

Research article

Participation of Duodenal enzymes in the adaptation of digestion and metabolism in poultry to soybean products

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Abstract: The role of trypsin secreted by the pancreas is not limited to its digestive function. Current data show that trypsin has hormone-like properties, involved in the neurohumoral regulation of digestion and metabolism and activates protease-activated receptors (PARs). The main pancreatic enzymes (amylase, lipase and trypsin) are not only secreted into the intestinal lumen but also enter the blood flow where they circulate in physiological concentrations. Soy products are widely used in poultry feeding but the mechanisms of adaptation of the digestive and metabolic systems of broiler chickens to various forms of soy supplements (soybean meal, whole soybeans and extruded soybeans) remain poorly understood. The aim of the study is to investigate the trypsin participation in the processes of adaptation of digestion and metabolism in broiler chickens to different soy products at the level of duodenal chyme and blood serum. We conducted a comprehensive assessment of the activity of trypsin, lipase and alkaline phosphatase in duodenal chyme (60 and 120 minutes after feeding) and in blood serum in our chronic experiments on fistulated broiler chickens of the Smena 9 cross. We established that replacing soybean meal with native soybeans leads to a significant decrease in trypsin activity both in the intestine and in the blood, as well as to a marked increase in lipolytic activity. When extruded soybeans are used, a partial normalization of protein metabolism is observed, accompanied by increased lipase activity. The results confirm the involvement of trypsin in poultry adaptation to the soy component of the diet at the intestinal and systemic levels and demonstrate the advantages of extruded soybeans as a more complete protein source compared to native soybeans.

Key words: broiler chickens; duodenal fistula; trypsin, taste in birds; digestion in birds; adaptation to soy products

1. Introduction

The pancreas secretes digestive enzymes and hormones, thereby regulating the digestion of macronutrients and, consequently, metabolism as a whole; it ensures energy homeostasis and is responsible for converting consumed food into energy for cells. The enzyme trypsin, secreted by the pancreas, is involved in the hydrolysis of dietary proteins. Scientific literature provides evidence that the hormone-like substance trypsin participates in the neurohumoral regulation of digestive processes together with classical hormones such as secretin and cholecystokinin [1]. It is considered an established fact that the major pancreatic enzymes, amylase, lipase, and trypsin, are not only secreted into the intestine as components of pancreatic juice, where they hydrolyze dietary nutrients, but also enter the bloodstream. Under normal physiological conditions, these enzymes constantly circulate in the blood, with their concentrations maintained within certain limits [2–4]. In recent years, research in this area has advanced significantly in the fields of physiology and pathophysiology [5].

Soybeans and their by-products differ from other feed crops due to their high protein and fat content, as well as the presence of enzyme inhibitors and other antinutrients that reduce the hydrolysis of feed nutrients and their assimilation by animals and poultry [6]. The high levels of proteins and fats in soybeans and their processed products inevitably affect lipid and protein metabolism. For this reason, soy products were selected as dietary components to investigate adaptation processes. In connection with the development of the new broiler cross “Smena 9” and the need to adapt it to industrial housing conditions, studying the metabolic characteristics of this bird is particularly relevant.

The mechanisms of adaptation to the diet involving digestive enzymes in the blood remain poorly understood. To address this, chronic experiments were conducted on fistulated chickens to determine the involvement of duodenal enzymes in the adaptation of digestion to soy products, with measurements performed in parallel in intestinal chyme and blood serum.

2. Materials and methods

The study was carried out on the basis of the scientific laboratory of the Department of Physiology, Ethology and Biochemistry of Animals of the Russian State Agrarian University - Moscow Timiryazev Agricultural Academy in 2024–2025. Approval for the research was obtained from the Bioethics Committee of the Russian State Agrarian University - Moscow Timiryazev Agricultural Academy (excerpt from minutes No. 46 dated May 5, 2025).

2.1. *Surgical method*

For the study, 20 broiler chickens of the Smena 9 cross (SGK Smena, FNTS VNITIP) aged 15 days were operated on to obtain the contents of the duodenum [7]. To obtain the contents of the duo-

denum, the bird was opened, and a cannula was implanted into the duodenum opposite the confluence of the pancreatic and bile ducts. Surgical operations were performed using sedatives and anesthetics. The chicken was fixed in a left lateral position in a special machine. An incision was made on the right side of the last rib along the edge of the lateral process of the sternum, 4–5 cm long. The duodenum was removed, the site of the ducts entering the duodenum was located, and a purse-string suture 0.5–0.6 cm long was applied opposite it. An incision was made inside the purse-string suture, a cannula was inserted, and the purse-string suture was tightened. The area around the implanted cannula was carefully treated, and if necessary, an additional purse-string suture was applied. The intestine was immersed deep into the thoracic cavity and the surgical wound was sutured with knotted sutures, capturing all layers. After the operation, the birds were given access to water for 16–18 hours, excluding feeding. Physiological experiments began 3–5 days after surgery, when the birds had fully recovered.

