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THE EXOCRINE PANCREATIC FUNCTION IN CHICKEN (*Gallus gallus* L.) FED DIETS CONTAINING DIFFERENT INGREDIENTS

V.I. FISININ, V.G. VERTIPRAKHOV, A.A. GROZINA

Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS, Federal Agency of Scientific Organizations, 10, ul. Ptitsegradskaya, Sergiev Posad, Moscow Province, 141311 Russia, e-mail fisinin@land.ru, vertiprakhov63@mail.ru (✉ corresponding author), alena_fisinina@mail.ru
ORCID:
Fisinin V.I. orcid.org/0000-0003-0081-6336
Grozina A.A. orcid.org/0000-0002-3088-0454
Vertiprakhov V.G. orcid.org/0000-0002-3240-7636
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Abstract

The ability of animal pancreas to adjust its exocrine function to the composition of a diet is still a controversial concept and open question. A bulk of experimental data is published to the present evidencing the ability of the pancreas to modify the composition of its secretion in accordance with the composition of the digested feed both in mammals (I.P. Pavlov, 1950; A.D. Sineshchokov, 1965) and poultry (P.P. Berdnikov, 1990; Ts.Zh. Batoev, 2001; V.G. Vertiprakhov, 2012). The supporters of the theory of non-parallel enzyme secretion argue that the pancreas can rapidly change enzymatic profile of the secretion in response to a change in the ingested feed composition (i.e., the ratio of protein, carbohydrates, and lipids). The problem of the pancreatic adaptation to feed composition for more effective digestion is especially urgent for intense commercial poultry production and highly productive commercial poultry crosses. The productive potential of these crosses cannot be effectively realized without detailed and comprehensive knowledge of the digestive function in poultry. There were the attempts of sampling of pure pancreatic juice by U.S. scientists (T.F. Degolier et al., 1999); however, their approach (cannulation of pancreatic ducts) did not find broad acceptance. The intestinal activity of the digestive enzymes is extensively studied in China (L.Q. Ren et al., 2012; H. Sun et al., 2013); however, their research did not elucidate the exact mechanism(s) of the adaptation of pancreatic secretion to feed quality. This paper was aimed at the investigation of exocrine pancreatic function in Hisex White chicken fed diets with different composition. The unique technique of transplantation of the pancreatic duct into the isolated duodenal section (allowing the collection of pancreatic juice during sampling periods and redirection of the juice into the duodenum at other times) enabled the sampling of pure pancreatic juice from live and healthy birds and the studying of its composition and changes induced by diet shifts. This technique of chronic fistulation provides new knowledge on the responses of enzymatic activity and chemical composition of the juice to the changes in diets. It was found that the pancreas can precisely adjust the enzyme-secreting activity to the composition of feed digested: large increase in dietary crude fat (by 20.8 %) resulted in a 33.8 % increase in the pancreatic lipase activity as compared to the basal level in starved birds. A moderate increase in dietary crude protein (by 3.3 %) and amino acids (by 2.1 %) resulted in substantial increase in proteolytic activity in the juice (by 28.1 %). The long-term adaptation of exocrine pancreas to a diet shift is related mostly to the basal levels of secretion, e.g. the basal level of lipase activity in pancreatic juice increases by 37.7 % after the increase in dietary crude fat. The short-term adaptation occurs in postprandial periods and involves both complex-reflex and neurochemical phases of the regulation of enzyme-secreting activity, e.g. the increases in lipase secretion at these phases after the shift to higher dietary crude fat level were 46.6 and 93.7 %, respectively. The concentration of total protein in pancreatic juice tended to increase in fed birds, in parallel to the increases in the secretion of individual enzymes.

Keywords: exocrine pancreatic function, chicken, Hisex White, adaptation of pancreas, feed composition, pancreatic enzymes, non-parallel secretion

Until now, the issue of animal pancreas ability to adapt to the diet com-

position is still controversial. There is a popular opinion about the identical changes in the activity of pancreatic juice enzymes. It is based mainly on findings for dogs which are being losing the pancreatic juice. In such animals, the pancreas ability to adapt the enzymatic composition of the secretion to the diet composition is largely impaired (1). In diseases of the digestive tract, as well as in overfeeding with certain food substances, in particular with fats, the pancreas ability to adapt its enzymes is also impaired [2-4].

