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# ACTIVITY OF DIGESTIVE ENZYMES IN DUODENAL CHYMUS AND BLOOD IN BROILERS OF PARENTAL LINES AND THE MEAT CROSS DEPENDING ON DIETARY BIOACTIVE ADDITIVES

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Abstract

The achievement of maximal productivity in poultry requires knowledge of the genetic productivity potential and adjustment of nutrition according to bird's physiological peculiarities. It is well established that early growth and development in poultry depend on the functional formation of the digestive system. Exocrine function of the pancreas plays one of the most important roles in this process. Earlier studies showed that the weight of the pancreas in broiler hybrid chicks is substantially higher compared to the parental lines till 35 days of age. Detailed comparative investigation of the corresponding distinctions in the digestion, however, requires physiological studies on fistulated chicks. The aim of the study presented was to determine and compare the activities of digestive enzymes in duodenal digesta and blood in hybrid broiler chicks (cross Smena 8) and in chicks of parental lines (Cornish and Plymouth Rock) when using bioadditives of several types, i.e. a spore forming probiotic, the mixture of low molecular weight organic acids, phytobiotic complex Intebio, feed additive with living bacteria (OOO Biotrof, Russia). It was found that amylase activity in the duodenal digesta was 22.0 % higher in the hybrid and 35.8 % higher in Cornish parental line as compared to Plymouth Rock line; lipase activity was higher 5.8- and 2.3-fold, respectively, due to the high dietary level of crude fat. The activity of proteases, however, was significantly lower in hybrids (by 9.0 %, $p < 0.05$) as compared to Plymouth Rock line and 32.3 % higher when compared to Cornish chicks ($p < 0.001$). The activity of blood amylolytic enzymes followed the reverse trend compared to the duodenal digesta; activity of other blood enzyme bore no differences between the genotypes. A diet formulation, as a proportion of protein, fat, and carbohydrates, can substantially influence the activity of the digestive enzymes in the intestinal lumen and in blood. Low molecular weight organic acids and the enzyme-based preparation in the diets for meat-type chicken stimulate activity of digestive enzymes in the duodenal digesta and blood with respect to the genotypic physiological peculiarities of the birds.

Keywords: intestinal digestion, digestive enzymes, blood pancreatic enzymes, broiler chicks, parental lines, meat cross, enzymes, probiotics, phytobiotics, organic acids

The achievement of maximal productivity in poultry requires knowledge of the genetic productivity potential and adjustment of nutrition according to bird's physiological peculiarities [1]. Study results [2, 3] show that growth and development in poultry depend on functional formation of the digestive system ensuring the initial metabolism, on which digestibility and digestion of food nutrients very much depends. Basic research of digestive physiology promotes development of the perspective systems and nutrition technologies for growing modern and newly created highly-productive poultry breeds and crosses.

Our previous findings showed [4, 5] that growth of chickens depends on weight of pancreas which performs not only the exocrine function, but also participates in metabolic regulation via production of hormones and bioactive substances. Obtained information evidences for significant superiority of hybrids over the chickens of parental lines by weight of pancreas at 35 days of age.

Presently, publications on digestive physiology studied using fistulated hens are sparse [6-9], and we completely failed to find similar comparative data for the youngsters of hybrids and their parental lines. It was found [10-13] that digestive ferments (amylase, lipase, and trypsin) enter the blood and constantly circulate in the blood stream. However physiological role of blood pancreatic ferments has not been studied enough and there are different views on entero-pancreatic circulation of the digestive enzymes [14].

It is evident that enhanced efficiency of feed conversion into animal products required digestive glands to effectively function of and their ability to adapt to varied nutritive conditions. Nowadays, this problem is more relevant due to the unprecedented extension of the list of regulated parameters of rations, and also due to necessity for replacement of the expensive highly nutritious components by low calorie alternatives with slow hydrolyzed ingredients, e.g. non-starch polysaccharides, trypsin inhibitors, erucic acid, glucosilonates, etc.). In recent years, a lot of biologically active additives (ferments, taste boosters, acidulants, phyto- and probiotics, etc.) are suggested which are marketed as the means for recovering from gastrointestinal problems. However, their impact on animal and human metabolism and health is poorly studied.

