bultka and Latvietis, 2001; Losyakova *et al.,* 2018) lining the bottom of some freshwater bodies. Sapropel is formed from the residues of aquatic vegetation, living organisms, plankton and soil humus particles, thus containing a significant amount of organic and mineral substances (Murunga *et al.,* 2020). The composition of sapropel depends on the physicochemical parameters of the reservoir and the composition of the biota during the entire period of its existence. Sapropel contains proteins, fats, carbohydrates, amino acids, humic substances, estrogen-like compounds, vitamins, enzymes and antibiotics (Stankevica *et al.,* 2016). Raw sapropel contains on average 6.8 g/kg of protein and 1.12 g/kg of nitrogen, 2.5 mg/kg of calcium, 0.7 mg/kg of phosphorus, 0.25 mg/kg of carotene, as well as B vitamins, copper, zinc, iron, cobalt and others macro- and microelements. Depending on the ash and organic substance contents, mineralized (the content of mineral substances from 70 to 85 %), mineral-organic (50–70 %), organo-mineral (30–50 %) and organic (up to 30 %) sapropels can be distinguished. In some cases, sapropel contains up to 50–60 % of organic substances and up to 30–50 % of minerals.

In Kenya, undocumented data showed the application of BioDeposit Agro (BDA) sapropel in different regions including Tala in Machakos. The BDA has been used in the production of banana, maize, vegetables, coffee and hydroponic cow feeds, as well as in poultry farming. The results indicate that the BDA contributes positively to the quality of produce, at the same time as increasing its yield (Murunga *et al.,* 2020). However, an increase in the content of ash and sand-clay admixtures above optimal values may worsen the quality of sapropel feed additives. Several studies showed that sapropel increases the productivity and meat quality of farm animals (Bultka and Latvietis, 2001; Losyakova *et al.,* 2018; Arzhankova *et al.,* 2017; Pozdnyakova *et al.,* 2019). The inclusion of sapropel in poultry diets contributes to an increase in the growth rate of chickens, improving their disease resistance and, consequently, food safety parameters (Slepukhina *et al.,* 2019). In particular, it was shown that the introduction of 3 to 5 % of dry sapropel into the diet of chickens not only reduced feed costs, but also increased their live weight and survival rate (Pozdnyakova *et al.,* 2019). The use of sapropel feed additives (SFA) allowed the gross live weight gain of broiler chickens to be increased by 1.7–2.0% while reducing feed costs per 1 kg of gain by 0.5% (Yurina *et al.,* 2020).

Assuming that the high survival rate of chickens obtaining sapropel supplements can be attributed to the bactericidal properties of sapropel, then the weight gain is likely to result from a greater activity of enzymes. The latter hydrolyze the protein components contained in the feed thereby making amino acids are more available for metabolism. This assumption is based on the information that a distinctive feature of young farm birds is the lack of digestive enzymes in their gastrointestinal tract, which leads to a decrease in the absorption of substantial elements (Robinson, 2015).

Despite the available knowledge about the positive action of sapropel feeds, the effects of sapropel on the activity of intestine peptidases in poultry birds require further elucidation. The aim of this work was to study the effect of dry sapropel on the activity of peptidases functioning in the intestine and carrying out the hydrolysis of proteins and peptides in the feed of Hisex white egg cross chickens.

MATERIALS AND METHODS

Materials

Effects of dry sapropel on the activity of peptidases, the growth and development of Hisex white egg cross chickens were studied at research departments of the Yaroslavl Zoo and the Papanin Institute for Biology of Inland Waters of the Russian Academy of Sciences.

