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Study of influence of proteolytic action fodder additive in the composition of feed on productivity and development of broilers chickens' internal organs

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Abstract. In the system of comprehensive assessment of feed nutritional value, protein plays a special role. With the correct selection of proteolytic enzymes in the diet and with appropriate conditions (pH, temperature, duration and quantity of enzymes), most feed proteins can be degraded to preferred degree in the body: either partially, limited by proteolysis, or deep and complete – to individual amino acids, which is accompanied by an increase in animal productivity.
The objective was to study the efficiency of using the exogenous enzyme Sibenza DP 100 in the diet on productivity and development of broilers chickens' internal organs. The experimental part of the work was carried out under the industrial conditions of private limited company 'LISKoBroiler' on Ross 308 broiler chickens. When the proteolytic enzyme was added to the birds' diet without decreasing the nutritional value of crude protein and digestible aminoacids, higher values of the average daily gain were observed among all experimental groups compared to the control group, while reducing feed output per 1 kg of gain. While reducing the nutritional diet value in accordance with the matrix by 2.5% for crude protein and digestible amino acids and with the addition of the Sibenza DP 100 enzyme (500 g t^{-1}), growing broilers was characterized by a slight lag in live weight compared to the control group, but with the most effective consumption feed per 1 kg of gain. The abovementioned system for the proteolytic enzyme use had the most positive effect on the morphological structure of the pancreas of broiler chickens.

Key words: enzyme preparation, protease, broiler chickens, pancreas, nutrient digestibility, intestinal length.

INTRODUCTION

It is a fact that the nutritional value of feed cannot be expressed by one indicator – it must be comprehensive. In the system of a comprehensive assessment of the nutritional value of feed, protein plays a special role. The presence of protein in the diet in disproportionate amounts and with an imbalance in amino acids significantly affects the growth and development of birds and many metabolic processes.

Enzymes have been used in world animal husbandry since the 1970s. They were the first to be used in Scandinavian countries – Finland and Denmark (Fomina, 2008). In foreign countries, Clickner F.H. and Follwell E.H. in 1926 for the first time reported an improvement in the growth of chickens and an increase in egg production as a result of the addition of enzyme preparation to bird ration. In numerous experiments carried out by foreign specialists in feeding birds with enzymes, we see that all available enzymes at the initial stage of study were implemented into bird rations, not taking into account their activity and only in experimental manner and then laboratory studies revealed substrates for each enzyme (Hennig, 1976).

Previously, all enzymes with several activities had a proteolytic effect as concomitant, but not basic. From 2012 to 2019, 4 enzymes of proteolytic action were registered in Russia. Currently, the use of enzymes with protease activity in feeding poultry is of great interest among specialists.

With the right selection of proteolytic enzymes and with appropriate conditions (pH, temperature, duration and amount of enzyme), most feed proteins can degraded to preferred degree: either partially, limited by proteolysis, or deep and complete – to individual amino acids (Tsyperovich, 1971; Tikhonov & Yudina, 2014; Booker, 2015). Proteases have a relatively broad specificity. This means that they can decompose a variety of bonds in the protein molecules, while certain bonds in the peptide chains can be rapidly degraded, but, in addition, they can also decompose many other uncharacteristic bonds. Therefore, in each case it is necessary to carefully select the used proteinase complex or a combination of enzymes. Usually, the suitability of the enzyme system chosen for this use can be determined using special control experiments (Tsyperovich, 1971; Angel et al., 2011; Ajayi, 2015).

Metabolic processes, occurring in the body of birds, largely depend on the morphological and functional features of the digestive system (Fisinin, 2010; Fisinin & Egorov, 2011; Cowieson & Roos, 2014). In this regard, the subjective on the proteolytic enzyme influence on the productive indicators of broiler chickens and on the development of internal organs is relevant and has practical interest for poultry producers (Yu et al., 2007). The objective is to study the effectiveness of use of the exogenous proteolytic enzyme Sibenza DP100 in the diet of broiler chickens.

The aim is to study the influence of exogenous proteolytic enzyme Sibenza DP100 in the ration for productive indicators and the development of internal organs of broiler chickens.

MATERIALS AND METHODS OF RESEARCH

Under industrial conditions of private limited company 'LISKoBroiler' of Cherkizovo Group of Companies, Liskinsky District, Voronezh Region.