2.2. Experimental protocol

By the method of analogues, three groups of broiler chickens with 5 chickens in each were formed. Group 1 (control) received a basic diet (RR) containing 19.05% soybean meal by feed weight. In group 2, soybean meal was replaced with soybeans (25.60% of feed weight), and in group 3, extruded soybeans (25.20% of feed weight) were used as a supplement (Table 1). All soy additives were introduced based on the norms of crude protein requirements in the diets of broiler chickens (VNITIP, 2021). The duration of the experiment was 7 days. Physiological experiments were carried out daily in the morning on an empty stomach after a 12-hour fasting diet. During the morning feeding, each broiler was given 30 g of feed; the remainder of the daily ration was provided after the experiment. At 60 and 120 minutes after feeding, samples of duodenal contents were taken in 1.0 ml eppendorf-type tubes, and the samples were centrifuged at 7000 rpm for 5 minutes, after which the supernatant was diluted with saline in a ratio of 1:10 for biochemical analysis.

The nutritional value of the diets in group 1 (control) and groups 2 and 3 (experimental) did not differ significantly: Crude protein was 19.0%, metabolized energy was 3050–3100 kcal/kg, and crude fat was 0.6% higher in experimental group 2 due to the excess fat content in soybeans compared to soybean products.

In the duodenal chyme, the activity of enzymes, pancreatic amylase, lipase, and alkaline phosphatase, and the content of triglycerides were determined. The analysis was carried out on a semi-automatic biochemical analyzer BS-3000M (Sinnowa, China) using biochemical reagent kits for veterinary medicine "DiaVetTest" (DIAVET LLC, Russia). Trypsin activity was determined using the method in [8] with the substrate N- α -benzoyl-arginine-p-nitroanilide (BAPNA).

At the end of each of the three series of experiments, a biochemical analysis of blood taken from the subclavian in the morning hours on an empty stomach was carried out. Blood was collected in tubes with a coagulation activator and centrifuged at 3500 rpm for 5 min. Biochemical parameters in blood serum were determined on an automatic biochemical analyzer BioChem FC-120 (High Technology, Inc., USA) using reagent kits from the device manufacturer.

Table 1. Composition of the diet of broiler chickens.

Ingredient, %	Group		
	1 Control	2 Experienced	3 Experienced
Wheat	42,87	38,58	43,77
Maize	18,30	18,00	13,21
Soybean meal 46 %	19,05		
Soybeans 36% Crude protein 18% Crude Fat		25,60	
Soybean extruded 36% Crude protein 18% Crude Fat			25,20
sunflower meal	10,00	10,00	10,00
Sunflower oil	5,70	3,80	3,80
Lysine monochlorohydrate 98 %	0,43	0,40	0,42
DL-methionine	0,34	0,32	0,32
L-threonine	0,16	0,16	0,16
Monocalcium Phosphate (MCP)	1,15	1,10	1,11
Salt	0,20	0,20	0,20
Limestone flour	0,87	0,92	0,89
Sodium sulphate, anhydrous	0,12	0,12	0,12
Baking soda	0,13	0,12	0,12
Choline chloride 60 %	0,18	0,18	0,18
Vitamin-mineral premix	0,5	0,5	0,5

2.3. Statistical processing of experimental data

For statistical processing of the obtained results, we used the method of pairwise comparison using Student's t-criterion. The mean value and standard error of the mean ($M \pm SEM$) were calculated; values at $p < 0.05$ were considered statistically significant.

3. Research results

The enzymatic composition of digestive juices is an important indicator of the adaptation of the digestive system of broiler chickens to the composition of the diet. The developed technique for obtaining duodenal contents made it possible to assess the activity of pancreatic enzymes, since the fistula was close to the confluence of pancreatic and bile ducts [7].

