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A study of the exocrinous function of the cattle pancreas after the introduction of feed with various protein source in rations

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Nowadays, the question on the ability of the pancreas to secrete pancreatic juice various in quantity and enzymatic composition under various components of the diet are not quite covered, there is evidence in the literature that indicates the ability of the pancreas to alter its secret in response to the nature of consumed food but this information is not frequent. The world practice faces methodological difficulties in obtaining pure pancreatic juice that is why such studies focus on researching the qualitative composition of the chyme, which does not allow to explore complex-reflex and neurohumoral mechanisms of pancreatic secreting adaptation to the quality of feed. As a result of our research, we obtained new knowledge on the exocrinous pancreatic function, nutrient digestion, biochemical blood indices in cattle under feed with various protein compositions in the diet due to a unique surgical operation on transplantation of the pancreatic duct into an isolated stretch of the intestine. We set phases of the pancreatic secreting regulation (complex-reflex and neurohumoral) and its relationship with major biosubstrates of the body. The obtained data reveal the mechanisms of adaptation of the digestive system to the nature of nurture; we proved the hypothesis of non-concurrent secretion, in which enzymatic composition of secretions adapts to not the nature of feeding, but to the ingredient composition of the feed.

Keywords: Pancreas; Enzyme activity; Adaptation; Fistula; Cattle; Blood

INTRODUCTION

Digestive enzymes are a necessary part of the digestion process for animals and are derived from exocrine glands (such as the salivary glands, the pancreas, etc.), cells in the mucosa of the gastrointestinal tract (Sheyda E, 2014, Batoev, 2016). They include amylases, lipases, proteases that are synthesized by the cells of the pancreas (pancreas exocrinous cells). For ruminants, the digestion process and use of nutrients, especially starch, may be restricted and those restrictions can be caused by an inadequate synthesis of digestive enzymes and release them into the small intestine (Popov, 1934, Batoev and Sanzhieva, 2012). Thus, a study of the circulation of pancreatic enzymes in animals is important to understand the process of their recycling and use in digestion as biochemical markers in assessing the body's need for nutrient substances and energy at different stages of postembryonic development (Harmon 2009, Felicildareynaldo and Kenneally, 2016).

The content of the digestive tract on an empty stomach and after the meal has a direct regulating effect on the digestive function of the gastrointestinal tract, the motor and secretory activity of cleavage and absorption of a food lump. The role of regulatory factors is acted by substances included in diets, as well as components of the digestive gland secretions, particularly of the pancreas.

For the first time, the connection of feeding with excretion and secretion of pancreatic juice was observed by N.F. Popov (1934). Replacement of bran with oatmeal or hay with silage in a diet increases digesting strength of the juice for protein, and vice versa, replacing bran by straw lowered the proteolytic activity of the juice. P.I. Zherebtsov, M.M. Serykh (1965), and T.E. Kostina (1958) proved that continuous flow of chyme from the maw to the intestine was a prerequisite for the continuous excretion of pancreatic juice.

Factorial dependence of pancreatic secretions on the amount and composition of feed consists of the ability of glandulocytes to respond to the composition of a food lump and recycling of enzymes from the blood. After termination, synthesis of hydrolases without repeated replenishment with absorbed from the intestine and circulating with the bloodstream enzymes cannot meet requirements for them in the process of intestinal digestion, as well as the formation of tissues “de novo”. It was proven that the pancreas secretes into its product not only synthesized “de novo” enzymes but pancreatic enzymes absorbed from the intestine into the blood and “secondary” excreted by the pancreas (Rothman et al., 2002).

The enzyme secretion rate in calves depends on the duration of the chyme passage through the small intestine, the limited proteolysis due to the presence of peptides and some free amino acids that stimulate the pancreozymin release, and lack of essential amino acids for protein synthesis. According to Rothman S.S. et. al., forming a single peptide protein link needs 3.5 J+3/g of energy, which provides a synthesis of only 4 to 15% of the actually allocated enzyme protein in the composition of the pancreatic exosecret. In this connection, the usefulness of diets of qualitative protein greatly reduces the energy consumption for the formation of tissues “de novo”. This energy-saving natural technology cannot be developed without knowledge of the true pancreatic secretion and the activity of the digestive enzymes in various phases of regulation. Research of such a level is connected to labor-intensive and unique methods, in particular, the removal of the pancreatic duct from an isolated intestine and returning the secret back to the intestine (Rothman et al., 2002).