There is a hypothesis that the pancreas responds to any food, regardless of its components, by releasing enzymes in the same proportions [5]. At the same time, there are a large experimental data pointing out the pancreas ability to change the composition of its secret according to the composition of consumed food [6-9], including data concerning poultry [10-14]. The supporters of such concepts postulate the possibility of an urgent change in the enzymes spectrum of the secret, depending on the type of consumed food. That is, carbohydrate food causes a predominant increase in exogenous amylase, protein food in proteases, and fatty food in lipase. In terms of morphology and physiology, the pancreas adaptation is ensured by changing the percent ratio of the enzymes in the course of synthesis, transportation and release of zymogenic granules in both individual acinar cells and in acini of topographically different areas of the gland [15]. The peculiarities of the induction of pancreatic secretion in birds as compared to mammals are discussed [16, 17]. Thus, basing on the analysis of the dose-dependent induction of amylase in vitro on isolated acini, the authors [17] have made the conclusion on the importance of the neuronal regulation, while the contribution of intestinal hormones, in their opinion, is not physiologically significant.

The studying of the adaptation of pancreas's secretion to the diet composition shall be based on the function of the nervous system and the humoral factors involved in the regulation of digestion. Thence, the optimal approach is to study the pancreatic secretion in a chronic experiment, conducted on a healthy animal pre-operated in a manner to exclude permanent loss of pancreatic juice. Due to the methodological difficulty of obtaining pure pancreatic juice in birds, the data on the adaptation of the pancreas secretory function in the scientific literature are scarce [18]. The studying of the digestive function basing on the activity of digestive enzymes is performed in the intestinal contents of fistulated bird [19-22], in pancreas tissue homogenates [23, 24] or by investigation of the digestibility of nutrients using the ileal availability method [25, 26], but such data do not allow studying mechanisms of complex reflex and neurohumoral phases of pancreas adaptation to the quality of food consumed.

Owing to the unique surgical transplantation of the pancreatic duct into the isolated piece of intestine, we have the possibility to obtain the pure pancreatic juice from the healthy chickens in the chronic experiment (Fig.) and to compare its properties as the diet changes.

The mechanisms of regulation of such process have not been studied fully, and further researches open up the perspectives for targeted enzymatic correction of the disorders of adaptability of the pancreas exocrine function in case of various diseases [27].

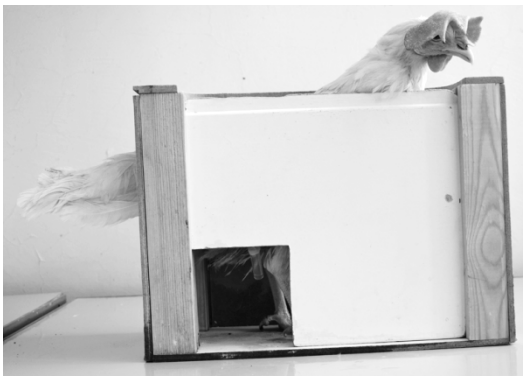
Our paper presents the first data on the chemical composition of the chicken pancreatic juice when using the diets with the different set of amino acids and other ingredients and on the indicators to character pancreatic enzyme activity.

The objective of the work was to study the exocrine function of the chicken pancreas under different dietary ingredients.

Techniques. The experiments were conducted on two 1-year old Hisex

White hens (*Gallus gallus* L.) which have been operated according to the method of Ts.Zh. Batoev and S.Ts. Batoeva [28]. For this, the 4-5 cm long piece was cut out from the duodenum and the main pancreatic duct was transplanted into it with the implantation of two L-shaped fistulas and formation of the external anastomosis which makes it possible to return the pancreatic juice to the duodenum in the period between the experiments.

The physiological tests were started in the morning when the hens were on an empty stomach after the 14-hour starvation. The birds were placed into the stall where they were kept for 3 hours. The microtube for collecting the pancreatic juice was attached to the fistula made of the isolated piece using the special rubber adapter. During the first 30 min, the juice was collected after the starvation, then the birds were given the portion of food (30 g) after that the secret was collected every 30 min during 180 min.