Adaptation of the digestive system to feed additives is crucial for poultry homeostasis and health, which determines relevance of sych studies. During physiological studies in fistulated hens, activity of amylase, protease, and lipase in gastrointestinal contents and blood plasma was studied for the first time in parental lines and their cross upon use of various bioactive additives in feeding.

Purpose of this research is to compare activity of digestive ferments in duodenal chymus and blood of hens at use of biologically active additives.

*Techniques.* Activity of the digestive ferments was studied in chronic experiment on 20-42-day old broiler chicken of cross Smena 8 and on young 49-70-day old birds of the parental lines of this cross (Selection and Genetic Center Smena, Moscow Province; B5 as the paternal line, B9 as the maternal line). Implantation of fistules in duodenum was performed with the use of sedative and anesthetic agents according to principles of human treatment of animals. T-shape fistula was installed in the ascending limb of duodenum close to the point of confluence of three pancreatic and two biliary ducts. In 3-5 days following surgical interference, birds recovered after surgery and could be involved in the study.

Physiological test was performed by group method, 5 heads per each group, where different types of the analyzed biologically active additives were included in main diet of poultry. Feed was prepared according to zootechnical regulations. Poultry was fed proportionally: 30 g per head in the morning on the empty stomach, and the rest daily feed was supplied during the day. Duodenal chyme (5 ml) was collected 1 hour after feeding, promptly centrifuged for 5 minutes at 5000 rpm, and diluted with cooled Ringer solution (1:10).

Chymus amylase activity was determined on a photoelectric photometer KFK-3 (Zagorskiy Optical Mechanics Plant, Russia) at $\lambda = 670$ nm by starch hydrolyze [15] and expressed in milligrams of the digested starch per 1 ml of chymus over 1 minute. Lipolytic activity was measured on a semi-automatic biochemistry analyzer BS-3000P with flow cell (Sinnowa Medical Science & Technology Co., Ltd, China) with the set of reagents for lipase (DIAKON-VET OOO, Russia). Protease activity was determined by decomposition of Hammerstein Grade

Casein (EMD Millipore Corp., Billerica, USA) under colorimetric measurement on a KFK-3 at $\lambda = 450$ nm [16].

Blood samples were collected from the axillary vein of birds, on the empty stomach, supplemented with sodium citrate and centrifuged at 5000 rpm for 3 minutes. Blood plasma had been studied for activity of amylase and lipase at a Chem well 2900 (T) device (Awareness Technology, USA) with relevant reagent kits (Human GmbH, Germany). Trypsin activity [17] was assessed on a BS-3000P analyzer.

Statistical processing of the results had involved calculation of the mean (*M*) and standard mean errors (±SEM). Reliability of differences was assessed by Student's *t*-test. Resulting differences were deemed statistically significant at $P < 0.05$.

*Results.* During the experiment, we tested effects of few types of feed additives, namely a probiotic supplement based on *Bacillus subtilis*, a mixture of low-molecule organic acids, a phytobiotic based on *Bacillus subtilis* with addition of volatile oils, and an enzymatic probiotic Cellobacterin-T (Biotrof OOO, Russia) (Table 1).

**1. Diets to study the effect of various feed additives on digestive function in meat broiler chickens of cross Smena 8 and young birds of their parental lines**

| Group | Diet composition |
|---|---|
| I | Basic vegetable diet (BD) balanced by all principal nutritional components |
| II | BD + sporous probiotic based on *Bacillus subtilis* (500 g/t) |
| III | BD + mixture of low molecule organic acids (1000 g/t) |
| IV | BD + phytobiotic based on *Bacillus subtilis* with addition of volatile oils (500 g /t) |
| V | BD + Cellobacterin-T (1000 g/t) |

Basic diets the parental lines and their hybrid were different accounting for feeding standards (Table 2).

