

КАЛЬЦИЙ КАК ИНГИБИТОР АКТИВНОСТИ ТРИПСИНА В ПАНКРЕАТИЧЕСКОМ СОКЕ КУР

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Представлены результаты изучения взаимодействия кальция и панкреатических ферментов у кур кросса Хайсекс белый 46-недельного возраста. Показано, что одновременно с всасыванием в желудочно-кишечном канале птиц происходит эндогенная экскреция кальция с пищеварительными соками. Определено влияние кальция на активность трипсина в эксперименте *in vitro*. В опыте вводили разные дозы кальция (5,0; 7,5; 0,5; 12,5 и 25,0 мг) в панкреатический сок кур, предварительно разведенный физиологическим раствором в 10 раз. Число повторов в каждом варианте опыта 20 раз. Для опыта применяли кальций хлористый 2-водный (ХИММЕД, РФ). Активность трипсина устанавливали кинетическим методом. Содержание кальция определяли биохимическим анализатором Sinnowa BS-3000P (КНР) и набором для определения кальция в крови животных ДИАКОН-ВЕТ (РФ). Установлено, что в панкреатическом соке кур содержится до $2,9 \pm 0,03$ ммоль кальция/л, что сопоставимо с уровнем кальция в сыворотке крови $1,99 \pm 0,10$ – $3,13 \pm 0,20$ ммоль/л. Определено, что кальций оказывает ингибирующее влияние на активность трипсина. Увеличение кальция в панкреатическом соке кур в 5 раз снижает активность трипсина на 34,7%. По данным дисперсного анализа, концентрация кальция в панкреатическом соке влияет на активность трипсина, сила влияния фактора достоверна и составляет 92%. Установлена устойчивая отрицательная корреляция между содержанием кальция в панкреатическом соке и активностью трипсина $r = -0.78$, что согласуется с корреляцией соответствующих показателей в крови. Это позволяет рассматривать кальций вместе с протеазами как элемент, регулирующий процессы метаболизма в организме кур.

Ключевые слова: панкреатический сок кур, кальций, трипсин, ингибитор

CALCIUM AS AN INHIBITOR OF TRIPSIN ACTIVITY IN PANCREATIC JUICE OF CHICKEN

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The results of studying the interaction of calcium and pancreatic enzymes in 46-week-old Hisex white chickens are presented. It is shown that intestinal absorption of calcium is accompanied by endogenous excretion of calcium with digestive juices. The effect of calcium on trypsin activity in an *in vitro* experiment was determined. In the experiment, different doses of calcium (5.0; 7.5; 0.5; 12.5 and 25.0 mg) were injected into the pancreatic juice of chickens, previously diluted with physiological solution 10 times. The number of repetitions in each variant of the experiment was 20 times. For the experiment, 2-aqueous calcium chloride (Chimmed, RF) was used. Trypsin activity was determined by the kinetic method. The content of calcium was determined with a Sinnowa BS-3000P biochemical analyzer (China) and a set for the determination of calcium in the blood of animals DIAKON-VET (RF). It was found that pancreatic juice of chickens contains up to 2.9 ± 0.03 mmol of calcium/l, which is comparable to the level of calcium in the blood serum of 1.99 ± 0.10 – 3.13 ± 0.20 mmol/l. The inhibiting effect of calcium on the trypsin activity was found. A five-fold increase in calcium in pancreatic juice of chickens reduces the activity of trypsin by 34.7%. According to

the analysis of variance, the concentration of calcium in pancreatic juice affects trypsin activity, the reliability of the effect is confirmed by 92%. A stable negative correlation was established between calcium content in pancreatic juice and activity of trypsin $r = -0.78$, which is consistent with the correlation of the corresponding parameters in the blood. These findings serve as evidence that calcium together with proteases can be regarded as an element that regulates metabolic processes in chickens.

Keywords: pancreatic juice of chickens, calcium, trypsin, inhibitor

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Конфликт интересов

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Conflict of interest

The authors declare no conflict of interest.