The hen with the chronic fistula of pancreatic duct during the experiment.

In biochemical analysis of the secret performed in 2 replications amylase was determined according to the Smith-Rhoy method in the modification for a high enzyme activity [29], proteases was determined according to Hammersten basing on the hydrolysis of purified casein under colorimetric control(λ = 450nm, KFK-3, Zagorsk Optic-Mechanical Plant OJSC, Russia) [11], and lipase was quantitated using a BS-3000P semi-automatic biochemical analyzer (SINNOWA Medical Science & Technology Co., Ltd., China) with a kit of veterinary diagnostic reagents for determining the lipase concentration in the blood of animals (DIACON-VET company, Russia).

1. Composition (%) and quality of used combined feeds

Ingredient, parameter	Control	Test
Wheat	58.224	55.781
Sunflower oilcake	5.000	21.026
Extracted soybean	19.784	8.912
Limestone (36%)	9.137	9.045
Soybean oil	1.936	3.026
Wheat bran	3.847	Not used
Monocalcium phosphate	1.149	1.233
Sodium salt	0.250	0.250
Lysine 98	0.073	0.214
Sodium sulfate	0.205	0.183
Fodder methionine 98	0.214	0.151
Mineral blend (0.08%)	0.080	0.080
Choline chloride	0.080	0.080
Vitamin blend (0.02%)	0.020	0.020
Nutritional value of 100 g of feed:		
metabolic energy, kcal	270.00	270.00
crude fat, g	6.72	8.12
crude fiber, g	4.89	5.92
crude protein, g	16.70	17.25

The amount of amino acids in the combined feed was determined by ion-exchange chromatography using postcolumn derivatization with the ninhydrin reagent and subsequent detection at λ = 570nm (λ = 440 nm for proline). A YL 9100 HPLC System for high-performance liquid chromatography (Young Lin Instrument Co., Ltd., Korea) which consists of YL9110 quaternary gradient pump, YL9101 vacuum degasser, YL9120 UV/VIS detector and YL9150 auto-sampler (Pinnacle PCX postcolumn derivator, ion-exchange column Na+ 4.0×150 mm, 5 μm, precolumn Na+ 3.0×20 mm, 5 μm) (Pickering Laboratories, Inc., USA) was used.

The statistical processing was performed with MS Excel software package; the mean values (*M*) and standard errors of the mean (±SEM) were cakculated. The significance of differences was evaluated by Student's *t*-test. The differences

were considered statistically significant at $p < 0.05$.

2. Amino acids content (%) in the used combined feeds

Amino acids	Control	Test	Change relative to control, %
Asparaginic acid	1.10	1.14	+3.6
Threonine	0.53	0.59	+11.3
Serine	0.67	0.69	+2.9
Glutamine	3.63	3.64	+0.3
Proline	1.09	0.98	–10.1
Glycine	0.69	0.81	+17.4
Alanine	0.63	0.69	+9.5
Cystine	0.28	0.28	Not changed
Valine	0.72	0.77	+6.9
Methionine	0.59	0.50	–17.3
Isoleucine	0.62	0.65	+4.8
Leucine	1.10	1.12	+1.8
Tyrosine	0.49	0.45	–8.2
Phenylalanine	0.74	0.75	+1.3
Lysine	1.15	1.14	–0.9
Histidine	0.41	0.43	+4.9
Arginine	1.06	1.10	+3.8
Total	15.50	15.83	

Results. The combined feed in the diets of hens (Table 1) differed in the content of crude fat and fiber, in test it was respectively 20.8 and 21.1% higher than in the control. The amount of crude protein in test differed from that in control insignificantly, but for protein quality the combined feed was selected to provide prevailing extracted soybean in the control, and sunflower oilcake in the test.

Total amino acids in the experiment were 2.1% more than in the control (Table 2). At the same time, the limiting methionine and lysine amino acids reduced by 17.3 and 0.9%, respectively, compared to the control but remained within the limits of relevant demands [30].

