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Dynamics of Free Amino Acids in the Serum of Chickens for the Treatment of Experimental Eymeriosis (Eimeria Tenella)

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ABSTRACT

Amino acids-the lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, methionine, valine, leucine, isoleucine, tyrosine and phenylalanine was identified in the blood serum of control, infected and treated black chicks. In the serum of infected and untreated and treated birds, on different days of invasion significant changes have taken place in the quantity of 10 statistically of the 17 amino acids that have been studied metabolism. On the 10th day of invasion the quantity of those amino acids of which there were changes in the metabolism decreased by half in the treated group. The qualitative changes in the quantity of most amino acids are accompanied by some increase, while others decrease their number. Quantitatively enlarged amino acids constitute 41.18 %, and reduced amino acids constitute 17.65% of all amino acids. Amino acids in an amount which has occurred statistically significant changes in comparison with those who have not revealed changes constitute 58.83% from all amino acids. This proves that coccidiosis seriously affect the metabolism of amino acids in the serum, and increases the number of amino acids. In the group treated with Baycox total number of amino acids is also increased. Total amount of amino acids on the 5th day, on the 7^{th} day, 10th day of invasion is 4,885 mkmol/ml ,– 4.924 mkmol/ml,– 4.872 mkmol/ml respectively . As seen in the treated group, the total amount of all the amino acids is close to the control group.

Eimeriosis affects metabolism of aspartic acid, and the amount of aspartic acid in control, infected and treated groups was the same. In the treated group the number of aspartic acid decreases during all the days of invasion ($P<0.01$).It follows that changes in the exchange of aspartic acid are the result of influence used for medicinal purposes Baycox. During treatment eimeriosis with Baycox, despite a slight positive effect of the drug on the metabolism of individual amino acids, in general, Baycox has positive effects on the regulation of metabolism of essential and nonessential amino acids.

KEY WORDS: Eimeriosis, chickens, amino acid, serum, metabolism, Eimeria tenella.

1.INTRODUCTION

Eimeriosis caused by protozoa of the genus Eimeria, is an extremely important problem mainly for broiler production [1, 2, 3, 4]. Most pathogenic for chickens are six species of coccidia: *E.acervulina, E.brunetti, E.maxima, E.mivati, E.necatrix and E.tenella* [5]. Only species *E.acervulina, E.maxima, E.necatrix, E.tenella* from them are relevant for economies in Azerbaijan [1]. 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 [2, 3, 4]. The special danger to farms represents association of coccidiosis with various diseases. Even the weakest form of coccidiosis together with worms, viruses, and bacterial diseases causes to increase of mortality of birds, loss of productivity, loss of feed, cause to decreased resistance of animals to other diseases. The cost of treatment and prevention leads to great economic losses [5, 6, 7, 8].

Among the drugs used in the treatment of eimeriosis specific drugs used for a certain species are not available. In most cases, several species of *Eimeria* is found in the intestines of birds, which complicates the treatment of the disease. Drugs used against coccidiosis have impact on individual stages of development of the parasite. Most of these synthetic drugs, which has costly production are highly toxic and accumulating in the body cause to various complications, affect the immunosuppressive state of the body, cause to contamination of poultry products and environment [8].

Prolonged use of the same drug in eimeriosis (*Eimeria spp*) causes to resistance to it [9, 10, 11, 12, 13].

Providing a wide assortment of different chemical products for the treatment and prevention of coccidiosis without the full application complicates the choice of the most promising ones. In this regard, search for new effective drugs, as well as the development of schemes application of these preparations together with a variety of plants remains important.

Research goal is to identify the changes in the dynamics of free amino acids in the serum at eimeriosis in 20-day black chicks of the local breed and its treatments with Baycox.

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2. MATERIALS AND THE METHODS OF STUDY

The experiments were carried out on chickens of local black breed, bred in the laboratory, "the biochemical basis of host-parasite relationships" of the Institute of Zoology of the National Academy of Sciences of Azerbaijan. Per diemlocal breed chickens were grown in the vivarium of the Institute up to 20days of age. Chickens were fed with standard bird combined feed for broilers. Upon reaching a specified age chicks were divided into 3 groups: control -uninfected(20 goals), control – infected untreated-infected untreated (50 goals) and infected treatment-experienced (50 goals). Chickens of the last two groups were infected by the introduction in goiter sporulated oocysts of *E.tenella* in dose of 20000 oocysts per bird.