**2. Recipes and nutritional value of combined feed in the basic diet** (BD) **used to study to study the effect of various feed additives on digestive function in meat broiler chickens of cross Smena 8 and young birds of their parental lines**

| Ingredient | Content in combined feed, % | |
|---|---|---|
| | BD of hybrids ("finish") | BD of parental lines |
| Wheat | 40.35 | 49,48 |
| Soybean meal | 2.95 | 0 |
| Sunflower cake | 25.00 | 18,58 |
| Wheat bran | 0 | 17,31 |
| Corn | 17.00 | 10,00 |
| Maize gluten | 4.06 | 0 |
| Soybean oil | 6.98 | 0 |
| Limestone (36 %) | 1.47 | 2,50 |
| Monocalcium phosphate | 0.62 | 1,04 |
| Culinary salt | 0 | 0,25 |
| Sodium sulphate | 0 | 0,18 |
| Feeding methionine | 0 | 0,10 |
| Mineral blend (0.08 %) | 0 | 0,08 |
| Choline chloride | 0 | 0,08 |
| Lysin 98 | 0.35 | 0,35 |
| Vitamin blend (0.02 %) | 0 | 0,02 |
| Premix | 1 | 0 |
| Nutritional value per 100 g of combined feed: | | |
| energy value, ccal | 320 | 255 |
| fat, g | 9.02 | 5,35 |
| fiber, g | 5.21 | 6,73 |
| protein, g | 19.79 | 15,59 |

Note. "Finish" means the third diet for growing broilers, from day 21to day 42 of age.

Our research found that duodenal chymus of meat chicken is characterized by high activity of digestive ferments (Table 3) that is in line with data of Chinese scientists [18].

Obtained results (see Table 3) showed that amylase activity in duodenal

chymus of hybrids is higher compared to the chicken of maternal line B9 by 22.0 % and of parental line B5 by 35.8 % ($P < 0.01$). It is mainly due to poultry feeding [19, 20], since in diet of broiler chickens as compared to that of the both parental lines the fiber content per 100 g feed was less by 1.52 %, whereas easier digested carbohydrates were higher. As to metabolic energy, diet of hybrid birds significantly (by 20.30 %) exceeded the combined feed of young birds of parental lines. Since broiler diet contained 9.00 % of raw fat while its content in the diet of parental lines was approximately 2-fold lower (5.35 %), lipase activity in duodenal chime of hybrids increased compared to the parents, namely being as much as 5.8 times higher in birds of the maternal line and 2.3 times higher in birds of the paternal line. Other pattern was noted in the stomach for activity of proteolytic enzymes. At high content of raw protein in diet of broiler chickens, their protease activity was 9.0 % lower ($P < 0.05$) than that of the maternal line, but 32.3 % higher ($P < 0.001$) as compared to the paternal line. This may be due to the fact that the hydrolysis of various components of the feed along the digestive tract occurs unevenly. Decomposition of fats is the first to occur be broken down, mainly in duodenum, whereas amylase and protease are mainly present in jejunum [21]. Hence, digestive system in meat chickens is developed adequately to the nutritional value of the provided feed depending on the genetic properties which define intensity of metabolic processes.

**3. Activity of digestive ferments in duodenal chymus and blood of meat broiler chickens of cross Smena 8 and youngsters of their parental lines depending on the type of dietary additives used** ($M$±SEM, $n = 25$, physiological test)