Methods

The cockerels were used at the age of 7 days old. At this age, 3 groups (30 in each group) were formed according to the principle of analogues. The first group served as a control. The dietary treatment contained the following ingredients: corn (45%), wheat (10%), soybean meal (10%), sunflower meal (15%), fish meal (9%), sunflower oil (3,5%), premix (7,5); One kg of the dietary treatment contained metabolic energy (2980 kcal), crude fiber (0.70%), crude fat (5.45%), crude protein (21.05%), lysine (1.48%), methionine + cystine (0.92%), tryptophan (0.27%), calcium (1.79%), phosphorus (0.79%), salt (0.5%). The content of trace elements in one kg of diet were: iron (50 mg); manganese (106.67 mg); iodine (1.0 mg); copper (9.2 mg); selenium (0.2 mg); zinc (70.83 mg). The chickens of group 2 and 3 received experimental diet, in which 3% and 5% of the feed weight was replaced with dry sapropel, respectively.

The chickens in all groups were kept in similar conditions, having free access to water and feed. Chickens were slaughtered at the age of 34 days old. The weight of the chickens was determined using a VLKT-500M electronic balance having an accuracy of 0.01 g. Subsequently, the intestines were removed and placed on an ice bath to be cooled to a temperature of 1–2 °C. The intestines were cut lengthwise. The chyme was removed with a sterile spoon followed by separation of the mucosa from the muscle layer with a special spatula. Chyme and mucosa samples of the small and large intestines were weighed separately on an analytical balance in 10 ml penicillin vials and filled with chilled saline in a ratio of 1: 9. The contents of the vials were homogenized using a piston-action Teflon tube homogenizer followed by additional dilution (1: 9).

The activity of casein-lytic and hemoglobin-lytic peptidases (the total activity of trypsin, EC 3.4.21.4 and chymotrypsin, EC 3.4.21.1) was evaluated by an increase in the tyrosine concentration using a Folin-Ciocalteu assay. As the substrate, 1% casein solutions or hemoglobin preparations on physiological saline (pH 7.4) were used. Prior to determination of the proteolytic activity of enzymes, the pH of the homogenate of each preparation and substrate was adjusted to 7.4 using a pX-150MI pH-meter. Homogenates and substrates (pH 7.4) were incubated at 20ºC for 30 min under constant stirring. The reaction was stopped by adding 1 ml of 0.3 N trichloroacetic acid (TCA). Following 10 minutes, the incubate was filtered using filter paper. Afterwards, 0.25 ml of the filtrate, 2 ml of 0.5 N NaOH, 0.25 ml of 0.025 N $CuSO_4$ and 0.75 ml of the Folin-Ciocalteu reagent, previously diluted 3 times, were added. In order to determine the initial content of tyrosine in the samples (background), TCA was added to the homogenate before adding the substrate. The other operations were identical. The color intensity of the samples was measured using a KFK-2 photocolorimeter at a wavelength of 670 nm 20 min after adding the Folin-Ciocalteu reagent. The level of enzymatic activity was judged by an increase in the reaction products following 1 min of incubation of the substrate and an enzymatically active preparation taking into account the background (the amount of tyrosine in the initial homogenate) per 1 g of fresh tissue mass, mmol / (g min). The enzymatic activity at each point was determined in 4 replicates taking into account the background (initial amount of tyrosine). The pH dependence of peptidases was determined at a pH range of 5–12 (Egorova *et al*, 1974).

Data Analysis

The data were statistically processed using Microsoft Excel 97-2003. The mean and error of the mean were determined. Differences between the mean values were considered significant at $P<0.05 – P<0.001$.

RESULTS AND DISCUSSION

In the control group, the average weight of chickens following 12 days of the experiment was

93.4 ± 1.8 g. In the 1st experimental group receiving 3% sapropel, this value equaled 98.9 ± 1.1 g, with the differences being statistically significant ($P<0.05$). At the end of the experiment (at the 34th day), the body weight in the control and two experimental groups was 291.1 ± 3.4 g, 311.2 ± 3.6 g and 297.0 ± 3.9 g, respectively ($P<0.01$). The smallest feed consumption per 1 kg of the weight gain was demonstrated by the chickens in the 1st experimental group (Table 1). At the end of the experiment the activity of peptidases functioning in the chyme and intestinal mucosa of chickens from these groups varied considerably (Table 2).