The material for carrying out scientific and industrial experiments was the broiler chickens of ROSS – 308 cross. Broilers breeding took place on a deep litter on the floor in specifically prepared mini insulators in windowless rooms with adjustable microclimate and with mechanization and automation of the drinking process. The litter is presented by husk of sunflower seeds 2–3 cm thick. All feeding and drinking equipment is presented and organized by BigDutchman. The microclimate in the housing is fully automated and controlled by viper computers by BigDutchman.

Technological parameters of keeping and feeding broiler chickens corresponded to cross breeding recommendations. Bird feeding was carried out manually, feed replenishing was done as it was eaten by birds. The live weight of chickens when starting the experiment in the daily age was on average 42 g. The landing density was 20, 22 heads m^{-2}.

Experimental birds were subjected to veterinary treatment according to the scheme of preventive measures adopted at the enterprise (annex 1).

For conducting all experiments chickens were selected in groups on the principle of analogues – the same in origin, age, general development. The bird intended for the experiment was individually weighed and distributed into groups by random sampling. The protease was introduced into the feed for the entire rearing cycle – 39 days. Feeding broiler chickens was carried out with full feed, which corresponded to the recommendations of Ross cross. All components of feed were subjected to zootechnical analysis immediately before preparation. Optimization of rations was carried out using программного 'CORMOPTIMA' complex. Rations based on corn, soybean protein meal, meat – feather meal (5.5% of each in the final ration) were used. Feeding broiler chickens was three-phase depending on the от period of cultivation:

Phase 1 – feeding aged 0–14 days – complete feed prepared on recipe PC-5-1.

Phase 2 – feeding aged 15–24 – complete feed prepared on recipe PC-5-2.

Phase 3 – of feeding aged 25–39 days – complete feed prepared on recipe PC- 6 1.

Table 1. Scheme of the scientific and economic experience

Groups	Amount of birds	Diet
Control	40	main diet (MD) – complete broiler fodder
1st experimental	40	nutritional value of MD is reduced in accordance with the matrix by 2.5% for crude protein and digestible amino acids without the inclusion of Sibenza DP 100
2nd experimental	40	MD + Sibenza DP 100 500 g t^{-1}
3rd experimental	40	nutritional value of MD is reduced in accordance with the matrix by 2.5% for crude protein and digestible amino acids with addition of Sibenza DP 100 in a dose of 500 g t^{-1}
4th experimental	40	nutritional value of MD is reduced in accordance with the matrix by 5% for crude protein and digestible amino acids with addition of Sibenza DP 100 in a dose of 500 g t^{-1}
5th experimental	40	nutritional value of MD is reduced in accordance with the matrix by 7.5% for crude protein and digestible amino acids with addition of Sibenza DP 100 in a dose of 500 g t^{-1}

According to the scheme of scientific and economic experience (Table 1), 5 groups of broiler chickens were formed: 1control group and 5 experimental groups, with 40 birds in each group. The control group received the main diet – complete fodder. In broilers of the 1st experimental group, the nutrition of the main diet was reduced by 2.5% for crude protein and digestible amino acids in accordance with the matrix recommended by the manufacturer, without the inclusion of the proteolytic Sibenza DP 100 enzyme. Chickens of the 2nd experimental group received the main diet with the addition of the Sibenza DP 100 enzyme in an amount of 500 g t^{-1}. The diet of broilers the 3rd experimental group was reduced in accordance with the matrix by 2.5% for crude protein and digestible amino acids with the addition of Sibenza DP 100 (500 g t^{-1}). Birds of the

4th experimental group was fed the main diet with a decrease in nutrition in accordance with the matrix by 5% for crude protein and digestible amino acids with the addition of the Sibenza enzyme DP 100 (500 g t^{-1}). In broilers of the 5th experimental group, the nutritional value of the main diet was reduced by 7.5% in crude protein and digestible amino acids with the addition of the Sibenza DP 100 enzyme (500 g t^{-1}).

The experimental chickens were reared on floor system in specially prepared mini isolators. The live weight of chickens averaged 42 g in the age of 1 day, when they were set for the experiment.

The digestibility of feed nutrients was studied in the course of balance experiments, according to the method of All-Russian Research and Technological Poultry Institute of Russian Academy of Sciences (Egorov et al., 2013).

