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Findings on the Calcium Metabolism in Organisms of Laying Hens

Research Article

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ABSTRACT

This study was conducted to investigate the effects of various amounts of calcium in the diet on the activity of digestive enzymes in the duodenum and blood plasma of laying hens. There was recorded a reverse relation between the activity of duodenal digestive enzymes with trypsin in plasma and the amount of calcium in the biological fluids. When the amount of calcium in the diet of birds increased from 3.0 to 3.4%, crude fat rose by 5.0%, and when increasing the calcium content up to 5.4%, the digestibility tended to decrease by 0.5% compared to the optimal value. Digestibility of crude cellulose decreased with the increase of calcium in the diet of laying hens. Despite the same digestibility of crude protein in the intestine, the optimal content of calcium in the diet of chickens (3.4%) was achieved by maximizing the use of nitrogenous substances exceeding values of other groups by 30.0 (3.0% calcium) and 25.7% (5.4% calcium). When increasing the amount of calcium in the diet, most of the calcium excreted with litter, there was also a growing excretion of calcium through eggs. Thus, the data obtained can serve as the basis for the development of feed additives with different levels of calcium in them.

KEY WORDS activity of digestive enzymes in blood, calcium, duodenal digestive enzymes, laying hens, phosphorus.

INTRODUCTION

The calcium metabolism in the body of an agricultural bird occurs the most intensely in comparison with mammals. Highly productive laying hens excrete so much calcium with eggs during the cycle of egg-laying, which is 20-30 times higher than total reserves of this element in the body of a chicken. Daily maintenance of a laying hen only for the shell formation is about 8-10 times higher (per 1 kg of body weight) than the daily requirement of a highly productive cow (Hester, 2017). The maintenance of animals in calcium is not achieved with ash elements in the feed (Dorigan *et al.* 2018).

Therefore, the deficit of calcium in the main rations in practice gets compensated by the inclusion of additives (shells, limestone, chalk, etc.) with a high content of well accessible calcium (Böswald *et al.* 2019).

In addition to the level of productivity, the maintenance of animals in calcium depends on the calorific value of the diet, environmental temperature, species characteristics. The maintenance in the calcium of laying hens at a high level of energy consumption is 10-11% higher than at an average level. The high temperature outdoors, sharp fluctuations of it, and increased humidity have a negative impact on the mineral metabolism in the body and require a higher level of calcium in the diet.

Therefore, calcium and phosphorus are the most relevant elements in the mineral nutrition of laying hens and all animals (Okan *et al*. 1998; Ayasan and Okan, 1999a; Ayasan and Okan, 1999b). In order to determine the optimal amount of calcium and phosphorus in the diet, knowledge of the mineral metabolism in connection with the general metabolism and activity of digestive enzymes should be broadened.

Over the last decade, there has been a trend to a gradual increase in the calcium level in combined feeds for laying hens (Fisinin *et al*. 2014; Adedokun *et al*. 2017). Reports from some authors on the positive impact of very high doses of calcium (up to 4-5% or more) on the quality of shell resulted in some passion for increased doses, but, in general, recommended standards remained at the level of 3.0 -3.5% in combined feeds of natural humidity or 3.3% - 3.8 in air-dried substance.

Regulations for the calcium level are calculated by the factorial method with the following parameters: the endogenous losses of calcium with feces and urine, calcium excretion with eggs, the level of egg production and calcium accumulation from the feed. We decided to expand the range of research and to study the level of calcium in chyme of the duodenum intestine of laying hens, litter, and blood plasma.

The aim of the research was to study the correlation of the level of calcium in the diet of laying hens with the activity of digestive enzymes in the intestine and blood plasma.

MATERIALS AND METHODS

Experiments were performed on 10 hens of the cross Hisex white aged 6 to7 months in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experiments or for Other Scientific Purposes (ETS No. 123, Strasbourg, 1986) (Batoev, 2001). The experiments involved chickens with a cannula in the duodenum (7 heads) and intact ones (3 heads). The birds were housed at the vivarium of the Federal Scientific Center "All-Russian Research and Technological Poultry Institute" of Russian Academy of Sciences (FSC "ARRTPI" of RAS) provided feeding and housing in line with the requirements for the particular age group and the cross of poultry (Vertiprakhov and Grozina, 2018).

Surgical operations on birds in order to obtain duodenal chime

Birds are deprived of feeding 12 to18 hours prior to surgery. Anesthesia on birds is performed using dipyrone and dimedrol and immobilization – by Xylazalum (0.4 mL).