In this work, we were the first to continue I.P. Pavlov’s, A.D. Sineschekov’s, and A.A. Aliev’s traditions and used a fistulous method which allows analyzing the regulation of organ’s activities without violating the natural physiological and biochemical processes and identifying the mechanism of influence of various factors (additives, feed, etc) at the level of a holistic organism (Vertiprakhov V.G., 2016). In our view, such a methodical approach could not set only the physiological thresholds of enzyme regulation, but also the prognostic factor of efficiency in the use of various nutrients in ration (Vertiprakhov et al., 2004, 2016).

MATERIALS AND METHODS

Welfare of animals

Ethical approval: All applicable international, national, and institutional guidelines for the care and use of animals were followed.

The object

All experimental studies were performed in accordance with the instructions and recommendations of the Russian Regulations, 1987 (Order No. 755 of 12.08.1977 the USSR Ministry of Health) and “The Guide for Care and Use of Laboratory Animals” (National Academy Press Washington, D.C. 1996).

Design of the study

Studying the external pancreatic function was conducted on four fistulated heads of cattle with an average weight of 210 ± 10 g+3 at the age of 12 months. The experiment was conducted in two replications using the Latin square 4 × 4 at the laboratory of biological tests and examinations of the Federal scientific center of biological systems of the Russian Academy of Sciences.

Animals were kept in individual metabolic cages (1.0 × 2.2 m) to collect urine, feces, and the pancreatic juice, with the optimal temperature and humidity settings for this species with free access to water. During the experimental period, the temperature of the environment was maintained between 23° C and 25° C.

Experimental diets were balanced up, considering the needs of animals, and differed by 4% in dry matter content and by 3.4% in cellulose. The difference of protein quality was achieved by

including soybean meal in the diet of group 1, and sunflower cake – in the second experimental group. The differences in protein content and metabolizable energy (MEn) were small (1.9%) (Table 1). Animals were fed twice a day, with equal parts, in the morning and evening.

Differences in protein quality were achieved by substituting soybean meal (group 1) for sunflower cake (group 2). The diets were balanced in the content of crude protein and metabolizable energy. Feces were collected within five days.

Table 1. The Structure of Formula and Diet Quality Indices

Indices	Control	Experiment
Mixed-herb hay	6.08	5.84
Concentrates	2.23	2.14
Soybean meal	1.01	-
Sunflower cake	-	1.36
Molasses	0.609	0.58
Vitamin and mineral premix*	0.06	0.06
Salt brick	0.02	0.02
Nutrition value of diet, g+3		
Dry matter	4.08	4.25
Crude cellulose	1.15	1.19
Crude fat	0.11	0.13
Crudeprotein	0.54	0.55
Calcium	0.01	0.01
Phosphorus	0.01	0.01
Crude ash	0.28	0.2
Nitrogen-free extractives (NFE)	1.97	2.09
Copper	0.014	0.015
Zinc	0.0114	0.0175
Lead	0.09	0.098
Cadmium	0.01	0.01
Cobalt	0.02	0.02
Iron	0.07	0.06
Manganese	0.52	0.51
M En, MJ	42.15	42.11
Carbohydrates/protein ratio	0.76	0.55
Glucose, g	395.1	300.2

* Microelement content in 1 g+3 of concentrates: Mn – 48 g-3; Zn – 36 g-3; Fe – 60 g-3; Cu – 10 g-3; I – 0.30 g-3; Se – 0.24 g-3; Co – 0.12 g-3. Vitamin content in 1 g+3 of concentrates: vitamin A (VA) – 2640 M; vitamin D (VD) – 302 ME; vitamin E (VE) – 17 g-3.

Surgery

For the implementation of our task, we held an original operation on the imposition of duodenal anastomosis on animals (Sineschekov, 1955). The essence of the surgery is in the dissection of a cut at the confluence of the pancreatic duct into the duodenum with a length of 4 to 5 m-2 and fixation of aplastic fistula in it. Another fistula was implanted below the cross-linked plot of the intestine, forming an outer anastomosis connected by a rubber tube to return the pancreatic juice into the duodenum.

Cannula tips get connected by a silicone tube. During the experiments, “the bridge” gets disjoined and the pancreatic juice, flowing from the delivery fistulous tube, is collected from the fistulous tube of the isolated segment of the duodenum. When there are no experiments, the ends of both fistulous tubes are connected to each other, and the pancreatic juice flows from the isolated plot of the duodenum into the small intestine.

