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The dynamics of free amino acids in chicken thigh muscle in experimental eimeriosis

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Abstract

The aim of research work was to learn the content of free amino acids in femoral muscle of local black rock chickens of Azerbaijan in the experimental infestation of chickens with the dose of 20,000 and 100,000 sporulated oocysts of *Eimeria tenella* per bird. The composition of 17 free amino acids in muscle of chickens has been studied. It was revealed that the total amount of amino acids of femoral muscle of chicks of the control group was 21.495 mmol/g, in the group with dose 20000 oocysts of *E. tenella* - 14.223 mmol/g at the 5th day of the invasion, at the 7th day -14.205 mmol/g, at the 10th day- 18.335 mmol/g. Consequently, the essential amino acids arriving into the muscle tissue, and are not used, and as pathological metabolic products in a form of free amino acids accumulated in the muscular tissue.

Keywords: *E. tenella*, coccidiosis, oocysts, muscles, chickens

Introduction

Eimeriosis, an intestinal disease caused by protozoan parasites of the genus *Eimeria* is an extremely important problem in poultry worldwide. In Azerbaijan republic it causes a huge economic loss to poultry industry, especially in the production of broiler chicken [1-4].

E. acervulina, *E. brunetty*, *E. maxima*, *E. mivati*, *E. necatrix*, *E. tenella* are the most pathogenic species of coccidia to infect chickens [5, 6]. There are 4 species of *Eimeria* Azerbaijan relevant for local farms - *E. acervulina*, *E. maxima*, *E. necatrix*, *E. tenella* [7]. Since each type of coccidia are localized in certain regions of the intestine, parasitizing of several species of *Eimeria* in organism of one host is possible [8, 9].

Pathogenic action of disease causative agent of parasitic diseases on animals is associated not only with the pathology of those organs where they are located, but also with the general influence on the organism. The biggest danger on a farm is association between coccidiosis and various diseases. The weakest form of coccidiosis, together with worms, viral and bacterial diseases leads to the great economic losses. This invasion causes to increase of mortality of birds, loss of productivity, loss of feed, decrease resistance of animals to other diseases. The cost of treatment and it leads to large economic losses [8, 10-12].

A spectral study of amino acids of animal tissues allows to some extent evaluate particular protein metabolism and physiological condition of the organism [13, 14, 15].

There is a complex process of asexual and sexual reproduction that goes coccidia in the intestinal mucosa. As a result, a large number of epithelial cells dies, violated the integrity of capillaries and blood vessels of the intestinal mucosa, causing profuse bleeding.

The aim of the research is to study changes in the dynamics of free amino acids in thigh muscle local black chickens, depending on the dose of infection in experimental eimeriosis (*Eimeria tenella*).

Materials and Methods

Experiments were carried out on local black rocks chickens bred in the laboratory of "biochemical basis of host-parasite relationships" of the Institute of Zoology of the National Academy of Sciences Azerbaijan. Day old chicks of local breed (*Gallus gallus*) were grown in the vivarium of the institute to 20 days of age. Chickens were fed with avian compound feed broiler. When the chicks reached specified age they were divided into 3 groups: control - uninfected (50 goals), and experienced -infested with dose 20,000 and 100,000 (50 goals each group). Chickens were infected by the introduction of a goiter with 20000 and 100000 *E.tenella* sporulated oocysts per bird.

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Chickens were infected with local strains. Oocysts from fecal samples from farms with a high percentage of infection have been purified in the laboratory of impurities. The separated oocysts were identified with microscope AXIO SCOPE A1 (Carl Zeiss Jena). Then they were sporulated in a thermostat to completely sporulate. Oocysts were separated from the potassium dichromate solution by centrifugation (Bio San LM-3000). The precipitate was suspended in the water taken in a quantity: 20,000 oocysts in 1 ml. Counting oocysts: the oocyst suspension in distilled water, thoroughly shaken, the suspension (0.01 ml) was mixed with sterile spell check calibrated to slide and counted all oocysts. The calculation was done 5 times and deduced average number for valid results.