| Ferment | Group | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| Broiler chickens of cross Smena 8 | | | | | |
| *Chymus enzyme activity* | | | | | |
| Amylase, $mg \cdot ml^{-1} \cdot min^{-1}$ | 341±27.4 | 354±33.3 | 270±34.5 | 309±37.3 | 462±28.2* |
| Lipase, IU/l | 1734±215.4 | 1455±161.8 | 1069±211.0* | 1462±212.7 | 1749±227.3 |
| Protease, $mg \cdot ml^{-1} \cdot min^{-1}$ | 33±1.0 | 31±0.9 | 32±1.4 | 33±1.3 | 36±0.6* |
| *Blood enzyme activity* | | | | | |
| Amylase, IU/l | 244±37.2 | 455±56.1* | 386±38.5* | 311±50.5 | 454±43.2* |
| Lipase, IU/l | 20±4.1 | 31±5.0 | 27±4.5 | 27±2.9 | 42±5.5* |
| Trypsin, IU/l | 29±2.0 | 21±2.9* | 53±5.3 | 41±3.8* | 35±2.3 |
| Chickens of parental line B5 | | | | | |
| *Chymus enzyme activity* | | | | | |
| Amylase, $mg \cdot ml^{-1} \cdot min^{-1}$ | 219±21.1 | 154±15.6* | 231±25.5 | 155±8.6* | 199±25.7 |
| Lipase, IU/l | 750±54.7 | 912±92.2 | 982±76.5* | 632±41.7 | 896±70.5 |
| Protease, $mg \cdot ml^{-1} \cdot min^{-1}$ | 22±1.8 | 27±2.0 | 30±1.7** | 22±1.7 | 28±0.9* |
| *Blood enzyme activity* | | | | | |
| Amylase, IU/l | 395±43.5 | 350±59.5 | 322±20.5 | 245±21.5* | 436±35.2 |
| Lipase, IU/l | 29±2.2 | 32±2.4 | 28±2.1 | 38±4.5 | 42±8.5 |
| Trypsin, IU/l | 35±5.4 | 41±1.0 | 34±3.5 | 46±6.5 | 43±9.1 |
| Цыплята материнской линии В9 | | | | | |
| *Chymus enzyme activity* | | | | | |
| Amylase, $mg \cdot ml^{-1} \cdot min^{-1}$ | 266±31.0 | 407±40.5* | 305±41.0 | 348±36.5 | 215±31.4 |
| Lipase, IU/l | 301±37.5 | 212±16.4* | 597±50.3** | 226±59.7 | 504±65.0* |
| Protease, $mg \cdot ml^{-1} \cdot min^{-1}$ | 36±0.8 | 37±1.1 | 36±1.0 | 34±1.5 | 35±1.0 |
| *Blood enzyme activity* | | | | | |
| Amylase, IU/l | 290±25.1 | 382±33.3* | 263±6.5 | 336±26.2 | 311±27.6 |
| Lipase, IU/l | 15±0.9 | 15±0.1 | 19±0.6* | 16±1.8 | 25±3.5* |
| Trypsin, IU/l | 29±0.5 | 27±0.5 | 30±0.9 | 27±3.2 | 25±1.7 |

N o t e. See description of groups in Table 1.
*, ** Differences from the control are statistically significant at $P < 0.05$; $P<0.001$, respectively.

Activity of blood amylolytic enzymes of meat chicken displayed the regressive trend as compared to such activity in stomach (see Table 3). In blood of broiler chickens this values decreased, as compared to these of young chickens, by 18.8 % for the maternal line and by 61.9 % for the parental line ($P < 0.01$). Lipase activity in blood varied in all studied groups, but insignificantly. We also did not observe any valid differences between the groups in activity of proteolytic ferments. These results are coherent with hypothesis of enteropancreatic circula-

tion of digestive ferments. Availability of pancreatic ferments in blood confirms the opinion that they may enter into the blood stream, flow with blood into the pancreas and be repeatedly secreted into the intestine without decaying in the intestine to amino acids [11, 23, 24].

In broiler chickens, dietary probiotic (see Table 3) had not led to significant changes in fermentative activity of duodenal contents. Herewith, blood amylase activity increased by 86.5 % ($P < 0.05$), and trypsin activity decreased by 37.6 % ($P < 0.05$). It appears that slowdown of proteolytic activity was due to competitive effect of sporous probiotic on digestive ferments, in particular proteolytic [24-27]. Probiotic had no significant effect on activity of the digestive ferments in small intestine and blood of paternal line B5 youngsters, whereas promoted a 53.0 % increase in intestine amylase activity ($P < 0.05$) of maternal line B9 youngsters. Similar changes were observed in blood, i.e. amylase activity increased by 31.7 % ($P < 0.05$). During tests, lipase activity in stomach decreased by 29.6 % as compared to the control.