Study of pancreatic secretion

The research was conducted after 16-h exposure on an empty stomach. Before the start

of the experiment, an animal was placed in a special machine for anchorage during the experiment. A fistula was implemented with a special container for collecting juice. Collecting the juice lasted for eight hours with an interval of 30 min. After taking the first sample, animals were fed (the amount of feed was 30% of the daily ration) and continued to collect the juice. Quantity and enzyme activity of the juice were determined conducted "incito".

The research was conducted at the laboratory of "Agroecology of man-made Nanomaterials" and The Test Center (FNC "Biological systems and agricultural technologies of the RAS", accreditation certificate RA.RU.21ПФ59 of 02.12.15.).

Blood samples were taken from the jugular vein into vacuum test tubes with the addition of anticoagulant, for biochemical indicators – into vacuum test tubes with a coagulation activator (Thrombin). The morphological analysis of blood was conducted on an automated hematologic analyzer URIT-2900 VetPlus ("URIT Medical Electronic Group Co., Ltd", China), the biochemical analysis of blood serum – on an automated analyzer CS-T240 ("DIRUI Industrial Co., Ltd", China) with commercial kits for veterinary medicine (JSC "DIAKON", Russia).

The amylase activity was measured with the Smith-Roe method in the modification for the determination of high enzyme activity (Vertiprakhov, 2004), the activity of proteases – by hydrolysis of purified casein in the Hammarsten method with calorimetric control (450 m-9 wavelength), the activity of lipases – on the automatic biochemical analyzer CS-T240 ("DIRUI Industrial Co., Ltd", China) with commercial kits for veterinary medicine DiaVetTest (Russia).

The statistical analysis

The statistical analysis was performed using ANOVA techniques (software Statistica 10.0, "StatSoft Inc.", United States) and Microsoft Excel. Statistical processing included the calculation of the mean value (m) and the standard error of the mean (± SEM). The significance of differences between the compared indicators was determined by the Student's t-test. The level of the significant difference was set at $p \leq 0.05$.

RESULTS

The pancreatic exosecretion is not only characterized with a large number of enzymes, but also with the ability of regulated changes in their number and their proportion in the secret under a wide variety of long-lasting diets (the slow adaptation), which is provided by the adaptive synthesis of relevant enzymes by acinar cells, and depending on the type of feed (the urgent adaptation) (Keller et al., 1958, Korotko, 2007).

The inclusion of sunflower meal in the diet was accompanied by an increase in the synthesis of the pancreatic juice by 30.7% and the lipase activity by 57.8%, which was a response to increasing cellulose and crude fat by 15.3% (Table 2). Since the closer the amino acid composition of feed protein is to the amino acid composition of the body of the animal, the less stress the body meets, the fewer juice the digestive gland has to provide for the synthesis of "its own" protein (Aliev, 1997).

The proteolytic activity decreased by 42.4% even against the background of a slight increase in crude protein in the diet by 1.9% and the amino acids by 1.2%. Therefore, the secretory function of pancreas clearly adapted to the quality of the received feed.

To understand the adaptation mechanisms of the enzymes of the pancreatic excretory system to various sources of protein, we studied the dynamics of pancreatic juice excretion and the enzyme activity for an 8-h period (Table 3).

The passage time and the stay duration of the content in the digestive canal in cattle depends on the quantity and the quality of feed, the ration structure, the index of feed granulation, the technology and frequency of feeding, physical exercise, season, the technology of treatment, age, physiological condition, etc.

The color feed method showed that passage of the first portions of the feed by the digestive tract of cattle weighing 200 g+3 took 10 to 15 hours, including on average passing the proventriculus for 3 h 25 min, passing the maw – 25 min, and the small intestine – 2 h 17 min (Aliev, 1997).

Based on the research, phases of the pancreatic secretion regulation were determined:

- phase 1 – complex-reflex – 0 to 90 min;
- phase 2 – gastric – 91 to 270 min;
- phase 3 – intestinal – 271 to 480 min

Dynamics of the pancreatic juice excretion correlated with the activity of enzymes.

Table 2; The Pancreatic Secretory Function in Calves with Using Various Ingredient Feed Composition in the Diet (n=5, M±m).