Biochemical studies have been carried out accordingly endogenous developmental stages of the parasite in the intestine, on the 5th, 7th and 10th days of the invasion. After decapitation of chickens for determining of free amino acids in a tissue homogenate was quickly taken 2-2.5 g comminuted femoral muscle and added 1% picric acid in a ratio 1: 10 and homogenized in a glass gomogenezatore. The resulting precipitate was separated by centrifugation (8000 rpm. during 10 min) (K-24). Then centrifuge liquid with chloroform was passed through APA-2n ion exchanger. After drying the collected eluate on a rotary evaporator under vacuum, it was dissolved in the lithium citrate buffer (pH 2.2) and free amino acid content in the femoral muscle of chickens was determined by ion exchange chromatography on an automatic amino acid analyzer AAA 881 (Czech Republic).

For statistical analysis of the results has been used statistical software IBM SPSS Statistics 20. The figures are expressed in micromoles per 1g of tissue (mkmol/g). The differences between the compared samples were considered significantly ($p \leq 0.05$).

Results

Amino acids entering the organism of humans and animals with food, occupy a central place in the nitrogen metabolism, providing the synthesis of its own proteins in the organism, nucleic acids, enzymes, many coenzymes, hormones and other biologically active substances. The physiological transport of amino acids in organism is an important condition for normal vital activity animals. The results of investigation are presented in the table 1 and 2.

The amino acid pool of all investigated chickens irrespective of the dose, and days of infestation contains 17 amino acid. However, there are the quantitative changes of all amino acids pool and certain separately taken amino acids. Thus, the total concentration of free amino acids in the investigated infected chickens is reduced in the control group. Table 1 shows that infection of chickens with 20,000 oocysts *E. tenella* contributes quantitative changes of free amino acids in the femoral muscle. In 12 of the 17 amino acids of the muscle tissue occur statistically significant quantitative changes on different days of infestation, representing 70.59% of the total amino acids (Table 1).

Table 1: Dynamics of free amino acids in the femoral muscle black chicks infected with 20,000 sporulated oocysts of *Eimeria tenella* (M±SD, mmol in 1g tissue, n =10)

Amino acids	Indicators of control chickens	Days after invasion		
		5-th	7-th	10-th
Essential amino acids				
Threonine	1.423±0.01	0.966±0.01***	1.580±0.01	1.992±0.01
Valine	0.345±0.01	0.430±0.01***	0.585±0.01	0.361±0.01
Methionine	0.088±0.01	0.154±0.01***	0.366±0.01*	0.202±0.01**
Leucine	0.228±0.01	0.320±0.01***	0.289±0.01	0.324±0.01
Isoleucine	0.114±0.01	0.250±0.01***	0.477±0.01***	0.232±0.01*
Phenylalanine	0.150±0.01	0.237±0.15	0.230±0.01	0.230±0.01
Lysine	1.557±0.01	4.177±0.01***	0.314±0.01***	0.590±0.01***
The amount of essential amino acids	3,905	6,534	3,841	3,931
Nonessential amino acids				
Cysteine	0.222±0.01	0.222±0.01	0.221±0.02	0.224±0.01
Aspartic Acid	0.621±0.01	0.426±0.01**	0.538±0.01***	0.713±0.01
Serine	4.796±0.01	0.582±0.40***	1.524±0.01***	4.119±0.01
Glutamic Acid	1.168±0.00	1.213±0.16	3.527±0.01	2.994±0.01
Proline	2.215±0.00	0.716±0.01***	1.361±0.01*	0.700±0.01*
Glycine	1.696±0.01	1.372±0.01	1.328±0.01	1.058±0.01
Alanine	1.949±0.01	0.946±0.09*	1.214±0.01	1.388±0.01
Tyrosine	2.257±0.01	0.285±0.01	0.289±0.01	2.262±0.01
Histidine	1.560±0.00	1.550±0.03	0.362±0.01	0.449±0.01
Arginine	1.106±0.02	0.377±0.02**	-	0.564±0.01
The amount of nonessential amino acids	17,590	7,689	10,364	14,710
Amount of all the amino acids	21,495	14,223	14,205	18,641

* - $P < 0.05$; ** - $P < 0.01$; *** - $P < 0.001$.