At 24th day of age, 3 broiler males with weight corresponding to the group average were selected from each group. The birds were slaughtered and microscopic examined for the pancreas condition. The material was prepared by fixing in a 10% aqueous solution of neutral formalin and then preparing histological sections of 5–7 μm thickness on the MHC-2 microtome and staining them with Mayer hematoxylin and eosin stain.

The length of the intestine and the weight parameters of the pancreas were taken into account by individual measurements during slaughter on the 24th day and 35th day (3 animals from each group). The pancreas was weighed on a BM-313 electronic laboratory balance; division value of 0.001 g.

RESULTS

The research results showed that the introduction of protease into the diet without reducing the nutritional value of crude protein and digestible amino acids from 1 day of age to the end of fattening has a positive effect on the bird weight. So, in the 2nd experimental group, the chicken weight at the end of fattening (39th day) exceeded the control group value by 0.68% (Table 2). In other experimental groups at the end of the technological cycle, this indicator was lower than in the control group: in the 1st experimental group by 2.6%; in the 3rd experimental group by 0.68%; in the 4th experimental group by 3.7% ($P \leq 0.05$); in the 5th experimental group by 7.2% ($P \leq 0.001$). At the same time, in the 3rd experimental group that received a diet with a minimum reduction in nutritional value for crude protein and digestible amino acids in accordance with the matrix by 2.5% and with the addition of Sibenza DP 100 in a dose of 500 g t^{-1} of feed, the lag in weight gain was the most minimal if compared to the control group indicator.

Table 2. Productive indicators of broiler chickens

Indicator	Group					
	Control	1	2	3	4	5
Weight at 39 day of age, g	2,345 ± 26.9	2,284 ± 25.85	2,361 ± 27.15	2,329 ± 26.58	2,258* ± 26.27	2,177*** ± 31.70
Daily average weight gain, g	59.04	57.49	59.46	58.64	56.82	54.74
Viability, %	95.00%	95.00%	95.00%	95.00%	92.50%	95.00%
Feed output per 1 kg of weight gain, kg	1.704	1.739	1.683	1.67	1.741	1.76

*Note: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.*

Analysis of feed output per 1 kg of weight gain of the experimental bird showed (Table 2) that they were the smallest in the 3rd experimental group. In broilers of the 2nd experimental group, this indicator was also lower than in the control group – by 0.02 kg, which amounted to 1.68 kg. The best results in terms of feed output in the 2nd and 3rd experimental groups indicate a higher digestibility and the use of nutrients of complete fodder, which ensured the greatest increase in the weight gain in these groups. In the 1st, 4th and 5th experimental groups, feed output were higher in comparison with the control group by 0.035–0.056 kg.

The results of the balance experiment showed that the chickens of the 4th experimental group differed in the best digestibility of the dry matter; the difference with the control group for this indicator was 1.9%. Broilers of the 2nd, 3rd and 5th experimental groups were also characterized by higher digestibility of dry matter in comparison with the control group by 1.6%, 1.2% and 1%, respectively. In the 1st experimental group there was a decrease in this indicator in comparison with the control group by 0.4%. Digestibility of feed protein in broilers of the 2nd, 3rd and 4th experimental groups was higher than in the control group by 3.2%, 2.1% and 1.1%, respectively. In chickens of the 1st and 5th experimental groups were no differences in this indicator compared to the control group. The digestibility of fat in birds of the 2nd and 3rd experimental groups was higher than in the control group by 0.86% and 0.26%, respectively, and in chickens of the 1st, 4th and 5th experimental groups was lower by 1.72%, 1.24% and 1.0%, respectively. Fiber digestibility in the 2nd and 3rd experimental groups was higher than in the control group by 1.71% and 0.44%, respectively. Chickens of the 1st, 4th and 5th experimental groups showed a decrease in fiber digestibility in comparison with the control group in the range of 0.3–0.66%. The highest consumption of calcium was observed in the 2nd, 3rd, 4th experimental groups. The 1st and 5th experimental groups were characterized by a decrease in calcium absorption by 0.6% and 0.2%, in comparison with the control group. The phosphorus consumption by broilers of all experimental groups was slightly lower than that of the control group in the range of 0.1–0.88%.