No.	Indices	Diets	
		Group 1	Group 2
1.	Amount of the pancreatic juice, 8 h/day l-3	356±34.3 1068±121	513±63.2 1539±147,3
2.	Enzyme activity in 1 ml of the juice		
2.1.	Amylase, g-3/(l-3 min)	4075±230	2825±237*
2.2.	Lipase, g-3/(l-3 min)	148.6±15.3	352±41.3*
2.3.	Protease, g-3/(l-3 min)	307±26.7	177±21.2*
3	Amount of juice/body weight, l-3/g+3	9.7	13.9
4	Amylase/body weight	37	25.6
5	Протеаза/body weight	2.7	1.6
6	Lipase/body weight	1.3	3.2

Note: * – statistically significant (p≤0.05)

Table 3; Dynamics of Juice Secretion and the Pancreatic Enzymes of Calves during the Period of Soy Meal Inclusion in Diet (n=5, M±m)

Period	Amount of Pancreatic juice, ml	Lipase	Amylase	Protease	Total protein	Phosphorus	Calcium
0-30 (before feeding)	17.5±8.3	60.30±4.5	4900±500	471.6±11.65	0.43±0.03	0.03±0.001	2.74±0.05
30-60 (after feeding)	27±1.5	68.95±5.25	3100±300	560.0±33.3	0.32±0.09	0.03±0.002	2.73±0.02
60-90	21±3.2	68.00±3.6	3600±400	543.3±16.65	0.44±0.08	0.03±0.005	2.66±0.07
0-90	21.5±2.0	65.7±7.3	3866±287	524.9±67.3	0.39±0.04	0.03±0026	2.71±0.04
90-120	18±1.5	89.30±9.9	4700±200	530.0±13.3	0.70±0.08	0.06±0.005	2.56±0.055
120-150	18±2.0	69.10±2.9	4500±100	676.65±33.35	0.42±0.04	0.06±0.005	2.66±0.135
150-180	24±3.1	84.10±6.8	800±75	588.3±55.0	0.45±0.05	0.04±0.005	2.76±0.045
180-210	22±3.1	89.3±5.4	1400±200	230.00±3.3	0.27±0.01	0.02±0.005	2.81±0.01
210-240	18.5±1.6	64.25±6.35	1800±130	238.30±45.0	0.13±0.01	0.03±0.005	2.67±0.045
240-270	42±3.8	86.5±0.1	3200±200	93.35±16.6	0.35±0.06	0.04±0.005	2.64±0.05
90-270	23.7±1.9	80.4±9.2	2733±256	392.7±45.7	0.38±0.04	0.04±0.005	2.68±0.043
270-300	14±1.8	96.65±9.25	1900±100	76.6±12.65	0.36±0.17	0.04±0.005	2.76±0.01
300-330	32±4.1	138.60±7.5	6100±500	121.7±55.0	0.55±0.13	0.05±0.005	2.81±0.01
330-360	13±1.2	77.90±3.4	4400±300	196.6±18.7	0.70±0.03	0.03±0.001	2.85±0.03
360-390	22±2.4	283.9±19.4	8000±600	138.3±28.35	0.50±0.03	0.04±0.005	2.63±0.025
390-420	20±2.2	314.9±20.9	4100±500	223.4±33.35	0.38±0.04	0.04±0.005	2.64±0.05
420-450	30±4.5	333.2±28.	4400±200	164.3±13.35	0.43±0.03	0.04±0.005	2.73±0.08
450-480	17±1.8	453.5±39.5	3300±400	58.3±8.35	0.29±0.04	0.06±0.004	2.80±0.01
270-480	21.1±2.2	242.6±29.5	4600±520	139.8±14.6	0.45±0.06	0.04±0.004	2.71±0.027

Note: * – statistically significant (p≤0.05)

The highest proteolytic and amylolytic activity was recorded in phase 1 of the pancreatic secretion regulation with a gradual decrease in the activity of proteases, and an increase in the lipolytic and amylolytic activities by 16.0% and 73.3% respectively in phase 3. The level of total protein, calcium and phosphorus had sustained values, the highest correlation coefficient was revealed between the number of secreted juice and the calcium content ($r = 0.64$, $p \leq 0.001$), between lipase and total protein ($r = 0.55$, $p \leq 0.05$), and between total protein and protease ($r = 0.55$, $p \leq 0.05$).