A bird gets fixed in the left lateral position on a special operating table, the operating field is prepared, a napkin gets imposed and conduction anesthesia is applied with 0.5% novocaine. Infiltration anesthesia is applied through the incision and into the abdominal cavity. The incision is carried out in layers on the right side behind the last rib caudally at a distance of 4 to 5 cm a few above the edge of the lateral process of the sternum. The duodenum is taken out, the ascending limb and the confluence of the pancreatic and bile ducts are to be found. Opposite this place, a 0.7 cm long purse-string suture needs to be imposed and an incision to be made which a cannula gets inserted into and the purse-string suture is quickly tied. Next, there is needed treatment around the cannula, optionally an additional suture to be applied. The wound is to be stitched up with nodular sutures sewing all layers. The wound heals within 3 to5 days and the birds can be used in physiological experiments.

Biochemical methods

Biochemical research was held with the following methods: measuring amylase - by Smith-Roy in the modification for the measuring high activity of the enzyme (Fisinin, 2014), proteases - by hydrolysis of casein purified by Hammerstein with calorimetric control (wavelength of 450 nm) (Fisinin, 2014), lipase - at the semi-automatic biochemical analyzer SINNOWA BS-3000P (PRC) with a set of veterinary diagnostic reagents for determination of the lipase concentration in blood of animals by the company "DIAKON-VET" (Russia). The content of calcium and phosphorus in biological samples and blood was measured at a semi-automatic biochemical analyzer SINNOWA BS-3000P (PRC) with a set of veterinary diagnostic reagents for the determination of these macroelements in the blood of animals by the company "DIAKON-VET" (Russia).

Blood was taken from the axillary vein in the amount of 2 to 3 ml. As the anticoagulant, we used 3.8% sodium citrate in volumetric ratio with a blood sample of 1:10. The sample was centrifuged at 5000 rpm for 3 minutes to separate the plasma from the formed elements. The trypsin activity in plasma was studied using nitroanilide benzoyl DL-arginine (BAPNA) as the substrate at the semi-automatic biochemical analyzer BS-3000P (PRC) by the kinetic method (Astrakhantsev, 2015). The activity of amylase and lipase in blood plasma was measured at an automatic biochemical analyzer Chem well 2900 (T) (the United States) with appropriate reagent kits - Human (Germany).

Physiological methods

The scheme of our experiments is presented in Table 1.

Table 1 The scheme of experiments on studying digestion in the intestine, digestibility of feed and absorption of mineral substances of the diet of laying hens

Group	Number of heads	Feeding
Control (intact hens)	3	The main ration–combined feed
Experimental 1 (duodenal cannula)	7	MR with the calcium level at 3.0%
Experimental 2 (duodenal cannula)	7	MR with the calcium level at 3.4%
Experimental 3 (duodenal cannula)	7	MR with the calcium level at 5.4%

Digestion trials on measuring the digestibility of nutrient and mineral substances were conducted using generally accepted methods.

Statistical methods

Statistical processing of results utilized JMP Trial 14.1.0 software that performed the calculation of average values (M) and standard errors of the mean (±SEM), the correlation coefficient. Significance of the differences was determined by Student's t-test, the differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

The results of our study found that a variable content of calcium in the diet of laying hens affected the activity of digestive enzymes in the duodenum (Table 2).

The data in Table 2 show that increasing amounts of calcium in the diet to 5.4% led to a decline of the lipase activity in the duodenum by 28.0%, the protease activity - by 16.9% compared to the control. The reduction of calcium in feed to 3.0% decreased the lipolytic activity by 37.4% and the activity of proteases by 18.4%. A decrease of the calcium content in feed resulted in reducing calcium in the duodenal chyme by 21.2% ($P \leq 0.05$), while an increase of it in the feed to 5.4% left calcium in the duodenum unchanged.

The calcium content in the blood of chickens had no significant differences when increasing it in the feed. The content of phosphorus in blood with a decrease of calcium in feed to 3.0% declined by 57.2% in comparison with the control (3.4% of calcium). Therefore, we can assume that the activity of digestive enzymes has a connection with the content of calcium in the diet of birds. The analysis of the correlation between the activity of digestive enzymes in the duodenum and total calcium of a diet showed that the optimal level of calcium in the diet of laying hens was characterized with a steady negative correlation between the content of calcium in the intestine and the activity of amylase ($r=-0.67$), lipase – $r=-0.45$, and protease – $r=-0.55$. There was also a steady negative correlation between the amount of total calcium in the blood and the trypsin activity which coefficient was -0.52.