Analysis of duodenal chyme sampled at the 60th minute of the postprandial period reflects the effect of feed on the complex reflex phase of pancreatic secretion associated with stimulation of the mechano- and chemoreceptors of the oral cavity [9,10]. The biochemical parameters recorded in this phase are influenced by the qualitative composition, degree of milling, and flavor characteristics of the feed, determined by the protein component of the diet [11]. The highest trypsin activity 60 min after feeding was observed in chickens of 1 control group fed soybean meal; it amounted to 1530 ± 58.9 units/L. The use of soybeans and extruded soybeans resulted in a 60.0% ($p < 0.05$) and 45.7% ($p < 0.05$) decrease in trypsin activity, respectively, compared to soybean meal (Table 2). The obtained data in-

dicating that soybean meal has the highest palatability for poultry compared to soybean and extruded soybean.

Table 2. Activity of duodenal chyme enzymes in broiler chickens when soybean by-products are added to their diet (M±SEM, n=5).

Index	Group					
	1 Control		2 Experienced		3 Experienced	
	60 min	120 min	60 min	120 min	60 min	120 min
Trypsin, U/I	1530.0±58.9	2092.0±136.3 ^b	611.0±40.5 ^a	909.0±55.2 ^{ab}	831.0±52.7 ^a	1237.0±103.3 ^{ab}
Alkaline phosphatase, U/L	5025.0±139.1	5045.0±160.6	4194.0±265.7 ^a	2621.0±131.5 ^{ab}	5201.0±187.2	3256.0±105.5 ^{ab}
Lipase, U/L	191.0±7.7	343.0±33.3 ^b	606.0±34.4 ^a	431.0±40.5 ^{ab}	468.0±45.8 ^a	599.0±37.9 ^{ab}

Note: ^a – differences with 1 control group are statistically significant at $p < 0.05$; ^b – the differences are statistically significant between the first and second hours within each group at $p < 0.05$.

Collection of duodenal chyme from broiler chickens 120 minutes after feeding corresponds to the peak of activity in the neurohumoral phase of pancreatic secretion regulation, when food masses flow from the stomach to the intestine and ensure the release of secretin and cholecystokinin hormones, which regulate the adaptation of digestive enzyme activity to the composition of the diet. Trypsin activity in this phase of regulation was highest in the 1st control group and reaches a value of 2092±136 U/I when soybean meal is used as a protein additive. When replaced with soybeans, trypsin activity decreased by 56.6% ($p < 0.05$), when replaced with extruded soybeans, by 40.9% ($p < 0.05$), compared to the control group. The higher activity of trypsin observed in the 1st control group with soybean meal was probably due to the highest content of crude protein in it (46.0%) relative to other soy products (Table 1). The lowest level of trypsin activity in both phases of pancreatic secretion (compound reflex and humoral) was recorded in chickens fed a diet with native soybeans characterized by a high content of protease inhibitors [7].

Differences between trypsin activity within the same group in duodenal chyme samples obtained at 60 and 120 minutes after feeding were significant ($p < 0.05$). Thus, in the control group, the enzyme activity increased by 28.3% ($p < 0.05$) 120 minutes after feeding, in the 2nd experimental group by 48.8% ($p < 0.05$), and in the 3rd experimental group by 48.8% ($p < 0.05$), relative to the first hour. This reflects the level of proteolytic activity of duodenal chyme in the neurohumoral phase of digestion regulation, and is probably due to the presence of trypsin inhibitors found in soy products [7]. Trypsin activity in the blood of broiler chickens decreased by 26.8% when replacing soybean meal with soybeans ($p < 0.05$) (Table 3). Consequently, the activity of trypsin when replacing soybean meal with soybeans in the diet decreased in the intestine and blood serum in parallel, showing adaptation at different metabolic stages (digestion and intermediate metabolism).

In the 2nd experimental group with soybeans, the activity of alkaline phosphatase (ALP) decreased in the intestine by 16.5% ($p < 0.05$) and 48.0% ($p < 0.05$), respectively, in the postprandial phases, and in the blood serum by 35.7% ($p < 0.05$) (Table 3), which may indicate a decrease in calcium-phosphorus metabolism. Lipase activity increased sharply in the intestine: After 60 minutes by 217.3% ($p < 0.05$),

after 120 minutes of the postprandial period by 25.6% ($p < 0.05$), in serum by 10.2 ($p < 0.05$) compared to the control group.

In the 3rd experimental group, when replacing soybean meal with extruded soybeans in the diet, the activity of duodenal trypsin decreased by 45.7% ($p < 0.05$) in the 60-minute interval after feeding, and by 40.9% ($p < 0.05$) after 120 minutes compared to the 1st control group. In the blood serum, on the contrary, an increase in enzyme activity by 43.0% was observed ($p < 0.05$) (Table 3). In this case, ALP activity decreased only in the second postprandial period by 35.5% ($p < 0.05$). Furthermore, after 60 minutes of the postprandial period, lipase activity increased in the duodenum by 145.0% ($p < 0.05$), after 120 minutes by 74.6% ($p < 0.05$), and in the blood serum by 37.3% ($p < 0.05$) (Table 3) compared to the control group.