The level of enzymatic activity in 2 experimental groups receiving sapropel was higher than that in the control. The highest level of enzymatic activity, as well as its greatest increased under the influence of sapropel, was found in the small intestine. Thus, in comparison with the control, when sapropel (3%) was added to the poultry diet (1st experimental group), the casein-lytic activity of peptidases increased by 51.3% ($P<0.05$), 70.3% ($P<0.01$) and 30.5% ($P<0.05$) and in the chyme, small intestine and large intestine, respectively. The addition of 5% of sapropel (2nd experimental group) led to an increase in the activity of casein-lytic peptidases by 10.3%, 45.6% and 21.3% in the chyme, small intestine and large intestine, respectively. Compared to the control, the activity of hemoglobin-lytic peptidases in the 1st sapropel group was higher by 48.8% ($P<0.55$), 65.5% ($P<0.01$) and 28.6% ($P<0.05$) in the chyme, the

Table 1. Feed Efficiency for Broiler Chickens during Experiment

Chicken Groups	Total Amount of Mixed Fodder, kg	Weight of Chickens, g	Feed Consumption per 1 kg of Live Weight, kg
Control	19.59	291.1±3.4	2.38
1-st experimental group	19.00	311.2±3.6	2.16
2-d experimental group	18.63	297.0±3.9	2.22
The cost of fed combined fodder, rub	1486.9	1442.3	1414.3
The cost of feed additive, rub.	-	8.88	14.4
The cost of combined fodder and and feed additive, rub	1486.9	1451.1	1428.7
Economic effect, rub.	-	+3.52	+1.03

Table 2. The Influence of Sapropel on the Activity of Peptidases in the Intestine of Chickens

Substrates	Variant	Activity of Ppeptidases, µmol/(g×min).		
		Control	Sapropel, 3%	Sapropel, 5%
Casein	Chyme	2.71±0.14	4.10±0.11*	2.99±0.07
	Intestinal mucosa 1	1.82±0.05	3.10±0.07**	2.65±0.07
	Intestinal mucosa 2	2.49±0.15	3.25±0.08*	3.02±0.05
Hemoglobin	Chyme	2.58±0.14	3.84±0.03*	2.69±0.14
	Intestinal mucosa 1	1.65±0.07	2.73±0.06**	2.25±0.07
	Intestinal mucosa 2	2.38±0.12	3.06±0.03*	2.62±0.14

1: small intestine, 2: large intestine, *: $P<0.05$, **: $P<0.01$

small intestine and the large intestine, respectively. For the proteolytic activity of enzymes in the 2nd sapropel group, these values were 4.3%, 36.4% (P<0.99) and 10.1%, respectively.

An assessment of the activity of casein-lytic and hemoglobin-lytic peptidases of the intestinal mucosa of chickens across a wide range of pH values (from 5 to 12) confirmed a greater effect of lower sapropel concentrations on enzymatic activity (Figure 1). Similar effects were observed when studying the pH-dependent influence of sapropel on the hemoglobin-lytic activity of chicken intestinal mucosa (Figure 2).

It is well known that the main source of digestive enzymes in the intestines of birds is the pancreas secreted into the lumen of the duodenum. Peptidases of pancreatic juice (trypsinogen, chymotrypsinogen, procarboxy-peptidases A and B, elastase), entering the intestine, are sequentially activated. Trypsin is activated by enterokinase, leading to activation of other peptidases. Impregnating the chyme, these enzymes hydrolyze proteins to the level of peptides. With a stream of water, a part of active enzymes, proteins and peptides enters the brush border of enterocytes, which form the basis of the mucosa epithelium. In the glycocalyx zone, the hydrolysis of proteins and peptides continues to the level of dimers, which are hydrolyzed by amino- and dipeptidases localized on the membranes of microvilli and cytosol, respectively, to the level of amino acids entering the transport systems (Ugolev, 1972). Typically, in vertebrates, digestion in the intestine occurs at slightly alkaline pH values. In comparison, bird digestion is characterized by lower or neutral pH values in all parts of the gastrointestinal tract, except for the ileum (Provorotova *et al*., 1967).