INTRODUCTION

Calcium in the body of chickens plays an extremely important role in the formation of the skeleton and shell of eggs. In addition, calcium is involved in the contraction of muscle fibers, affects the automation, processes of excitation and contraction of the heart, in blood coagulation, activates a number of enzymes and stabilizes the trypsin of pancreatic juice [1]. Calcium is found in bone tissue, muscles, and biological fluids in the body of animals. Simultaneously with absorption in the gastrointestinal canal of birds, endogenous excretion of calcium with digestive juices occurs. Studies have established that the pancreatic juice of chickens contains up to 2.9 ± 0.03 mmol of calcium / 1, which is comparable to the level of calcium in the blood serum - 1.99 ± 0.10 - 3.13 ± 0.20 mmol / 1 [2]. The norms of calcium in the diet of chickens at different age periods vary depending on the need for a macronutrient. With a high level of calcium, the degree of its assimilation by hens decreases both in absolute and in relative terms [3]. An increase in the diet of layers of calcium above the norm is impractical. Instead of the expected improvement in shell quality, the opposite results are often obtained. Excess calcium inhibits the absorption of trace elements (zinc, manganese, iron, possibly copper) and impairs the absorption of plant (phytate) phosphorus. The introduction of excessive amounts of calcium into compound feed (in Russia, usually in the form of chalk) impairs their taste and eatability by poultry.

The aim of the research is to determine the effect of calcium on the activity of trypsin in the digestive juice of chickens in an in vitro experiment.

Objectives of the study - to study the activity of trypsin against the background of different doses of calcium in the pancreatic juice of Hisex white hens; to carry out an analysis of variance of the influence of the amount of calcium on the activity of trypsin in pancreatic juice, to calculate the correlation between the signs in the pancreatic juice and the blood plasma of chickens.

MATERIALS AND METHODS

The studies were carried out in the conditions of the physiology laboratory of the Federal Scientific Center "All-Russian Research and Technological Institute of Poultry" of the Russian Academy of Sciences in 2020. The object of the study is pancreatic juice obtained in chronic experiments from 46-week-old Hisex white chickens operated on according to the method of Batoev TS. ZH. [4]. Trypsin activity was determined by the kinetic method [5]. The calcium content was determined using a semi-automatic biochemical analyzer Sinnowa BS-3000P (China) and a kit for determining calcium in the blood of animals by the company "DIAKON-VET" (RF). First, the basic activity of trypsin, which was in the pancreatic juice of chickens, was determined. For the experiment, the juice was preliminarily diluted with physiological solution 10 times so that its proteolytic

activity corresponded to the duodenal chyme in chickens. Then, different doses of calcium were injected into the tubes with juice, starting from 5.0 mg, increasing the amount of the mineral by 0.25 mg in each next tube, up to 12.5 and 25.0 mg. For the experiment, we used calcium chloride 2-water (HIMMED, RF). Studies of the activity of trypsin in each of the tubes with calcium were repeated at least 20 times.

For statistical processing of the results, Excel was used to calculate the mean (M), mean square deviation ($\pm m$), correlation, and also used the Microsoft Office analysis package to perform the ANOVA. The significance of the differences was established by the Student's t-test; the differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The results of the study, obtained *in vitro*, showed that the activity of trypsin in the pancreatic juice of chickens changes with the addition of different doses of calcium (see table. 1). The data in the table indicate that calcium has an inhibitory effect on the activity of trypsin. The sharpest periods of decrease in enzyme activity were noted between the first and second test tubes (by 17.0%, $p < 0.05$), the third and fourth - (by 15.6%, $p < 0.05$). In general, with an increase in calcium in the pancreatic juice of chickens by 5 times, the activity of trypsin decreases by 34.7%.

The conducted analysis of variance showed that there is a relationship between the content of calcium in pancreatic juice and the activity of trypsin (see Tables 2, 3). The strength of the

factor's influence (the amount of calcium) is reliable and amounts to 92.0%.

Correlation analysis showed that between the calcium content and the activity of trypsin in the pancreatic juice of chickens, there is a stable inverse relationship, the coefficient of which is minus 0.78.

According to the results of a biochemical study of biochemistry in the blood of laying hens, a stable inverse correlation between calcium and trypsin activity was noted (see figure), $r = -0.51$.