Oocysts required for the infection of chickens were separated from the solution by centrifugation of potassium dichromate. The precipitate was suspended in water, collected in such an amount that the concentration was about 20,000 oocystsin 1 ml. Oocysts required for invasion by chickens, were separated from the solution by centrifugation of potassium dichromate. The precipitate was suspended in water, collected in such an amount that the concentration of oocysts was about 20,000 in 1ml. Counting oocysts were produced as follows: the oocyst suspension in distilled water thoroughly was shaken. With sterile micro pipette the graded micropipette 0.01ml of the suspension was applied to a glass slide and counted all oocysts. In order to obtain reliable results count were produced 5 times and deduced average number.

Treatment of chickens was started the day after infection with the use of 2.5%th Baycox at 2ml /liter of drinking water for two days. Biochemical studies were conducted in the course of treatment, respectively endogenous developmental stages of the parasite in the intestine, that is on the 5th, 7th and 10th days of the invasion.

Blood collected from chickens decapitated accordingly invasion days was centrifuged (BioSan LM-300) for 5 minutes at 300 R /min, was then determined the amino acid composition of serum collected, and the free amino acid content was determined by ion exchange chromatographyon an automatic amino acid analyzer AAA881 (Czech Republic). For statistical processing the results used the statistical program IBM SPSS Statistics 20. Data obtained from analytical tests and *in vivo* experiments were expressed as mean ± Sd from at least thirty experiments. The mean value for each group was analysed and compared with other groups using Student's *t*-test. *P*-Values less than $P \leq 0.05$ were considered statistically significant.

3. RESULTS AND THEIR DISCUSSION

The impact of parasite on the biochemical processes in organism of the host- animal is implemented primarily by end products of metabolism, as well as produce toxins. Both processes occur in parallel, which leads to serious disturbances in metabolism in the host until death. During eimeriosis in the organism of animal - host have been serious violations in the exchange of nucleic acids, amino acids, fat, water, carbonates, vitamins, minerals [14, 15, 16, 17].

The various metabolic processes, differing by structural and morphological features and functional purpose in tissues and organs, also fundamentally differs from each other. Metabolic processes in the organism of birds, as in other animals, are characterized by complex biochemical reactions. Healthy organism, continuously receives metabolites – of various substances metabolic process, number of which is balanced very quickly and remains fairly constant (homeostasis). Any changes in metabolic processes affect the amount of blood metabolites and the number of end-products of metabolic processes.

In the blood serum of control, infected and treated black chicks amino acids-the lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, methionine, valine, leucine, isoleucine, tyrosine and phenylalanine are identified. By table data of the free amino acid composition of the serum of control, the infected *E.tenella* and treated with Baycox, it has been seen that on the 3rdday of invasion in serum of infected, untreated chicks in an amount of aspartic acid, serine, methionine, valine and isoleucine in comparison with the corresponding data in the control group, statistically significant changes were not revealed ($P>0.05$). It should be noted that the statistically significant changes in the amount of methionine, valine, leucine and histidine were not observed in all groups, i.e., the changes were within the physiological range.

The qualitative changes in the quantity of most amino acids are accompanied by some increase, while others decrease their number. In the group of infected, untreated amount of lysine, arginine, glutamic acid, proline, alanine and tyrosine, as compared with the corresponding data of the control group in creased by 0.043, 0.052, 0.041, 0.120, 0.191, 0.120 and 0.011 mkmol/ml, the amount of threonine, glycine, and phenylalanine decreases accordingly 0.047; 0.064 and 0.010 mkmol/ml.

Thus, the total number of increased amino acids is 0.578 mkmol/ml, reduced - 0,121 mkmol/ml. Quantitatively enlarged amino acids constitute 41.18 %, and reduced amino acids constitute 17.65% of all amino acids. Amino acids in an amount which has occurred statistically significant changes in comparison with those who have not revealed changes constitute 58.83% from all amino acids. This proves that coccidiosis seriously affect the metabolism of amino acids in the serum, and increases the number of amino acids.

On the 5th, 7th and 10th days of invasion in serum of chickens treated with Baycox statistically significant changes in the number of lysine ($P<0.05$) occur. Although on these days of invasion amino acid indices in the

treated group relatively higher appropriate indicators of the control group, on the 10th day these were lower (P<0,05).

As compared with the infected group, the amount of lysine was lower. At this time, the changes were statistically significant (P<0.05). On the 10th day of the invasion there was a strong increase in the number of arginine (P<0.01). On this day, the difference between these indicators and indicators of the control group was 0.071 mkmol/ml. These changes in comparison with the indicators of infection in the treatment group were not statistically significant (P>0.05).

Analysis of the indicators aspartic acid shows that eimeriosis affects metabolism of this acid, and the amount of aspartic acid in both groups is 0.433 mkmol/ml. In the treated group the number of aspartic acid decreases during all the days of invasion(P<0.01). It follows that changes in the exchange of aspartic acid are the result of influence used for medicinal purposes of Baycox and mechanical impact parasite or metabolic products to get her with Baycox.