Table 3. Biochemical parameters in the blood serum of broiler chickens when using soy additives in the diet (M \pm SEM, n=5).

Index	Group		
	1 Control	2 Experienced	3 Experienced
Trypsin, U/L	71.3 \pm 6.1	52.2 \pm 1.9 ^a	102.0 \pm 14.0 ^a
Alkaline phosphatase, U/L	931 \pm 46	599 \pm 90 ^a	649 \pm 56 ^a
Uric acid, mmol/l	108.5 \pm 4.9	58.3 \pm 0.8 ^a	83.7 \pm 2.2 ^a
Total protein, g/l	42.1 \pm 0.8	36.2 \pm 0.5 ^a	39.8 \pm 1.0
Lipase, U/L	5.9 \pm 0.2	6.5 \pm 0.1 ^a	8.1 \pm 0.5 ^a
Triglycerides, mmol/l	0.32 \pm 0.02	0.11 \pm 0.01 ^a	0.24 \pm 0.04

^a – differences with 1 control group are statistically significant, at $p < 0.05$.

High levels of trypsin in the duodenal chyme at 60 and 120 minutes of the postprandial period indicate significant biological activity of the protein in the supplement. A high level of trypsin in the blood serum indicates the maximum anabolic effect [12].

Trypsin activity in the blood when used in the diet of broiler chickens decreased by 26.8% ($p < 0.05$), and when extruded soy was added to the feed, it increased by 43.0% ($p < 0.05$) compared to the control. This indicates that proteases adapt not only to the quantity but also to the quality of protein in feed. The decrease in alkaline phosphatase activity in experimental groups 2 and 3 indicates a decrease in metabolism, which is consistent with low levels of uric acid and lipid metabolism. In experimental group 3, there was a tendency toward higher indicators, indicating the use of a more complete soy product after technological processing of soybeans.

The results of the lipolytic activity in duodenal chyme indicate that in terms of the amount of lipids remaining after processing in extruded soybeans and soybeans, the lipase enzyme adapts to lipids and shows a higher result than that of soybean meal (Figure 1).

In the second experimental group (soybeans) the lipase activity in the duodenal chyme increased by 217.3% ($p < 0.05$) in 60 minutes after feeding, and by 25.6% ($p < 0.05$) after 120 minutes, compared to the control group received soybean meal. In the second experimental group the lipolytic activity in the blood serum was higher than the control by 10.2% ($p < 0.05$). In group 3 (extruded soybeans) a statistically significant increase in lipase activity was observed relative to the control group. In the duodenal chyme it increased by 145.0% ($p < 0.05$) in 60 minutes after feeding and by 74.6% ($p < 0.05$)

after 120 minutes, and by 37.3% ($p < 0.05$) in the blood serum. These changes reflect the adaptation of the enzymatic system of broiler chickens in the duodenum and blood to the higher intake of lipids from raw and extruded soybeans.

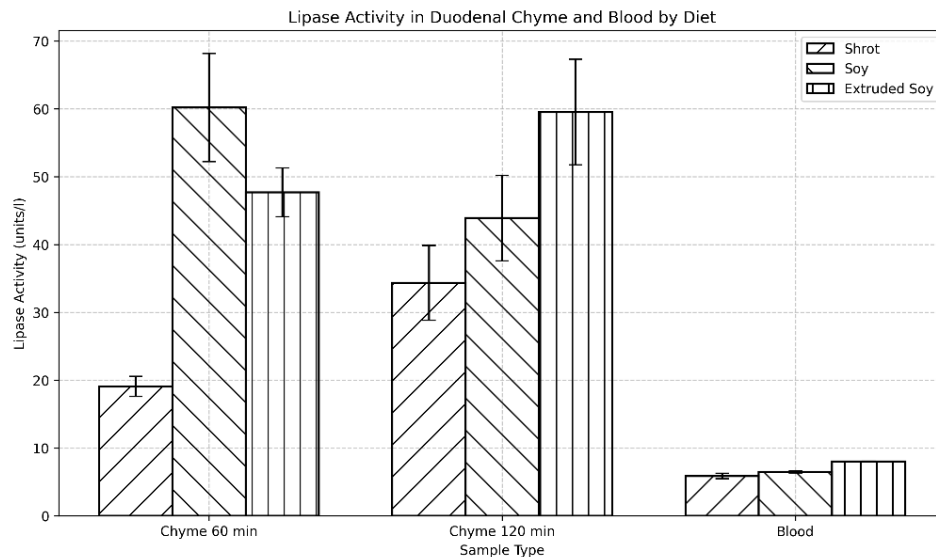


Figure 1. Lipase activity in different biological media (duodenal chyme and blood).