Table 3 presents the results of investigations of the pancreas secretory function. The obtained data show that upon the replacement of the control feed with the test sample, the lipase activity increased by 33.8% compared to control. This was due to a 20.8% increase in the amount of crude fat in the diet. The content of proteases increased by 28.1% as resulted from respectively 3.3% and 2.1% increase in crude protein and total amino acids in the feed. That is to say that the secretory function of the pancreas accurately adapts to the quality of the consumed food..

3. Secretory function of the pancreas of Hisex White chickens (*Gallus gallus* L.) under different compositions of feeds in the control and test periods of chronic experiment (M±SEM, n = 20)

Index	Control period	Test period	Change as to control,%
Amount of pancreatic juice during the experiment, ml	8.4±0.32	7.6±0.24	–9.5
Activity per 1 ml of pancreatic juice:			
amylase, mg/(ml · min)	4620±253.1	4855±290.0	+5.1
lipase, μmol/(ml · min)	6.5±0.51	8.7±0.62*	+33.8
proteases, mg/(ml · min)	267±17.9	342±61.3*	+28.1
Total protein, g/l	31.4±0.83	33.0±1.70	+5.1
Calcium, mmol/l	2.7±0.03	2.8±0.03	+3.7
Phosphorus, mmol/l	0.9±0.06	0.9±0.05	Not changed

* Differences with the control are statistically significant at $p < 0.05$.

To understand the mechanism of adaptation to the new feed, it is necessary to consider the time-course of the pancreatic juice secretion and of the enzyme activity after feeding (Table 4). The experimental data demonstrated that the time-course of the pancreatic juice secretion when using different feeds in the diet is different in the first (0-30th minute) and fourth (90-120th minute) periods of the chronic experiment. This corresponds to the concepts of the complex-reflex and neurochemical phases of regulation of the pancreas's exocrine function [11]. The matters of interaction of nerve and humoral mechanisms including those with the participation of trypsin in combination with nitrosyl iron complexes are still studied little and are the subject of our researches [31].

The obtained data show that the changes of lipase activity is most pronounced (see Table 4), There was a 37.7% increase before feeding, 46.6% in-

crease during the third period that was conditioned by the complex reflex phase of regulation of the pancreatic secretion, and 93.7% increase during minutes 120-150 which is related with the neurochemical phase of the regulation of secretion. This points out to the long-term adaptation to the second feed which causes the increase of lipolytic activity mainly in the phase of neurochemical regulation, i.e. in the period when the food masses digested in the stomach enter the duodenum thereby stimulating the release of secretin and cholecystokinin. When using the second feed, the proteolytic activity increased in the second, third and fourth periods of the experiment which correspond to the complex-reflex phase of the regulation of pancreatic secretion. During these periods, a stronger secretory response of the pancreas to the feed was observed (proteolytic activity increased 3.8 times compared to tthe basal level) that is apparently due to better taste of the second feed.

4. Release of pancreatic juice and enzyme activity in Hisex White chickens (*Gallus gallus* L.) under different compositions of feeds in the control and test periods of chronic experiment (M±SEM, n = 20)

Stage of the experiment in minutes	Pancreatic juice, ml	Activity		
		amylase, mg/(ml · min)	lipase, μmol/(ml · min)	proteases, mg/(ml · min)
0-30 (before feeding)	1.0±0.07	2570±434.0	5.3±0.65	108±17.5
	0.6±0.06*	3070±441.7	7.3±0.58*	92±20.8
30-60 (feeding)	1.4±0.13	4600±266.7	8.1±1.24	240±16.5
	1.3±0.09	4880±372.3	8.8±0.57	347±25.3*
60-90	1.5±0.07	5101±216.5	7.3±0.95	291±20.2
	1.6±0.09	5240±494.3	10.7±0.92*	416±37.7*
90-120	1.6±0.06	4954±398.5	6.6±1.35	308±22.7
	1.3±0.08*	4880±377.0	9.7±1.16	417±31.5*
120-150	1.5±0.08	5018±453.2	4.8±1.60	308±24.5
	1.5±0.08	5181±353.3	9.3±0.69*	394±32.5
150-180	1.4±0.07	5479±246.0	6.7±1.32	344±25.2
	1.3±0.07	6300±363.7	10.2±1.08	422±28.3

N o t e. Indices in the control and test periods are shown above and below the line, respectively.
* Differences with the control are statistically significant at $p < 0.05$.