The inclusion of sunflower meal in the diet was accompanied by a pronounced increase in the lipolytic activity by almost two times in comparison to the previous diet, raising its activity up to the intestinal phase of regulation (Table 4) with the oppression of the protease activity. This may be connected with the reaction of pancreatic secretions to the higher fat content in the diet.

It is known that the lipolytic activity of the pancreatic juice in ruminants is significantly lower

than that in monogastric, but there are no differences in molecular mass and amino acid composition (Koturay, 2012). It is connected with the activity of microflora, under the influence of the lipolytic activity of which the lipolysis of the principal forage quantity occurs (Tricarico et al., 2008, Lebedev et al., 2018). An important element in the stimulation of secreted enzyme activity is the evacuation speed of feed masses from the stomach, so, based on studies G.F. Korotko (2008), "carbohydrate" feed leaves the stomach faster, "protein" one – slower, and "adipose" one – even slower. Thus, we observe an increase in the activity of amylase in almost all phases of regulation, and for lipase – only in the intestinal phase (Korotko, 2008).

It is worth noting that the level of total protein in the pancreatic juice with background of a decline in the proteolytic activity increased with the highest values in the intestinal phase of the regulation, as evidenced by the previously revealed postulates on the connection of the feed quality with the activity of the digestive enzymes, so replacement of bran to oatmeal or hay to silage increases the digestive power of the juice and vice versa, replacing bran to straw lowered the proteolytic activity of the juice – these processes were studied by Popov N.A. (1930).

The level of the amylase and protease activity was lower than that of group 1, but the maximum values during this period were the same as in the intestinal phase of the pancreatic secret regulation, they correlated with the phosphorus content ($r = -0.65$, $p \leq 0.05$) and ($r = 0.62$, $p \leq 0.05$), respectively.

The enzyme excretive activity of the digestive glands and the small intestine adapts to a prolonged flow of particular nutrient composition, structure, and properties of the gastrointestinal tract contents, having a slow and fast (urgent) adaptation of various hydrolase ratios in the composition of the digestive gland secretions in its luggage (Rothman, 1980).

Also, the protease-amylase ratio, according to Batoev Ts.Zh. (2012, 2016), reflects the adaptation of enzymes to the quality of species food, the content of food components of animal and vegetable origin. In our experiment, the protease-amylase ratio declined by 30.8% in period 2 followed by an increase of 43.8%. The lipase-amylase ratio decreased when soybean meal was replaced to sunflower cake from 1:27 to 1:16. On the contrary, the lipase-protease ratio increased and peaked to period 3 that perhaps reveals an imbalance both in amino acid and fat metabolism (Table 5).

Blood, in addition to participating in the oxygen supply for cells, takes part in the transfer of the enzymes of the digestive glands, which depends on the performance of glandulocytes. Enzyme synthesizing glandulocytes of the exosecretive digestive glands transport most enzymes into the cavity of the digestive tract, and some number of them get transferred into the interstitium, where they enter the lymph and blood flow from. The second mechanism of origin of digestive gland hydrolases in blood is resorption – suckback of the enzymes and their zymogenes from the ducts and the small intestine (Korotko, 2010).

It is known that metabolic processes are directed and adjusted in accordance with the physiological state of an organism, the nervous system, as well as enzymes and hormones circulating in the blood. The presence of interceptors in the blood-forming organs and the body systems serves as proof that the blood-forming organs are included in the system of reflex interactions and through them in the activities of the entire organism as a whole (Zha, 2007).

Amylase being in blood catalyzes the formation of glucose and dextrin. Typically hydrolysis of polysaccharides occurs under the participation of several amylases at a time. Our results show that the animals of group 1 had the amylase concentration of 90 ± 23.1 u/l and increased by 50 u/l ($p \leq 0.01$) at the end of the study against the background of lipase decline by 2.4 u/l ($p \leq 0.01$). The lipase activity reduction based on its proteolysis under the influence of proteases and, firstly, chymotrypsin.

Metabolic processes occurring in the gastrointestinal tract largely determine the degree of digestibility and usability of nutrients from the feed. During the experiment, we conducted digestion trials using a generally accepted method. Digestibility coefficients of nutrients of three digestion trials are presented in Table 6.

So, in the digestibility of protein, animals of group 2 surpassed the indicators of group 1 by 4.5%. At the same time, the amount of fat in the diet had no effect on its digestibility (0.4%), against the backdrop of its superiority in the control diet by 15.4%.

The relationship between bacterial crude protein in the duodenum and the digestibility of organic matter is important.