Table 3. Digestibility and use of feed nutrients by broilers with the introduction of Sibenza DP 100 in the diet

Indicator	Group Control	1	2	3	4	5
Digestibility:						
of dry matter	74.6	74.2	76.2	75.8	76.5	75.6
proteins	89.6	89.7	92.8	91.7	90.7	89.4
fats	88.84	87.12	89.7	89.1	87.6	87.84
fibers	29.16	28.3	30.87	29.6	28.86	28.5
Consumption of:						
Calcium	51.5	50.9	52.54	52.13	51.8	51.36
Phosphorus	46.64	45.9	46.1	46.54	45.76	45.8

Based on the analysis of the nutrient digestibility, we can conclude that the best nutrient uptake was in the 2nd and 3rd experimental groups (in comparison with the control group), where the protease was added in addition to the main diet without reducing the nutritional value of the diet and with a minimum decrease in the nutritional value of the diet by 2.5% in crude protein and digestible amino acids. A tendency towards a decrease

in the digestibility of food nutrients in chickens in the 4th and 5th experimental groups was observed, which indicates that a decrease in the nutritional value of the diet by 5.0% and 7.5% while feeding protease in an amount of 500 g/t is impractical. This statement is confirmed by a lower weight of chickens in these groups and an increase in feed output per 1 kg of gain.

The weight of the pancreas in broiler chickens is usually 3.55–3.81 g (Shneiberg et al., 1987). According to Somova (2012) the most active growth of this organ is recorded in chickens at age of 1 to 14 days (Somova, 2012). In general, the highest rates of pancreas growth in birds are recorded according to various authors up to 30 days of age, after which there is a gradual slowdown in the increase in the absolute weight of the organ (Bodrova, 2011; Matveev & Zhambulov, 2017).

When analyzing autopsy of chickens at 24 days (the period of changing from grower to finale diet) and before including meat and feather meal in the diet, in the conditions of this poultry farm, we see the following differences between the groups.

The pancreas weight in chickens of the 2nd and 3rd experimental groups was higher than the control by 2.79 and 9.22%, respectively, amounting up to 3.68 and 3.91 g. The pancreas weight in broilers of the 1st, 4th and 5th experimental groups was less than of the control group value by 0.84%, 7.2% and 12.29%, respectively (Fig. 1).

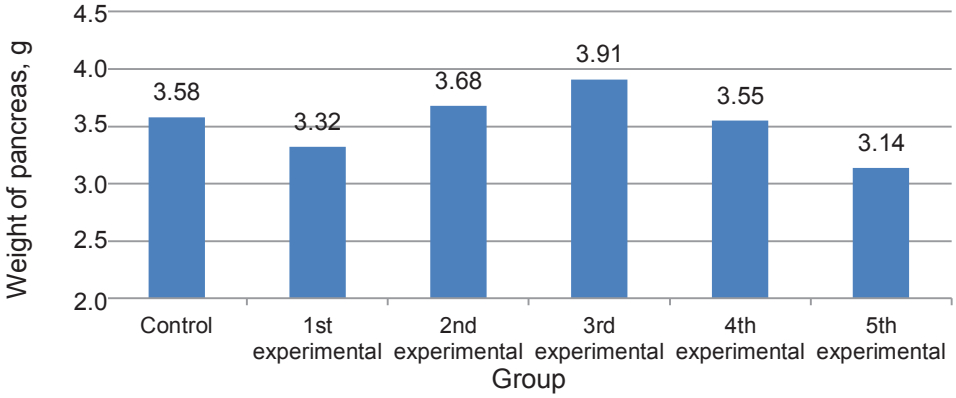

Figure 1. The weight of the broiler pancreas on 24th day, g (n = 3).

Hydrolysis of food nutrients occurs in all parts of the digestive tract, however, its highest intensity is observed in the small intestine. The feed uptake in broiler chickens is more intensive mainly due to the greater intestinal length, which ensures rapid growth and high meat yield of birds (Shestakov et al., 2012; Korsakov et al., 2019; Kablucheeva, 2000).

The intestinal length in broilers of the 2nd, 3rd, 4th experimental groups was 2.26%, 3.95% and 3.39% higher than that of the control group, and was estimated to 181–183 cm, compared with the length of the intestines of the control group, which was 177 cm (Fig. 2). The 5th experimental group showed no differences in the intestine length compared with the control group. In birds of the 1st experimental group, which received a nutritionally reduced diet in accordance with the matrix by 2.5% for crude protein and digestible amino acids and did not receive Sibenza DP 100, the intestinal length was 168 cm, which was 5.08% lower than in the control group. The established difference

negatively affected the assimilation of nutrients, as evidenced by the results of the balance experiment.