The data obtained are consistent with the results of studies by A.G. Mikhailova et al. [6] on the possibility of regulation with the help of calcium ions of undesirable side hydrolysis when using enteropeptidase. Changes in the content of calcium in the blood of broiler chickens and their progenitors in ontogenesis have been established, which may have an effect on metabolism [7]. Thus, in 1-day-old chickens, the amount of calcium in the blood plasma was markedly low, but already in the first week of postembryonic life, it increased by 38.7% and remained at this level up to 35 days. In the blood plasma of 1-day-old chickens, a high activity of trypsin was observed, which could be associated with the production of a sufficient number of inhibitors that inhibit the activity of the enzyme during embryogenesis. By the age of 7 days, trypsin activity decreased in broiler chickens by 11.8 times. Subsequently, a gradual decrease in this indicator was observed both in absolute terms and in relation to live weight. In general, the relative indicator of trypsin activity per unit of live weight decreased by 501 times over the 35-day study period in broiler

Табл. 1. Активность трипсина на фоне разных доз кальция в панкреатическом соке кур
Table 1. Trypsin activity depending on the doses of calcium in chicken pancreatic juice

The amount of calcium in the test tube, mg / (mmol / l)	Trypsin activity, units / l				
	First test tube	Second test tube	Third test tube	Fourth test tube	Fifth test tube
5,0/3,95	124,8 \pm 3,35				
7,5/5,87		103,7 \pm 2,82			
10,0/8,07			100,0 \pm 0,70		
12,5/10,30				84,4 \pm 0,47	
25,0/20,38					81,5 \pm 2,21

Табл. 2. Однофакторный дисперсионный анализ взаимосвязи кальция и трипсина

Table 2. One-way ANOVA of the relationship between calcium and trypsin

Group	Count	Sum	Mean	Dispersion
Calcium concentration	5	1,2	0,24	0,02425
Trypsin activity	10	989,689	98,9689	304,5126677

Табл. 3. Дисперсионный анализ влияния количества кальция на активность трипсина панкреатического сока

Table 3. ANOVA of the influence of calcium amount on the activity of trypsin in pancreatic juice

The source of variation	The sum of squared deviations (SS)	The degree of freedom (Df)	Mean square (Ms)	Actual distribution criterion (F)	p – value*	Theoretical distribution criterion (F-critical)
Between groups	32491,32	1	32491,32	154,1159011	1,38935E-08	4.667192732
Within groups	2740,711	13	210,8239			
Total	35232,03	14				

The power of influence 92,0%

* The probability that the variance reproduced by the equation is equal to the variance of the residuals.

chickens. In addition, calcium ions are markers of the state of the pancreas in its pathology [8, 9]. Pancreatitis is currently a common and severe disease that has no specific therapy and is characterized by an insufficiently studied pathogenesis. Calcium (Ca (2+)) is a universal carrier of signals that regulate many aspects of cellular activity. It controls the secretion of digestive enzymes in the acinar cells of the pancreas. The Ca (2+) reload is a key early event in the pathogenesis of many diseases. In pancreatic acinar cells, abnormal Ca (2+) calcium ions are a key factor in initiating cell damage. Due to the long and constant action of Ca (2+), trypsin is activated, vacuolization and necrosis, which are crucial in the development of pancreatitis. Consequently, in pancreatitis, calcium, on the contrary, is an activator of trypsin, and not its inhibitor, as under normal conditions. Research by O. Gryshchenko et al. [10] also found that Ca (2+) signals in stellate cells provide an amplification loop that promotes the death of acinar cells. The initial release of kallikrein and trypsin proteases from dying acinar cells may, through the generation of bradykinin and protease-activated receptors, induce CA (2+) signals in stellate cells. They can then damage acinar

cells and thereby induce additional release of proteases [10]. The authors point to the possible participation of nitric oxide in this process, the connection with which trypsin was established in studies on poultry [11].