Table 4; Dynamics of the Juice Secretion and the Pancreatic Enzymes in the Third Period (Sunflower cake) (n=15, M±m)

Period	Amount of pancreatic juice, ml	Lipase	Amylase	Protease	Total protein	Phosphorus	Calcium
0-30 (before feeding)	37±4.2**	232.4±22.3***	3000±160*	226.3±23.7*	0.43±0.08	0.09±0.001	2.73±0.05
30-60 (after feeding)	28±3.3	227.9±20.6**	2900±300	251.6±38.4**	0.26±0.05	0.08±0.002*	2.68±0.015
60-90	27±3.4	200.6±19.7**	2400±600***	480.0±44.3	0.14±0.02*	0.07±0.001	2.50±0.055
0-90	30.6±4.5*	220.3±2.8***	2766±353*	319.3±35.4*	0.27±0.05	0.08±0.001	2.63±0.04
90-120	17±2.3	259.9±26.9***	2600±400**	83.3±3.8***	0.29±0.11*	0.08±0.001	2.44±0.01
120-150	33±3.8	342.8±22.2**	1400±400*	385.0±58.3*	0.39±0.03	0.09±0.002	2.50±0.11
150-180	48±5.2*	256.3±27.3**	2100±500***	171.7±25.0**	0.18±0.07*	0.13±0.005*	2.72±0.01
180-210	26±3.2	295.0±29.6***	3800±400**	166.7±14.2	0.22±0.08	0.12±0.005*	2.53±0.125
210-240	27±3.5	182.8±45.7*	3200±200*	116.6±13.3	0.30±0.05	0.12±0.005*	2.62±0.095
240-270	23±3.4*	196.5±37.5**	2300±300	233.3±16.6*	0.51±0.09	0.17±0.005*	2.69±0.045
90-270	29±3.48	255.2±31.5*	2566±366	192.7±21.8*	0.31±0.07	0.10±0.0026	2.58±0.065
270-300	45±5.2*	104.7±3.45	4000±200*	30.0±8.2*	2.28±0.05*	0.09±0.005	2.74±0.05
300-330	37±4.2	932.1±1.95*	2900±300*	46.6±3.35*	2.27±0.11*	0.08±0.01	2.72±0.045
330-360	32±3.5*	540.6±31.6***	3800±200	113.4±13.4	2.43±0.09*	0.08±0.005	2.59±0.045
360-390	30±2.8	112.6±11.0	1900±300***	113.4±3.35	1.98±0.08*	0.04±0.005	2.54±0.005
390-420	33±4.3	817.3±93.4*	3200±400*	105.0±5.0	2.22±0.06**	0.08±0.001	2.64±0.035
420-450	31±3.7	479.8±58.2	3400±600	141.7±15.0	2.42±0.01**	0.08±0.002	2.70±0.025
450-480	39±5.1**	463.0±31.0	2300±200*	180.0±30.2**	2.52±0.01***	0.08±0.005	2.65±0.035
270-480	35.2±4.1	492.8±32.9*	3071±314*	104.3±11.2	2.3±0.05***	0.07±0.002	2.64±0.035

Note: * – statistically significant (p≤0.05), comparison to the control group (soybean

Table 5; The ratio of the Main Proteolytic Enzymes in the Pancreatic Juice of Calves

Periods	Protease/Amylase	Lipase/Amylase	Lipase/Protease
Group 1	1:13	1:27	1:2
Group 2	1:16	1:16	1.9:1

Table 6; Indicators of Digestibility of Diets in Calves

Indicator	Group 1	Group 2
Dry matter	61.0±0.96	59.4±1.4
Organic matter	63.0±0.84	62.0±0.65*
Crude protein	79.0±0.72	75.5±0.85
Crude fat	54.0±1.03	54.2±0.58
Crude cellulose	53.8±1.07	54.9±1.53
NFE	70.0±0.98	64.2±1.43*

Note: * – statistically significant (p≤0.05)

The level of bacterial crude protein intake in the duodenum increases with the increase in organic matter digestibility in diets for ruminants.

It is known that regardless of the protein content in the diet, the duodenal chyme contains about 1% protein due to protein release with the digestive juices. Alignment of the protein concentration in chime occurs largely due to nitrogen release with the digestive juices.

Hence, the digestive system clearly adapted to the new feed. For a more detailed consideration of the adaptive mechanism, we carried out an estimation of the daily dynamics of the digestive enzyme activity in duodenal chyme.