Accordingly, the dietary probiotic had selective effect on activity of the digestive ferments in meat chicken. Activity of blood enzymes in broilers changed, and amilolythic activity in blood and stomach increased in maternal line, whereas activity of the stomach lipase decreased.

Low-molecule organic acid is one of the most effective antibiotic surrogates. These compounds, including those in form of salts, are characterized by high antibacterial activity. In our test, they had significantly changed the lipase activity in duodenal contents: this activity rate was high in control broiler chickens, but it had been decreased by 38.4 % upon addition of organic acids in the diet. In blood, such values went up: upon addition of organic acids amylase activity increases by 58.2 %, protease activity increases by 82.7 % ($P < 0.05$). In chickens of parental lines, organic acids had promoted higher activity of lipase (by 30.9 % for line B5, by 98.3 % for line B9) and protease (by 36.4 % for line B5) in duodenal contents as compared to control chicken. Accordingly, one may assume that organic acids promote secretion of the intestinal juice which stimulates activity of the pancreatic ferments, mainly lipase, in gut, increases activity of blood amylase and trypsin in broilers, and activates blood lipase in youngsters of maternal line.

Results obtained upon addition of dietary phytobiotic confirm its stimulating effect on the digestive process in broiler chickens and young birds of paternal line, which had been manifested by the increase of blood trypsin activity by 41.4 % ($P < 0.05$) and 31.4 %, respectively. In such cases, better appetite and protein metabolism could be expected, taking into account the role of trypsin in regulation of the metabolism and increase of blood vessel diameter.

Fermentative preparations as feed additives are widely used in chicken farming, but the mechanism of their effect on digestive processes had not been studied in full. Our tests with dietary fermentative preparation Cellobacterin-T had shown (see Table 3) that Cellobacterin-T increased gut amylase activity in broiler by 35.5 % and protease activity by 9.1 % ($P < 0.05$) as compared to the control. At the same time, blood activity of pancreatic ferments increased: by 86.1 % for amylase, by 110.0 % for lipase, and by 20.7 % for trypsin ($P < 0.05$) as compared to the control. Lipolythic activity (in line B9) and proteolythic activity (in line B5) in duodenal contents increased due to the fermentative preparation supplement (see Table 3), with similar changes observed in blood.

It should be noted that used additives affect activity of the digestive ferments in poultry gastrointestinal tract interacting with a large number of physical and chemical factors, as well as with rapidly changing microbiota. Thus, the design of formulations and schemes of their application in poultry must be focused

on various properties and mechanisms of action of probiotics, phitobiotics, acidifiers, ferments, etc., to use them as the improvers of sustainability of gut microflora ecosystem and gut health, and also as the additional energy sources [28].

Therefore, hybrid broiler chickens overcame young birds of parental lines by activity of digestive ferments in duodenal chymus, except protease in maternal line. By blood amylase activity, hybrids were inferior to chickens of parental lines due to type of poultry nutrition. Use of probiotic did not have significant effect on duodenal enzymatic activity in healthy poultry, except for chickens of maternal line in which amylase activity increased and lipase activity decreased. Herewith, blood amylase activity increased in broiler chickens and chickens of maternal lines. Organic acids affect digestion by increasing gut lipase activity in chickens of parental lines, and also gut proteolytic activity in paternal line, whereas blood activity of digestive ferments increased in broilers and chickens of maternal line. Phytobiotics did not have significant effect on gut digestive ferments, but after entering blood, they increased trypsin activity in broilers and chickens of paternal line. Used fermentative preparations promoted higher activity of gut and blood digestive ferments in hybrids, except for gut lipase, higher duodenal protease in chickens of paternal line, and higher gut and blood lipase in chickens of maternal line.

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