P-Values less $P \leq 0.05$ were considered statistically significant.

The amount of free amino acids, except phenylalanine, glutamic acid, tyrosine, cysteine, glycine and histidine of chickens infected with 20,000 *E. tenella* oocysts varies considerably in prepatent period. The amount of threonine,

aspartic acid, serine, proline, alanine and arginine is decreased, and the amount of valine, methionine, leucine and isoleucine is increased.

At infection with a dose of 100,000 oocysts quantitative changes occurring from 17 certain amino acids invasion in 12 on day 5, and in 5 on the 7th, in 4 amino acids on the 10th day of ($P<0.05-0.001$). In general, the changes occur in the number of 13 amino acids, which represents 76.47% of the total amino acids (Table 2).

The contents of all amino acids except valine, glycine, tyrosine, and histidine in chickens infected with 10,000 *E.*

tenella oocysts are also changed in prepatent period. The amount of methionine, leucine, isoleucine, phenylalanine, lysine, and glutamic acid are increased. This dose of chickens infection causes a decrease of amount of threonine, aspartic acid, serine, alanine and arginine. The observable quantitative change in the prepatent period in amino acids in the patent period becomes more significant.

Table 2: Dynamics of free amino acids in the femoral muscle black chicks infected with 100,000 sporulated oocysts of *Eimeria tenella* (M \pm SD, mmol in 1g tissue, n=10)

Amino acids	Indicators of control chickens	Days after invasion		
		5-th	7-th	10-th
Essential amino acids				
Threonine	1.423 \pm 0.01	0.178 \pm 0.01*	0.170 \pm 0.01	1.211 \pm 0.03
Valine	0.345 \pm 0.01	0.333 \pm 0.01	0.330 \pm 0.02	0.340 \pm 0.01
Methionine	0.088 \pm 0.01	0.228 \pm 0.01*	0.288 \pm 0.02*	0.299 \pm 0.01
Leucine	0.228 \pm 0.01	0.373 \pm 0.01*	0.367 \pm 0.01	0.350 \pm 0.02
Isoleucine	0.114 \pm 0.01	0.316 \pm 0.01	0.320 \pm 0.02	0.249 \pm 0.01
Phenylalanine	0.150 \pm 0.01	0.268 \pm 0.01*	0.266 \pm 0.01	0.268 \pm 0.02
Lysine	1.557 \pm 0.01	3.641 \pm 0.01*	1.640 \pm 0.03*	1.010 \pm 0.01*
The amount of essential amino acids	3,905	5,337	3,381	3,727
Nonessential amino acids				
Cysteine	0.222 \pm 0.01	0.212 \pm 0.01	0.223 \pm 0.03	0.221 \pm 0.02
Aspartic Acid	0.621 \pm 0.01	0.403 \pm 0.03*	0.503 \pm 0.01**	0.619 \pm 0.01
Serine	4.796 \pm 0.01	0.503 \pm 0.01*	1.500 \pm 0.09**	2.070 \pm 0.02*
Glutamic Acid	1.168 \pm 0.02	1.802 \pm 0.01*	1.987 \pm 0.01	1.987 \pm 0.01
Proline	2.215 \pm 0.01	0.276 \pm 0.01**	2.000 \pm 0.02	2.109 \pm 0.02**
Glycine	1.696 \pm 0.01	1.248 \pm 0.01	1.236 \pm 0.01*	1.271 \pm 0.01
Alanine	1.949 \pm 0.01	0.742 \pm 0.01*	2.071 \pm 0.03	2.080 \pm 0.04
Tyrosine	0.257 \pm 0.01	0.266 \pm 0.01	0.300 \pm 0.03	1.337 \pm 0.02
Histidine	1.560 \pm 0.01	1.289 \pm 0.01*	1.373 \pm 0.03	1.297 \pm 0.01***
Arginine	1.106 \pm 0.02	0.749 \pm 0.01***	0.700 \pm 0.01*	0.617 \pm 0.02
The amount of nonessential amino acids	15,590	7,490	11,893	14,608
Amount of all the amino acids	19,495	12,827	15,274	18,335

* - $P<0.05$; ** - $P<0.01$; *** - $P<0.001$.