Threonine is one of the amino acids subjected metabolic changes under the influence of coccidiosis. The changes in this acid differ from metabolic changes in the other acids. Thus, the amount of threonine in the treatment group compared with the control group on the 5th and 10th days of the invasion are normal, and on the 7th day the amount is lower than in the control group as 0.085 mkmol/ml (P<0.01). When compared to indicators of the infection group the opposite picture can be seen. That is, when compared with the corresponding indicators of the infected, untreated group on the 5th and 10th days of the invasion the amount of threonine is 0.054 and 0.030 mkmol / ml higher (P<0.05). In comparison with indicators of the 7th day, statistically significant changes were not observed (P>0.05).

Table: Dynamics of free amino acids of serum of blood of chickens infected *E.tenella* and treated with Baycox (mkmol / ml, M±Sd, n=5)

Amino Acids	Indicators uninfected chickens	Indicators infected chickens	The experimental treated chickens		
			5-th day	7-th day	10-th day
Lysine	0.206±0.08	0.249±0.12 P<0.05	0.247±0.07 P<0.05	0.232±0.10 P<0.05	0.173±0.12 P<0.01
Histidine	0.082±0.17	0.090±0.13 P<0.05	0.092±0.10 P>0.05	0.092±0.11 P>0.05	0.083±0.03 P>0.05
Arginine	0.263±0.17	0.315±0.15 P<0.001	0.263±0.08 P>0.05	0.276±0.27 P>0.05	0.334±0.18 P<0.001
Aspartic Acid	0.433±0.16	0.433±0.21 P>0.05	0.377±0.27 P<0.01	0.377±0.27 P<0.05	0.381±0.29 P<0.01
Threoine	0.320±0.35	0.273±0.09 P<0.05	0.327±0.11 P>0.05	0.235±0.08 P<0.01	0.303±0.08 P>0.05
Serine	0.352±0.18	0.368±0.18 P>0.05	0.302±0.17 P<0.01	0.456±0.24 P<0.001	0.352±0.20 P>0.05
Glutamatic Acid	0.410±0.11	0.451±0.13 P<0.01	0.457±0.14 P<0.01	0.434±0.23 P>0.05	0.437±0.14 P>0.05
Proline	0.168±0.03	0.288±0.17 P<0.05	0.330±0.11 P<0.01	0.328±0.09 P<0.01	0.328±0.11 P<0.001
Glycine	0.556±0.14	0.492±0.04 P<0.05	0.483±0.05 P>0.05	0.454±0.07 P<0.01	0.468±0.14 P>0.05
Alanine	0.530±0.11	0.721±0.18 P<0.001	0.718±0.09 P<0.001	0.690±0.14 P<0.001	0.742±0.12 P<0.001
Cysteine	0.538±0.23	0.378±0.15 P<0.01	0.379±0.18 P>0.05	0.379±0.18 P>0.05	0.350±0.13 P>0.05
Methionine	0.158±0.18	0.160±0.10 P>0.05	0.161±0.14 P>0.05	0.174±0.17 P>0.05	0.163±0.15 P>0.05
Valine	0.174±0.07	0.177±0.06 P>0.05	0.120±0.06 P>0.05	0.172±0.13 P>0.05	0.171±0.13 P>0.05
Leucine	0.315±0.09	0.319±0.19 P<0.001	0.312±0.11 P<0.001	0.335±0.20 P>0.05	0.274±0.11 P>0.05
Isoleucine	0.015±0.01	0.013±0.01 P>0.05	0.018±0.01 P<0.05	0.018±0.01 P<0.05	0.014±0.02 P>0.05
Tyrosine	0.150±0.09	0.161±0.10 P<0.001	0.159±0.12 P<0.05	0.152±0.15 P>0.05	0.152±0.14 P>0.05
Phenylalanine	0.154±0.17	0.144±0.13 P<0.01	0.140±0.16 P<0.01	0.140±0.16 P<0.05	0.147±0.21 P>0.05
Total	4.824	5.032	4.885	4.924	4.872

P-Values less P≤0.05 were considered statistically significant.

Similar changes have occurred in the metabolism of serine and aspartic acid. At that time although statistically significant changes were not observed in amount of serine under the influence of diseases in the treated group, the amount thereof on the 5th day invasion was 0,050 mkmol/ml lower, and on the 7th day 0.104 mkmol\ml above corresponding indicators of the control group (P<0.01).

In the treated group, statistically significant changes in the amount of glutamic acid were observed on the 5th day of invasion ($P<0.05$).

In serum, as infected and treated with Baycox chickens an increase in the amount of proline was observed. In comparison with the control group the amount of proline in the infected group were 1.7 times higher, in the treated group on 5- th day of the invasions -1.96 times, on the 7th and 10^{th} days, respectively it was 1.95 times higher. At this time, any changes were statistically significant.