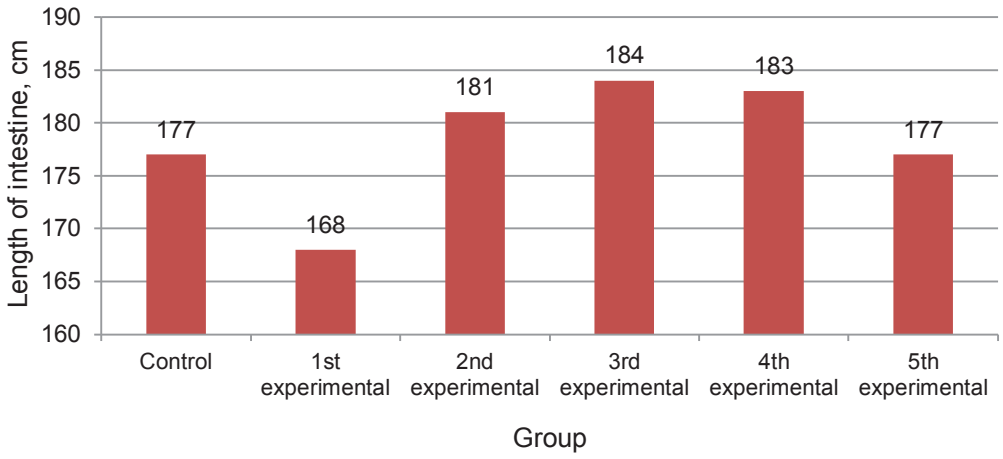

Figure 2. The length of the intestines of broilers on 24th day, cm (n = 3).

At 35 days of age, the weight of the pancreas in chickens of the 1st, 2nd, 3rd, 4th and 5th experimental groups was higher by 4.80%, 6.47%. 1.25%, 4.38% and 0.21%, respectively, than in the control group (Fig. 3).

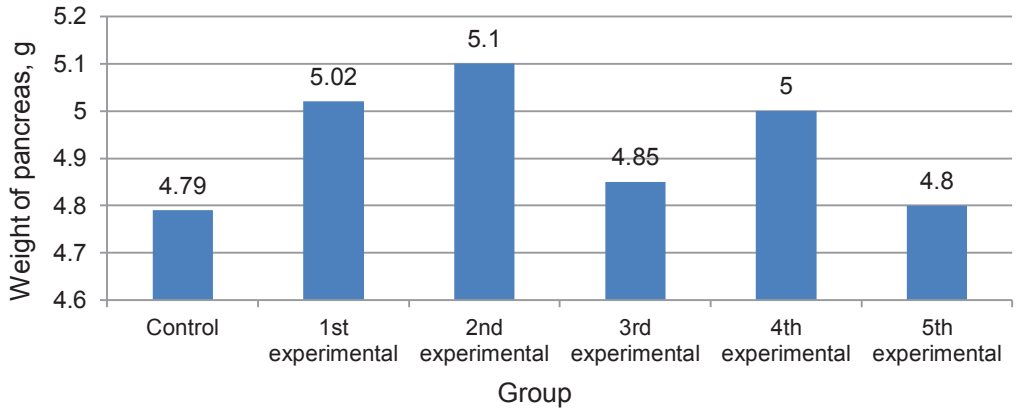

Figure 3. The weight of the broiler pancreas on 35th day, g (n = 3).

The intestinal length in broiler chickens of the 1st, 3rd, 4th and 5th experimental groups was lower by 13.68%, 1.35%, 8.97% and 13%, respectively, than in the control group, and amounted to 192.5, 220, 203 and 194 cm, respectively, while intestinal length of the control group was 223 cm. The length of the intestine in broilers of the 2nd experimental group, by the 35 day of age, exceeded the control group by 2.24%. The experimental group that consumed a diet with a minimal decrease in nutritional value for crude protein and digestible amino acids with the addition of protease had a slightly lower intestinal length by 1.35% compared with the control group (Fig. 4).