The mechanisms of sapropel action on the activity of broiler intestinal peptidases require a particular study. It should be noted that both direct and indirect action of sapropel components on enzyme molecules is possible. According to our observations revealed, casein-lytic and hemoglobin-lytic peptidases showed a higher activity in the large intestine as compared to the small intestine of chickens. In all likelihood, the enzymes of pancreatic juice cannot be fully adsorbed at the structures of the brush border of the anterior intestine due to fast intestinal motility. At the same time, this phenomenon can be considered as an adaptation mechanism that promotes a more complete protein catabolism among animals having a relatively short intestinal tract.

The differences in the characteristics of casein-lytic and hemoglobin-lytic peptidases can be explained by the fact that these enzymes hydrolyze peptide bonds in different parts of the protein molecule. The sorption of the substrate in the active center of trypsin is optimal for binding the residues of aliphatic basic amino acids, namely arginine and lysine. The sorption of the

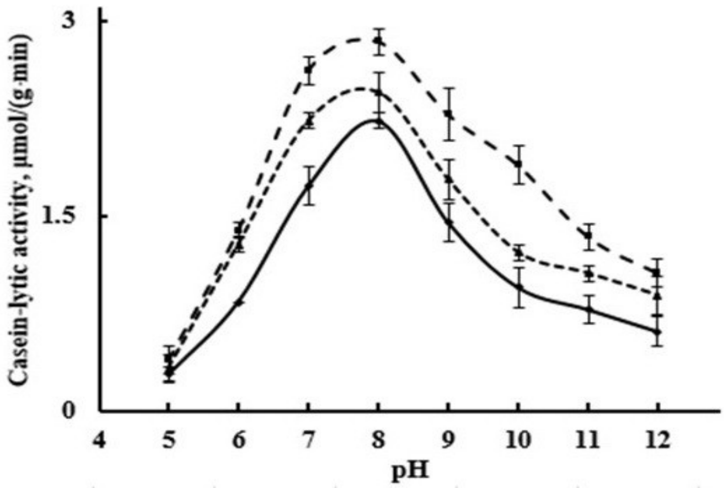

Figure 1. The Effect of Sapropel on the pH-dependence of Casein-lytic Activity of Chicken Intestinal Mucosa

———: control group , - - -: 1st experimental group (3% sapropel), – – – –: 2nd experimental group (5% sapropel)

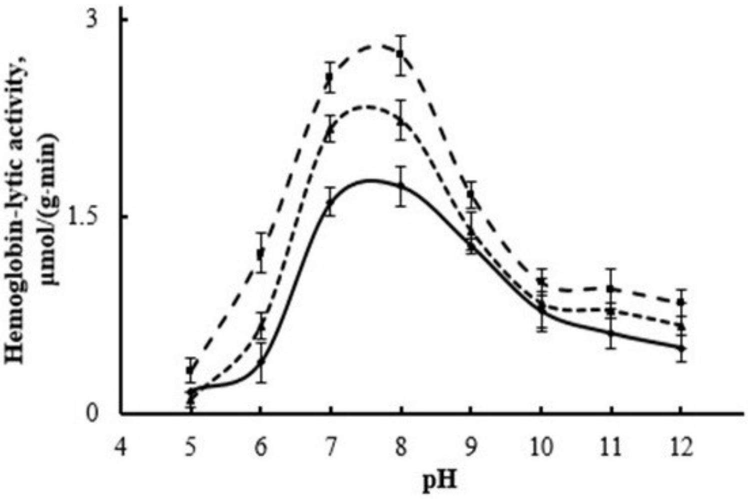

Figure 2. The Effect of Sapropel on the pH-dependence of Hemoglobin-lytic Activity of Chicken Intestinal Mucosa.
———: control group , - - -: 1st experimental group (3% sapropel), – – – –: 2nd experimental group (5% sapropel)

substrate in the active center of chymotrypsin is optimal for binding the side chains of hydrophobic amino acid residues, such as tryptophan, phenylalanine and tyrosine (Dixon and Webb, 1964). The latter mechanism allows a more complete hydrolysis of the protein components of the feed.