P-Values less $P\leq 0.05$ were considered statistically significant.

The amount of essential amino acids during infection with a dose of 20,000 oocysts compared with the control was increased in prepatent period; this index decreases particularly at the fifth and the seventh days, and tenth day to the control group.

The total number of amino acids on the 5th day falls, on the 7th day of its number begins to rise and this rise continues until the 10th day, but this index is lower than the control group. It was found that eimeriosis contributes to lowering the total number of amino acids in thigh muscles. This pattern was observed in the group infected with *E. tenella* dose of 100,000 oocysts.

During all the disease changes in the concentration of free amino acids caused by *Eimeria tenella* infection with a dose of 100,000 oocysts were more profound than during infected with a dose of 20,000 oocysts. Dynamics of free amino acids in the thigh muscles of chickens at coccidiosis indicates a violation of the functions of enzyme systems that are involved in their transformation in the organism.

Discussion

Amino acids are the major substrates of the metabolism of nitrogenous substances in living organisms [16]. It is known that there are half of the detected amino acids included in the

protein can be synthesized in the birds. Alanine, glutamic acid, aspartic acid, hydroxyproline, proline, serine and glycine are the nonessential amino acids. It should be noted that the last amino acid is replaceable only for adult birds, young stock are not able to synthesize it in the required amount [17].

The main criteria of the evaluation of the physiological state of the organism are the level of protein- amino acid metabolism and the content of individual free amino acids in different tissues of the organism. Study of the amino acid composition of various tissues of animals enables to estimate the intensity of protein metabolism, as well as to determine the features of the metabolic processes in different periods of the life cycle of the parasite [18].

The content of free amino acids in the liver, blood and other tissues is the most plastic indicator. At various diseases, this figure is rapidly changing [19, 4, 15].

There are unitary data for the free amino acid composition in blood and muscle at chicken coccidiosis. When studying individual free amino acids in the chicken coccidiosis was investigated only blood and plasma. Previously, we and other researchers have identified certain quantitative changes in the content of free amino acids of serum and muscle tissue in birds of different age groups, experimentally infected with certain species of *Coccidia* [20-26, 4, 15].

Alanine is the precursor of a large number of natural amino acids. Alanine through the Krebs cycle is converted into a serine, valine, leucine and isoleucine. Leucine is essential for building and development of muscle tissue. Leucine and isoleucine can be as a source of energy on a cellular level. On the 5th day of invasion amount of leucine in the thigh muscles of chickens infected with *Eimeria tenella* was significantly increased ($P<0.001$), and on the 7th and on 10th days was return to normal level.

Isoleucine is essential for the formation of muscle tissue, can serve as a source for its cells. Isoleucine deficit expressed by loss of muscle mass, as it plays a significant role in obtaining energy due to glycogen breakdown of muscles, deficiency isoleucine also leads to the manifestation of hypoglycemia. On the 5th day of invasion amount of isoleucine increased by 0.135 mmol/g ($P<0.001$), and on the 7th day at 0.333 mmol/g ($P<0.001$). On the 10th day of invasion, although there is some reduction in the amount of isoleucine unlike the previous day, but it is still at 0.110 mmol/g more control group (Table 1).

The cells involved in the biosynthesis of serine glycine, sulfur containing amino acids tryptophan and ethanolamine, sphingolipids. It was revealed sharp reduction of the amount of serine in the femoral muscle infected chickens of the 5th and 7th day infestation. In these days of invasion number of serine accordingly on 4.214 and 3.272 mmol/g less than in the control group ($P<0.01$). On the 10th day of invasion the number of lysine returned to normal.