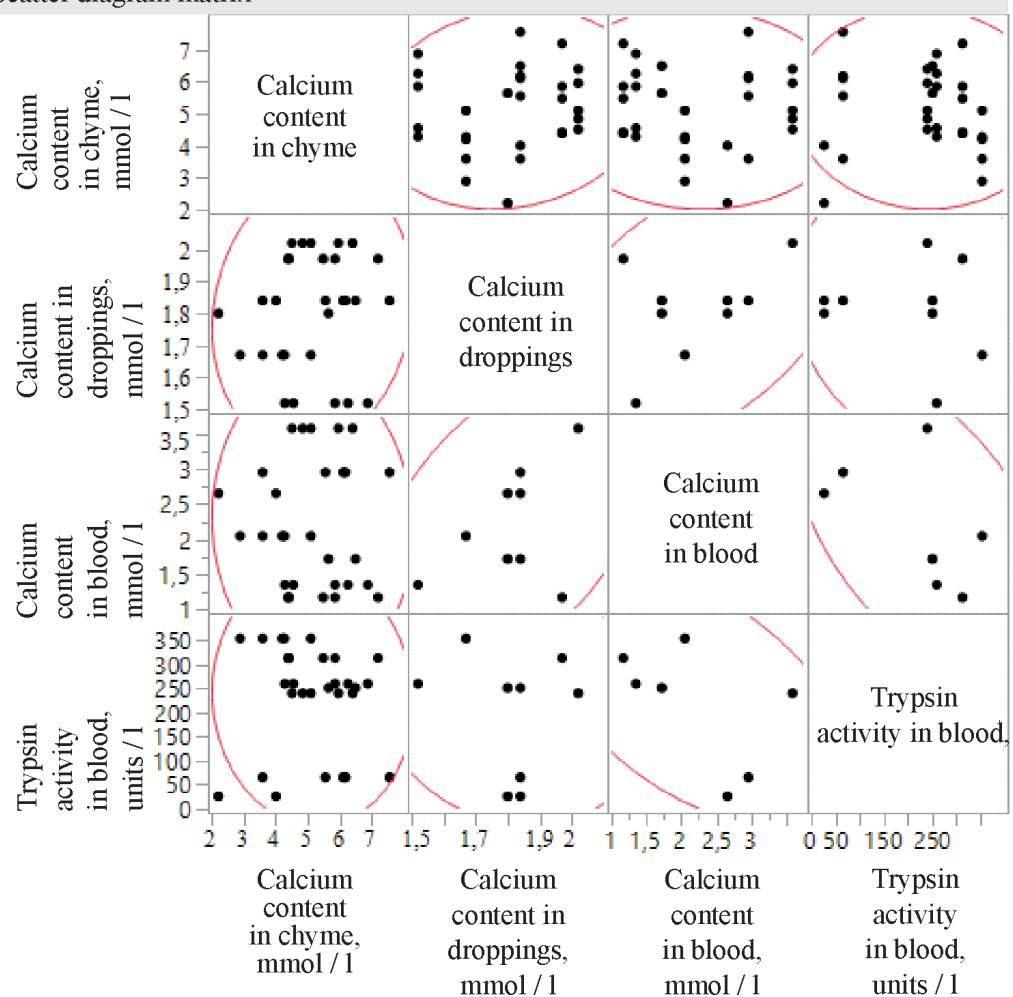
Magnesium is a natural calcium antagonist [12]. An increase in the concentration of calcium in the blood and its introduction into the duodenum enhances the secretion of all pancreatic enzymes, which is not consistent with the results of our study. However, in chronic pancreatitis, the concentration of calcium in pancreatic juice decreases in parallel with the debit of enzymes [12]. Magnesium is also excreted in the pancreatic juice simultaneously with calcium, which indicates its important role in the secretory process of the pancreas. This is confirmed by data on chickens [13].

Thus, calcium in in vitro experiments in chicken pancreatic juice has an inhibitory effect on trypsin activity. The analysis of variance carried out showed that between the content of calcium in pancreatic juice and the activity of trypsin, there is a strong relationship equal to 92.0%. In this case, an inverse correlation is observed, which is characteristic of the indicators of trypsin activity and the calcium content in

Correlation between the activity of trypsin and calcium in biological matrix of chickens

Correlations	Calcium in chyme	Calcium in droppings	Calcium in blood	Trypsin activity in blood
Calcium in chyme	1,0000	0,1267	-0,0420	-0,0413
Calcium in droppings	0,1267	1,0000	0,4718	-0,1496
Calcium in blood	-0,0420	0,4718	1,0000	-0,5143
Trypsin activity in blood	-0,0413	-0,1496	-0,5143	1,0000

Scatter diagram matrix



Корреляция между активностью трипсина и кальцием (Ca) в биологических средах кур (рассчитана в программном обеспечении JMP Trial 14.1.0, производитель SAS Institute, USA Carolina)

Correlation between trypsin activity and calcium (Ca) in biological media of chickens (calculated using JMP Trial 14.1.0 software, manufactured by SAS Institute, USA Carolina)

the blood. The new knowledge gained on the interaction of calcium and trypsin activity allows us to make an assumption about the regulatory function of trypsin and calcium in the metabolism of poultry.

CONCLUSIONS

1. According to the research results, the concentration of calcium in the pancreatic juice of

birds affects the activity of trypsin, the strength of the factor's influence is reliable and amounts to 92%.

2. The correlation between the calcium content in pancreatic juice and the activity of trypsin has a stable negative character with a coefficient of minus 0.78, which is consistent with the correlation of the corresponding signs in the blood.

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