It is important to note that the addition of sapropel to the poultry feed increases the activity of casein and hemoglobin-lytic peptidases, which function in the chyme and mucosa of both parts of the intestine. Indeed, when sapropel is included in the diet of chickens in the amount of 3% of the daily feed weight, in comparison with the control, the casein-lytic activity of peptidases increases by almost 2, 1/3 and 2/3 times in the chyme, large intestine and small intestine, respectively. This undoubtedly has a positive effect on the processing and subsequent assimilation of protein hydrolysis products, which is evidenced by an almost 7 % increase in the body weight in the 1st sapropel group compared to the control. At the same time, a further increase in the concentration of sapropel in the feed does not lead to a proportional increase in the activity of peptidases and the weight of chickens, which increases only by 2%. This occurs largely due to a worsening assimilation of sapropel. It should be emphasized that sapropel, increasing the activity of casein-lytic and hemoglobin-lytic peptidases of the chicken intestine mucosa over a wide range of pH values (from 5 to 12), does not change the pH optimum. Our data agree well to the observation that the crude enzyme extract of the intestine and pancreas of chickens demonstrates a high proteolytic activity with an optimum of pH 7.5 (Srisantisaeng *et al*., 2013).

At present, it is not clear which components of sapropel improve the peptidase synthesis and the conditions of their functioning. Since sapropel contains important macro- and microelements involved in various processes – 4.41 mg/kg of copper, 15.75 mg/kg of zinc, 339 mg/kg of manganese, 3.84 mg/kg of cobalt and 24.2 mg/kg of chromium (Pozdnyakova *et al*., 2019), there is no doubt that these mineral supplements can affect the metabolism of chickens (Robinson, 2015). Particularly noteworthy is the fact that the mineral components of sapropel enter the body of birds with food in organic form. A previous study comparing the effect of inorganic minerals (sulfate form) and organic minerals (peptide-chelate form) on the productivity of broilers of the Ross 308 cross showed that substitution of inorganic minerals with organic minerals increases bird productivity and enhances their immune response (Abdallah *et al*., 2009). This may be largely connected with the dominance of chelated metal transport among vertebrates, from fish to mammals (Bakke *et al*., 2011). At the same time, an increase in the proportion of sapropel leads to a decrease in its positive effect on the activity of peptidases. It can be assumed that increased concentrations of biogenic metals can affect both the rate of various biochemical reactions and the amount of metals entering the intestine during recycling.

In addition, sapropel may contain metabolic stimulants of unknown nature. It is of particular importance that all these substances are present in their native form, which contributes to their better assimilation. In this case, the products of the vital activity of microflora can play a significant role. Thus, all layers of sapropel deposits in the eastern part of the Mediterranean Sea demonstrate a pronounced activity of bacterial exoenzymes, aminopeptidase and alkaline phosphatase, and a weak activity of β-glucosidase (Coolen and Overmann, 2020). There is a reason to believe that sapropel will have similar effects on the activity of peptidases in other bird species, since the structure of the active sites of enzymes is conservative (Dixon and Webb, 1964).

CONCLUSION

Dry sapropel (3% of the daily feed weight) may increase the average live weight of broiler chickens, as well as the activity of casein-lytic and hemoglobin-lytic peptidases in the chyme and intestinal mucosa, compared to the control. An increase in the sapropel concentration in feeds of up to 5 % does not lead to an increase in the peptidase activity. Sapropel increases the activity of intestinal peptidases particularly significantly in the 7.0–9.0 pH range. In order to stimulate digestive processes and increase the weight of chickens, dry sapropel should be introduced into the poultry diet at an optimal level of 3% of the feed weight.

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