Thus, our experimental data obtained from laying hens of the cross Hisex white aged 180 to 200 days allowed concluding that there was an inverse relationship between the activity of digestive enzymes in the duodenum and trypsin in plasma with the amount of calcium in the appropriate biological fluids. It is known that the activity of digestive enzymes has a connection with digestibility and availability of nutrients in the body of a bird. So, one of the research tasks was to study the nutrient digestibility and availability of mineral elements of feed pending on the level of calcium in the diet of chickens. The results of the study are presented in Table 3.

The data in Table 3 show that digestibility of crude protein did not reveal significant differences between the groups, therefore changing the content of calcium in the diet had no effect on the protein digestibility.

The results show that an increase of the calcium content in the diet of birds from 3.0 to 3.4% affected the digestibility of crude fat resulting in a 5.0% increase while increasing the calcium content up to 5.4% tended to decrease the digestibility by 0.5% compared to the optimal value. Bauman (1968) believes that calcium absorption in the body of the bird is correlated with the amount of fat in the diet. It is possible that an increase of calcium, reducing the activity of the lipase, raises the fat content in the intestine, thereby affects its absorption.

The data helped discover that the digestibility of crude cellulose changed with the increase of calcium in the diet of laying hens. The most significant decline of digestibility of cellulose was observed between the minimal and maximal levels of calcium in the diet, the difference was 9.5%, but as high fluctuations in values were observed, such a difference was not considered significant. The same could not be said about the values of the experimental groups where, between groups 2 and 3, the decline of the cellulose digestibility was 69.5%, $P \leq 0.001$. Therefore, when calcium in diet got increased, digestibility of crude cellulose greatly declined.

The analysis of the nitrogen balance indicates that the optimal calcium content in the diet of chickens was achieved by maximizing the use of nitrogen substances exceeding the performance of other groups by 30.0 (3.0% of calcium) and 25.7% (5.4% of calcium).

Table 2 The activity of digestive enzymes in the duodenum and plasma with various amounts of calcium in the diet of chickens

Index	Calcium content in the diet, %		
	3.0	**3.4 (control)[1]**	5.4
Indices in the duodenum			
Amylase, mg/(mL/min)	916±45.6	**966±43**	696±3 4.5***
Lipase, u/L	1037±108.7*	**1656±201.4**	1794±171.8
Proteases, mg/(mL/min)	43.6±1.8*	**53.4±3.2**	44.4±1.4*
Calcium, mmol/L	41±2.1*	**52±1.9**	53±1.2
Indicesin plasma			
Trypsin	150±44.2	**216±38.1**	174±45.0
Calcium in blood, mmol/L	1.9±0.4	**2.1±0.3**	2.8±0.7
Phosphorus in blood, mmol/L	0.3±0.05*	**0.7±0.06**	0.8±0.04

[1] Differences are significant in comparison with the optimal level of calcium in the diet (bolded).
* (P≤0.05) and *** (P≤0.01).

Table 3 Digestibility of the main nutrients and the nitrogen balance with different amounts of calcium in the diet of laying hens

Indices	Calcium in feed 3.0%		Calcium in feed 3.4%		Calcium in feed 5.4%	
	Control	Experimental 1	Control	Experimental 2	Control	Experimental 3
Crude protein, %	88.7±1.4	90.2±0.6	90.9±0.5	91±0.4	91.2±0.1	91.1±0.3
Crude fat, %	89.2±0.9	90.1±2.5	94.2±0.2*	91.8±0.5	93.7±1.5	92.8±0.7
Crude cellulose, %	20±9.1	25±1.7	17.2±3.4	22.3±4.4	10.5±4.1	6.8±2.2*
Nitrogen balance, %	16.6±3.5	18.6±7.3	46.6±5.8*	39.1±6.0	20.9±3.1	23.6±5.2

* (P≤0.05).

Given the same level of digestibility of protein in all groups, it can be concluded that the optimal level of calcium in the diet of laying hens affects the protein metabolism, increasing the intermediate metabolism and better absorption of protein. Moreover, indicators of the activity of the duodenal proteases and trypsin in blood confirmed this hypothesis.

Data on the balance of calcium in the body of laying hens are presented in Table 4. Table 4 shows that increasing the amount of calcium in the diet led to the leveraging of the excretion of it with litter, there was also a growing excretion of calcium with eggs. As a result, the balance of calcium greatly reduced at both the minimal content of it in the diet (3.0%) and the excessive one (5.4%). Hence, the research results were consistent with the recommendations on the regulation of calcium in the diets of laying hens (Batoev, 2001).

However, today there are practically no data on the effect of calcium, as one of the most important mineral elements, on the digestive system, and in particular, on the enzymatic activity in the gastrointestinal tract of birds. It is known (Bauman, 1968) that absorption of calcium is influenced by many factors, and primarily vitamin D, the concentration of hydrogen ions in the intestine, some carbohydrates, fats, proteins, as well as the content of salts of potassium, phosphorus, sodium, and magnesium in the diet. However, the relationship of calcium in the diet with digestive enzymes has not been studied.