Compared to indicators of the control group in treated group an amount of glycine reduced 0.102 mkmol/ml ($P<0.05$). Out of all investigated amino acids sharpest decrease is observed in the amount of alanine. In comparison with the infected, untreated, in the infected, treatment group amount alanine was higher 0.191 mkmol ($P<0.01$).

In the infected group on the 5th, 7th and 10th days of the invasion weight gain was: 0.188, 0.160 and 0.212 mkmol/ml. In comparison with indicators of the infected group, on days of invasion the difference was 0.003, 0.031 and 0.019 mkmol/ml below respectively. It is explained by even the minor influence of Baycox in regulation the metabolism of alanine.

Al though, in an amount of cysteine in the infected group a statistically significant change have occurred, in the treated group, under the influence of used for medicinal purposes drug the metabolism of this acid is not regulated.

In the treated group on the 5th, 7th days of invasion statistically significant changes were observed in the amount of leucine and isoleucine. On the 5^{th} day of invasion the amount of leucine ($P<0.05$) decreased, the same picture observed in the amount of tyrosine and phenylalanine. Generalizing it can be noted that different days of invasions in amount amino acids differ from each other. That is a quantity of amino acid varies depending on days of invasion. For example, the quantity of alanine at the 5^{th} day invasions rises, at the 7th lowers, and on the 10 th day increased to level saga in on day 5.

Cysteine is important for manifestation of biological activity of many enzymes and protein hormones. In the organism, it is easily converted to cystine. Cysteine is synthesized from methionine through homocysteine with participation of serine, and this process is irreversible. Leucine relates to proteinogenic essential amino acids. Leucine is required for constructing and development of muscle tissue.

Isoleucine is found in many proteins and is in a free state. Deficiency of isoleucine expressed in loss of muscle mass. As leucine, isoleucine may be a source of energy on a cellular level.

Being a essential amino acids - cysteine, leucine and isoleucine should be obtained from food or by synthesis from the other amino acids. The development the parasite in the intestinal epithelial cells during coccidiosis is the cause of functional disorders as the digestive system, as well as absorption of proteins, leading to a decrease in the amount of amino acids entering the organism. In connection with the positive effect of Baycox at the treatment of birds on the function of intestinal epithelial cells the amount of cysteine, leucine, and isoleucine in the treated group is higher than those of the control group.

Metabolism of free amino acids in the blood serum is exposed to changes accordingly invasion days and under the influence of the drug, used for medicinal purposes. In both groups, the most significant changes in the amount of free amino acids are observed on the 5th day of invasion. Thus, comparing obtained results it has been revealed that in the serum of infected and untreated and treated birds of the 17 amino acids that have been studied metabolism on different days of invasion in the quantity of 10, statistically significant changes have taken place. On the 10th day of invasion in the treated group the quantity of those amino acids decreased by half which there were changes in the metabolism.

Comparing the total quantity of amino acids it becomes clear that the total amount of amino acids in the control group amounted 4.824 mkmol/ml, and in the group of infected, untreated -5.032 mkmol/ml. As seen, under the influence of eimeriosis total number of amino acids increases in the serum. In the group treated with Baycox total number of amino acids is also increased. Total amount of amino acids on the 5th day ofinvasionis4.885 mkmol/ml, on the 7thday– 4.924 mkmol/ml, 10th day– 4.872 mkmol / ml. As seen in the treated group, the total amount of all the amino acids is close to the control group.

From this it follows that the treatment eimeriosis with Baycox, despite a slight positive effect of the drug on the metabolism of individual amino acids, in general, Baycox has positive effects on the regulation of metabolism of essential and nonessential amino acids.

4.CONCLUSIONS

1. Coccidiosis, exerting a strong effect on the exchange of amino acids in blood serum is the cause of increase of the quantity of most amino acids. 41.18 of amino acids,% are those that have increase in the amount and 17.65% - in which the number has dropped.

2. In the group infected with 20,000 *E.tenella* oocysts, and treated 2ml/ L dose of Baycox decreases the quantity of aspartic acid at all days of the invasion. It was revealed that the changes in metabolism of aspartic acid, manifested as a result of the influence of Baycox, used for medicinal purposes.

3. Metabolism of free amino acids in the blood serum undergoes changes in different days of invasion, and under the influence of the drug. The greatest changes in the quantity of free amino acids are observed on the 5th day of invasion.

4. Despite a slight positive impact 2ml / l dose of Baycox of at metabolism of the individual amino acids in the treatment eimeriosis, in general it has a positive influence on the regulation of the metabolism of all essential and nonessential amino acids.

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