4. Discussion

The issues of adapting digestion to the composition of the diet have been discussed in the literature for more than a century [13–15]. However, the results of acute animal experiments do not enable us to fully reveal the mechanism of action of additives on the secretory function of the pancreas and the activity of duodenal enzymes. The results of studies performed on chickens with chronic pancreatic duct fistula indicate that even with a slight (0.5%) increase in crude protein in the diet, the activity of proteases increases by 28.1% ($p < 0.05$) [16]. The principle of feedback in the regulation of pancreatic secretion was discovered by French scientists in rats in the last century [17] and supplemented by the results of studies on cockerels with data that in the postprandial phase, trypsin in the intestine is secreted in parallel with the activity of the enzyme in the blood serum and correlates with nitric oxide metabolites, taking part in the regulation of metabolism through the autonomic nervous system [18,19]. Soybeans contain a number of components that negatively affect the nutritional value of protein. Factors that are destroyed during heat treatment include protease inhibitors and lectins. Protease inhibitors have an anti-nutritional effect, causing hypertrophy/hyperplasia of the pancreas, which leads to growth retardation. Lectin, due to its ability to bind to glycoprotein receptors on epithelial cells lining the intestinal mucosa, slows growth by inhibiting the absorption of nutrients [20]. The results of studies on broiler chickens confirm the participation of duodenal enzymes in the regulation of digestion and metabolism in poultry when replacing the protein components of the feed in the diet. In this work, the mechanism of the effect of soybeans on metabolism was studied for the first time using modern statistical methods of assessment. Additionally, the participation of not only trypsin activity, but lipase and alkaline phosphatase in adaptation processes is shown. The novelty of the work lies in the use of the

assessment of the taste and nutritional properties of feed components [21], which emphasizes the role of the pancreas in animals in the regulation of metabolism at the digestion level, which, due to pancreatic enzymes, triggers intermediate metabolism, which is shown in the correlation between the activity of enzymes in the intestine and blood in poultry. It has been established that taste sensations and nutritional value, digestive function, and absorption of nutrients by the body are not always coordinated in different soy products. The inverse correlation is especially expressed in the activity of trypsin when using soybean meal, which confirms the hypothesis of pancreatic secretion regulation according to the feedback principle.

5. Conclusions

Replacing soybean meal with raw soybeans in broiler chicken diets leads to a significant decrease in trypsin activity (by 56.5–60.0%) and alkaline phosphatase (by 16.5–48.0%) in duodenal chyme, as well as proteolytic enzyme activity, accompanied by suppression of protein metabolism in the blood. Furthermore, there is an increase in lipase activity (by 25.6–217.3%) associated with an increase in the crude fat content in the diet and enhanced lipid metabolism.

These changes are due to the presence of trypsin inhibitors in raw soybeans, which reduce protein digestibility and absorption.

When extruded soybeans are used, similar trends in the activity of duodenal chyme enzymes are observed, but blood biochemical parameters show normalization of protein metabolism at the level of the control group (with soybean meal) and superiority in terms of lipid metabolism intensity.

Extrusion of soybeans effectively inactivates anti-nutritional factors, making them a promising alternative to soybean meal in broiler chicken diets without negatively affecting metabolism.

Compliance with ethical standards

All applicable international, national and/or institutional guidelines for the care and use of animals have been complied with. All procedures performed in animal studies complied with the ethical standards approved by the legal acts of the Russian Federation, the principles of the Basel Declaration and the recommendations of the Commission on Bioethics of the Russian State Agrarian University-Moscow Timiryazev Agricultural Academy (extract from the protocol No46 05.05.2025).

This article does not contain any studies involving human subjects.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Contribution of the authors

A significant contribution to the development of the concept or design of the work was made by K.S.V., V.V.G., S.N.A., the receipt, analysis or interpretation of data for the work belongs to K.S.V., S.N.A., V.V.G., participation in the writing or editing of the article with a significant intellectual con-

tribution was made by V.V.G., K.S.V., S.N.A., all co-authors approved the final version of the article to be published, and agreed to be responsible for all aspects of the work, and also give a guarantee that that all questions regarding the validity and reliability of any part of the work have been properly analyzed and resolved.

Conflict of interest

The authors declare the absence of obvious and potential conflicts of interest associated with the publication of this article.

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