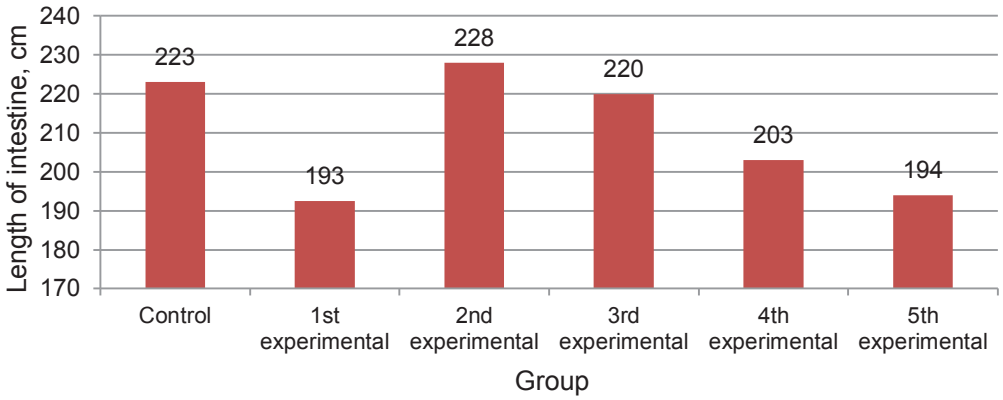

Figure 4. The length of the intestines of broilers on 35th day, cm (n = 3).

The pancreas is one of the vital organs of the digestive system, which produces enzymes that hydrolyze all the essential nutrients to monomers that can be absorbed into the blood and lymph. Digestive cells produce hydrolytic enzymes according to the general principles of protein synthesis. The endocrine part of the pancreas is represented by islets of Langerhans (Somova, 2007; Cyganova et al., 2008; Fisinin, 2017).

In a histological study of the pancreas in broiler chickens of the control group, we saw that the ducts are well functionally (Fig. 5), the islets of Langerhans were small and also had no physiological deviations (Fig. 6); the islets of Langerhans in one of the birds were larger with intensification functional activity.

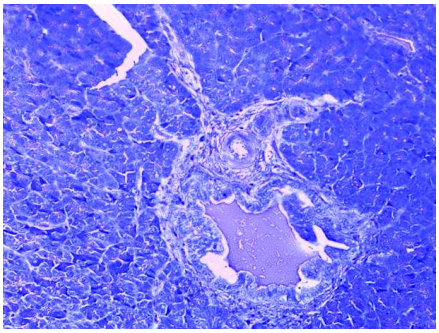

Figure 5. Histologic pattern of the pancreatic duct in chickens of the control group. Age 24 days. H&E stain. x400.

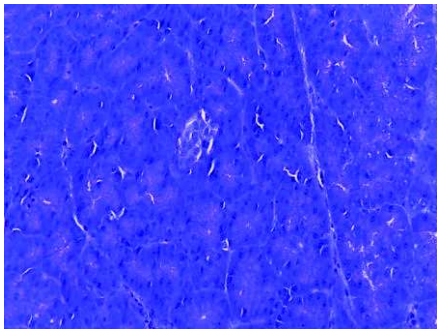

Figure 6. Histologic pattern of islets of Langerhans in broilers of the control group. Age 24 days. H&E stain. x400.

Birds of the 1st experimental group, which consumed a diet with a reduced nutritional value without protease administration, had inflammatory infiltrate in the pancreatic ducts (Fig. 7). Also, hyperemia of the vessels in the pancreas and an enhanced reaction of pancreatocytes was observed. The islets of Langerhans were activated.

When examining the pancreas of broiler chickens of the 2nd experimental group, we observed that the islets of Langerhans were small, normally functioning; the vessels were moderately blood-filled (Fig. 8). No inflammatory processeswere observed.

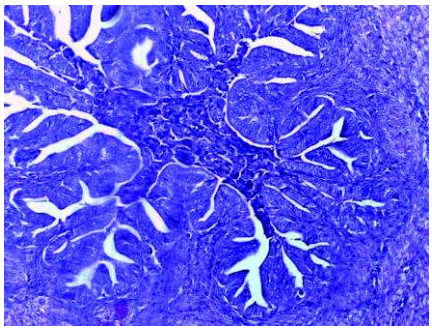

Figure 7. Histologic pattern of the pancreatic duct in chickens of the 1st experimental group. Age 24 days. H&E stain. x400.