Arginine is important in the metabolic processes of the liver. Conditionally essential amino acid arginine is capable of forming ionic and hydrogen bonds stabilizing secondary and tertiary structure of the protein. On the 7th day of invasion, changes in the exchange of arginine in the femoral muscle of sick chickens were pronounced and to determine the amount of them was impossible. On the 10th day of the invasion quantity of arginine was more than in the control group at 0.542 mmol/g and 0.564 mmol/g.

From the results in table 1 see that as compared with the control group, eimeriosis is accompanying by a decrease in the number of amino acids femoral muscle of chickens. The total number of amino acids on the 5th day of invasion is lower at 7.272 mmol/g than in the control group. On the 7th day of the invasion this difference was 7.290 mmol/g.

In connection with the completion of the developmental stages of the parasite in the intestine, this difference decreases and it is 2.854 mmol/g. This indicates that the completion of the developmental stages of the parasite is accompanied by a reduction of metabolism of most amino acids. If on the 5th day infestation changes occur in the metabolism of 11 amino acids, on the 10th day were statistically significant changes in the metabolism four amino acids (methionine, isoleucine, lysine, and proline).

It was founded that a dose of 100,000 oocysts infection causes changes in the metabolism a greater number of amino acids (Table 2). There is disrupted exchange of all essential amino acids in the femoral muscle of chickens infected with 100,000 oocysts on 5th day infestations. The change in exchange phenylalanine does not occur during infection 20,000 oocysts, and 100,000 sporulated oocysts contribute to the development disorders in phenylalanine metabolism. It is necessary to note that in compare with the infection dose of 20,000 oocysts, dose 100,000 oocysts leads to a drastic reduction in the total amount

of essential amino acids. On the 5th day of the invasion amount of essential amino acids at infection with 20,000 oocysts is 6.534 mmol/g, and with 100,000 oocysts amount is reduced to 5.337 mmol/g. This figure on the 7th and 10th days of invasion is less than the 5th day in the 1.956 and 1.610 mmol/g, respectively.

The changes in an amount of amino acids except histidine, tyrosine and cysteine are statistically significant at an infectious with 100,000 oocysts. In this period, amount of some increases, and the amount of other reduced.

As noted, these fluctuations are clearly expressed at infection dose 100000. On the 7th day of invasions, the number of amino acids having statistically significant quantitative changes, such as were the same on the 5th day infestation. On the 10th day of the invasion of the corresponding last stage of the parasite exchange of most amino acids is beginning to recover. On the 10th day of the invasion of the total number of amino acids at infection with 100,000 oocysts are 18.335 mmol/g, and with infection of 20,000 oocysts are 18.641 mmol/g.

From the table 1 and 2 can see that dose of 20,000 and 100,000 oocysts *E. tenella* lead to greatest quantitative changes in amino acids in the femoral muscle on the 5th day infestation. Methionine and cysteine are the two primary sulfur-containing amino acids, so their pool reflects supply of the organism with sulfhydryl groups. Studies have shown that high cysteine levels in the muscles are a result of lack of protein in the diet.

The comparison of free amino acid spectra in the muscles at both doses of infection reveals that changes of the amount of cysteine are not statistically significant, and the amount of methionine is increases.

So was founded that quantitative changes of amino acid begin in second generation meronts and their quantity are restored by the end of the prepatent period, and the quantitative changes in certain amino acids take place in the latent period. The infection dose of 20,000 and 100,000 causes changes in the amount of lysine, methionine and proline during the entire invasion, and even after the completion of developmental stages of the parasite it will not be returned to normal.

It is known that increasing concentrations of aromatic amino acids indicates a violation of the essential amino acids utilization tissue cells by blocking the enzyme systems (9). This leads to the formation of an amino acid imbalance. Consequently, the pathological process caused *Eimeria* and their metabolic products, influences the entire amino acid exchange in birds, apparently through activity disorders corresponding enzymes liver and muscles involved in amino acid metabolism that leads to discoordination, inhibition of biosynthesis and updating tissue proteins.

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