So, for the first time, the research reveals the correlation between the enzymatic systems of the intestine and minerals affirming the unity of metabolic processes in the body, which has scientific and theoretical value for improving the nutrition of poultry (Kvan *et al*. 2018).

It is known (De Barboza *et al*. 2015) that many factors influence calcium absorption, primarily vitamin D, the concentration of hydrogen ions in the intestine, individual carbohydrates, fats, proteins, as well as the content of potassium, phosphorus, sodium, and magnesium salts in the diet. The results of our studies are consistent with the data (Cufadar *et al*. 2011; Manangi *et al*. 2018), which showed that physiological experiments performed on both operated and intact birds did not affect the absorption of calcium and phosphorus, their excretion in urine and egg laying. Studies conducted (Jiang *et al*. 2013) indicate that different doses of calcium in the diet of chickens lead to changes in the eggshell and poorer quality of bones than in chickens receiving a control diet.

High dietary calcium increases the concentration of serum calcium, osteoprotegerin mRNA and osteocalcin protein and inhibits the activity of serum alkaline phosphatase and reduces its mRNA compared to low or control dietary calcium.

A high-energy and high-calcium diet significantly reduces egg production. Compared to the control energy diet, high- and medium-energy diets increase fat deposition, but adversely affect bone metabolism homeostasis.

Table 4 The balance of calcium in the body of laying hens with different levels of it in feed

Indices	Calcium in feed 3.0%		Calcium in feed 3.4%		Calcium in feed 5.4%	
	Control	Experimental 1	Control	Experimental 2	Control	Experimental 3
Consumed with feed, g	1.7±0.40	1.6±0.20	2.0±0.20	2.2±0.07	3.4±0.20	3.7±0.20
Extracted with litter, g	1±0.10	1±0.10	1.2±0.08	1.8±0.05	1.9±0.20	2.4±0.20
Absorbed by organism, g	0.7	0.6	0.8	0.4	1.5	1.3
Extracted with eggs, g	1.63	1.25	0.76	1.5	2.17	1.67
Balance, g	- 0.93	- 0.65	+ 0.04	-1.1	- 0.67	- 0.37

Dietary calcium does not affect fat deposition, a high-calcium diet promotes bone homeostasis, while a low-calcium diet is associated with a deterioration in the quality of eggshells and bone homeostasis. Our experiments showed that with a decrease in calcium in the diet of hens, the lipolytic activity of the duodenal chyme and digestibility of fat decrease, which is confirmed by the work of foreign authors (Cufadar *et al*. 2011; Manangi *et al*. 2018).

However, the relationship between calcium in feed and digestive enzymes has not been studied previously. A significant novelty of our work is the determination of the correlation between operated and intact laying hens between the level of calcium intake and the activity of digestive enzymes such as amylase, lipase, and protease in the duodenum. The experiments also took into account the level of calcium in the chyme of the duodenum, litter and blood plasma. This allows you to study the mechanism of calcium metabolism in the body of chickens to develop new methods for assessing the dynamics of macronutrients and extend the use of poultry.

CONCLUSION

1. There is an inverse connection between the activity of the duodenal and plasmatic digestive enzymes with the amount of calcium in the appropriate biological fluids, which may be used in further studies to identify clinical and physiological indicators of mineral nutrition of poultry.
2. An increase of the calcium content in the diet of birds from 3.0 to 3.4% affects the digestibility of crude fat resulting in a 5.0% increase while increasing the calcium content up to 5.4% tends to decrease the digestibility by 0.5% compared to the optimal value.
3. Digestibility of crude cellulose decreases with the increase of calcium in the diet of laying hens.
4. The optimal calcium content in the diet of chickens is achieved by maximizing the use of nitrogen substances exceeding the performance of other groups by 30.0 (3.0% of calcium) and 25.7% (5.4% of calcium) despite the same digestibility of crude protein in the intestine.
5. Increasing the amount of calcium in the diet leads to a rise of its amount in the liquid part of the duodenal chime and growing excretion with litter and egg.

Thus, the results of our first research on the enzymatic activity of the intestine allow us to suppose about the correlation between the enzymatic systems of the gastrointestinal tract and minerals (in particular calcium). We found an inverse correlation between the concentration of calcium in the feed and the activity of digestive enzymes. That is, we can state that these studies prove the unity of the ongoing processes in the body, which serves as a theoretical justification for creating technologies for to control the growth, development, and productivity of poultry.

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