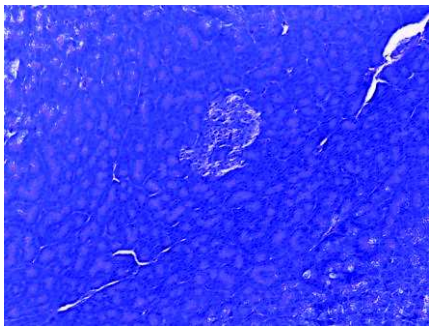

Figure 8. Histologic pattern of islets of Langerhans in chickens of the 2nd experimental group. Age 24 days. H&E stain. x400.

When examining the pancreas of the 3rd experimental group, we saw that the pancreas was somewhat activated, one of the ducts was without pathology (Fig. 9), in other – the epithelium was smoothed, outgrown; lumen of the duct there had a complex thick secret, islets of Langerhans were without pathology.

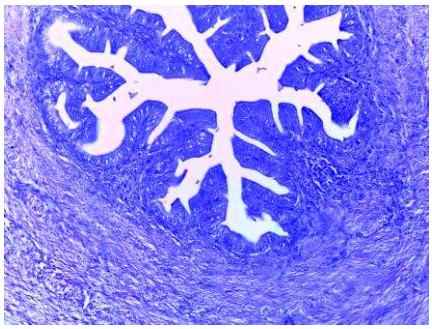

Figure 9. Histologic pattern of the pancreatic duct in chickens of the 3rd experimental group. Age 24 days. H&E stain. x400.

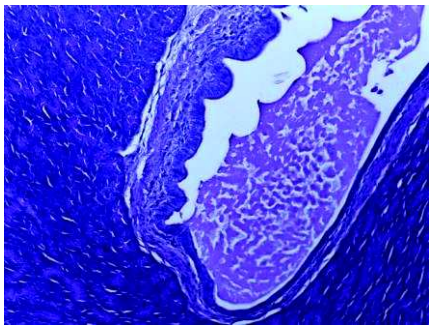

Figure 10. Histologic pattern of the pancreatic duct in chickens of the 4th experimental group. Age 24 days. H&E stain. x400.

The 4th experimental group that consumed a diet with reduced nutrition in accordance with the 5% matrix for crude protein and digestible amino acids and with the protease administration, had vascular hyperemia in the pancreas, the epithelium of ductswas smoothed, the wall was overgrown and there was a large amount of secretion in the lumen (Fig. 10), the islets of Langerhans had moderate secretion.

The 5th experimental group that consumed a diet with reduced nutrition in accordance with the matrix of 7.5%

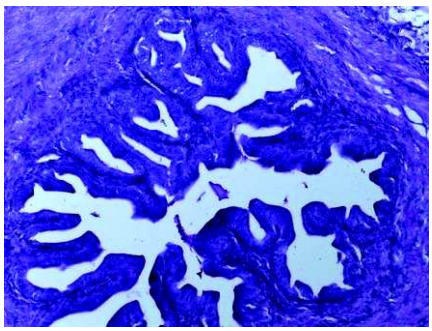

Figure 11. Histologic pattern of the pancreatic duct in chickens of the 5th experimental group. Age 24 days. H&E stain. x400.

in crude protein and digestible amino acids and with the protease administration, had the following changes in the pancreas: the duct epithelium was hyperplastic (Fig. 11), there was an increase in the number of islets of Langerhans per unit area.

CONCLUSION

On the basis of the conducted studies, it can be concluded that the introduction of the Sibenza DP 100 protease in addition to the main diet of broiler chickens, without reducing the nutritional value of crude protein and digestible amino acids, has a positive effect on the weight, feed output per 1 kg of weight gain and nutrient absorption. An analysis of the internal organs development indicates more intense metabolism in the body of broiler chickens in this experimental group. That is confirmed by the results of a morphology study of the pancreas, characterized by the best condition of the organ ducts and the absence of inflammatory processes.

The experimental group, which received a diet with a minimum decrease in nutritional value for crude protein and digestible amino acids in accordance with the matrix by 2.5% and the addition of Sibenza DP 100 – 500 g t^{-1}, was characterized by the smallest lag in weight gain against the background of the control group indicator, compared with groups where the nutritional value of the diet was reduced by 5% and 7.5% with the enzyme administration, while the best feed output per 1 kg of weight gain were noted.

The use of Sibenza DP 100 in broiler diets, with a decrease in nutrition in accordance with the matrices by 5% and 7.5% for crude protein and digestible amino acids in total of the studied parameters, was not effective, accompanied by a decrease in bird productivity.

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