Considering phase 3 (the intestinal), we concluded it was characterized by food passage from the maw to the duodenum and greater secretion of the pancreatic juice. The amount and the composition of the pancreatic secretion depend on the quality and the quantity of food and are controlled by the receptive cells of the intestine and, firstly, the duodenum (Table 7).

Recorded on the first day when using soybean meal, with a gradual decline to the third day.

Replacement to sunflower cake in the diet was accompanied by a decline in the activity of protease in duodenal chime by 22.5% (the first day), with a gradual decrease on the third day of the accounting period, which demonstrates the adequacy of the response of the digestive system on the changing range of synthesized digestive enzymes to secure the supply of protein to the body.

It is worth noting that the activity of lipase increased in both groups on the first day with a decrease on the second day and equalization of the indices on the third day of sampling, but with a difference of about 50% between the groups.

Total protein of the chyme synthesized by proventriculus microorganisms in parallel decreased by 8.3% with an increase of the indices after feeding and correlated with the enzymatic activity. The phosphorus content increased by 31.2% and coincided with the dynamics in the pancreatic juice, which is confirmed by the results of research by Prof. Smolin S.G.

The correlation analysis indicates the existence of a positive relationship between the level of lipids in post-pyloric chyme, the level of the pancreatic juice secretion, and its lipolytic activity ($r = 0.86$, $p \leq 0.001$). A higher coefficient of correlation was observed between the absorption of fat in the small intestine and the lipolytic activity of the pancreatic juice ($r = 0.91$, $p \leq 0.001$).

Table 7; Daily Activity of the Digestive Enzymes in Duodenal Chyme with Different Sources of Protein in the Diet of Calves, u/l (n=15, M ±m)

Time of sampling, day	Time of sampling	Control			Experimental		
		Amylase	Protease	Lipase	Amylase	Protease	Lipase
1	Before feeding	373±28.2	56.6±6.5	1.3±0.08	301±23.4*	49.1±5.7*	2.2±0.3*
	1 h after feeding	378±32.1	67.5±7.4	1.1±0.2	353±22.3	44.6±8.6*	2.3±0.18*
	6 h	310±27.2	64.1±7.2	1.5±0.4	530±34.2*	52.3±6.4*	2.01±0.4
2	Before feeding	371±28.4	61.9±5.8	1.2±0.1	615±54.2*	44.48±5.2*	0.3±0.02*
	1 h after feeding	174±15.5	52.4±5.6	1.3±0.08	280±16.3*	39.03±4.2*	0.8±0.05
	6 h	610±56.4	55.8±5.8	1.2±0.08	190±20.0*	41.51±5.6*	0.11±0.05*
3	Before feeding	189±20.2	41.7±4.3	1.43±0.07	105±10.4*	32.67±5.4*	2.1±0.3
	1 h after feeding	192.8±8.7	34.9±3.7	1.5±0.05	340±42.5*	24.52±3.4*	2.12±0.2
	6 h	133.3±14.6	26.5±3.7	1.35±0.12	210±35.3*	35.22±2.9*	0.7±0.08*

Note: * – statistically significant ($p \leq 0.05$)

The inclusion of sunflower meal in the diet was accompanied by an amylase decrease on the first and the third days of sampling by 10.5% and 21.6% respectively. The maximum activity of amylase was

In our experiment, a high coefficient of positive correlation was recorded between the amount of lipase and total protein ($r = 0.94$, $p \leq 0.001$), which can be considered as a mechanism of the protein metabolism regulation due to the recretion from blood flow and the inclusion of substances without significant changes in the exosecret.

CONCLUSION

Thus, there takes part a decrease of protein and carbohydrate metabolism due to the high content of anti-nutrient substances in sunflower meal against the background of the mineral imbalance (Chaudhary et al., 2002, Coverdale et al., 2004, Fisinin et al., 2017, 2018), which indicates an adequate response of the enzymatic system to the quality of feed expressed in an increase of the pancreatic juice, the amylolytic and proteolytic activities, amid an increase of the lipolytic one.

CONFLICT OF INTEREST

The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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AUTHOR CONTRIBUTIONS

LSV, SHEV wrote a manuscript. VVG, GIA, KOV performed an experiment. GIZ, RVA, MIS designed experiments and reviewed the manuscript. All authors read and approved the final version.

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