S.S. Rothman [32] for the first time morphologically described the phenomenon of differences in secretion after the effect of nervous, humoral or food stimuli and called it non-parallel secretion, however many researchers did not agree with his findings. Meanwhile, as pointed out by S.S. Rothman [33, 34], the studying of this problem would not only contribute to the disclosure of the regulation mechanisms, but also would made it possible to manage the specific secretion of enzymes in various states of the organism. Later, in a number of studies performed on animals, such type of enzyme secretion in response to the endogenously released or exogenous stimulating or inhibiting substances has been confirmed [35-37]. Currently, the concept of “non-parallel” postprandial secretion of pancreatic enzymes has been recognized by most scientists [38-40]. Our experimental data are also in line with the hypothesis of such type of pancreatic enzyme secretion by. It should be noted that bulls showed the decrease of proteolytic activity of the pancreatic juice upon the increase of dietary protein has been discovered and described [41].

In our research, the pure pancreatic juice of chickens has been obtained owing to the unique operation for transplantation of the pancreatic duct to another place of the intestine; therefore, in the scientific literature similar data on the physicochemical properties of pancreatic juice are few [42]. In this work, we estimated several indices of pancreatic secretion. The analysis of total protein, calcium and phosphorus in the pancreatic juice has not revealed any differences between the effect of the control and the test feed (see Table 3), but studying dynamics of these indices after the feeding has made it possible to disclose some reg-

ularities of the secretion (Table 5). It is clear from the presented data that the total protein of the pancreatic juice tends to increase after the food intake (by 15.2-19.2%, $p < 0.05$). Similar changes occur in the activity of pancreas's enzymes. Cognate pattern of the content of organic matters in chicken pancreatic juice has been detected by S.G. Smolin [42]. In our test, the phosphorus content, on the contrary, slightly decreased ($p > 0.05$) 30 min after the feeding.

5. Changes in the content of total proteins, calcium and phosphorus in pancreatic juice of Hisex White chickens (*Gallus gallus* L.) under different compositions of feeds in the control and test periods of chronic experiment (M±SEM, n = 20)

Stage of the experiment in minutes	Control period			Test periods		
	total protein, g/l	calcium, mmol/l	phosphorus, mmol/l	total protein, g/l	calcium, mmol/l	phosphorus, mmol/l
0-30 (before feeding)	25.9±0.93	2.7±0.08	1.0±0.17	28.3±2.73	2.9±0.03	1.0±0.15
30-60 (feeding)	30.8±1.50*	2.6±0.06	0.7±0.06	32.6±1.57	2.8±0.03	0.8±0.09
60-90	31.3±0.87*	2.7±0.03	0.7±0.08	36.1±2.24*	2.8±0.03	0.9±0.21
90-120	32.3±1.22*	2.8±0.08	0.7±0.11	34.4±1.97*	2.8±0.04	1.0±0.19
120-150	33.7±1.02*	2.7±0.04	0.8±0.08	33.1±1.87	2.9±0.03	0.9±0.09
150-180	34.3±1.91*	2.7±0.03	1.4±0.19	33.7±1.56*	2.9±0.04	0.9±0.10

* Differences with the value prior feeding are statistically significant at $p < 0.05$.

Thus, basing on the obtained experimental data, we can conclude the following. The secretory function of chicken pancreas accurately adapts to the quality of the consumed food. Upon the increase of the crude fat content in the diet by 20.8%, the activity of the pancreatic juice lipase increases by 33.8%; a slight increase in crude protein (by 3.3%) and in the amino acids (by 2.1%) in the feed causes the 28.1% increase in proteolytic activity vs. the background level. The long-term adaptation of the pancreatic secretion to a new feed is mainly reflected in the basal secretion which increases by 37.7% upon the increase of the crude fat content in the feed. The urgent adaptation is well expressed in the postprandial period (complex reflex and neurochemical phases of the regulation of pancreatic secretory activity) when the lipase activity increases by 46.6 and 93.7%, respectively. The tendency to the increase in the total protein content in the pancreatic juice after feeding are noted that occurs simultaneously with the increase in the activity of pancreatic enzymes in the juice.

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