RATIO OF THE CONTENT OF FREE AMINO ACIDS IN THE BLOOD AND SPERM OF ROOSTERS

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Abstract

In this paper are presented the experimental results obtained after the administration of polyphenols of plant origin extracted from green walnuts and their influence on the ratio of free amino acids content in blood serum and reproductive material of roosters. It was demonstrated that the total content of free amino acids in the blood serum of roosters is 437.14±45.23 mcm/100 ml. The highest share belongs to proline – 31.34%, glycine – 10.63%, alanine – 7.73%, threonine – 7.62% and glutamine – 7.37% of the total content of all amino acids. Seminal fluid is characterized by a 3 times higher content of free amino acids than in blood serum, which constitutes - 1885.42±130.43 mcm/100 ml. For seminal fluid, it is characteristic that the highest share is represented by the content of asparagine – 16.3%, glutamine 40.16%, glutamic acid – 9.64% of the summary content of amino acids in the seminal plasma. The total content of free amino acids in the reproductive cells of roosters constitutes 110.69±3.28 mcm/100 ml. In this biological material the highest share belongs to taurine with a value of 37.28%, followed by arginine with a value of 17.68%, glycine – 15.19%, alanine – 6.55%, proline – 6.43%, glutamine – 5.80%.

Key words: *free amino acids, roosters, reproductive material, plasma, spermatozoa*

INTRODUCTION

The functional state of the body is determined by the nature of metabolic processes, their stability and balance, and the loss of any link of metabolism leads to a chain reaction of interrelated disorders. Amino acids that enter in the body of humans and animals with food occupy a central place in nitrogen metabolism, providing the synthesis of their own proteins, nucleic acids, enzymes, many coenzymes, hormones and other biologically active substances (Ciochină et al., 2011).

Each tissue and physiological fluid of the body is characterized by its own fund of amino acids, their different content and ratio, which reflects the specifics of amino acid metabolism in them (Garaeva et al., 2009).

The blood constantly contains a certain amount of free amino acids. Some of them are of exogenous origin, the other part of blood amino acids is formed as a result of the breakdown of tissue proteins. The amount of amino acids that circulate in the blood is small, because, entering the blood, they are quickly extracted by tissue cells, where they are used to build proteins and other nitrogen-containing compounds. The content of free amino acids in serum and blood plasma is almost the same; the total dispersion of the fund of amino acids in plasma is determined: firstly, by the influence of the intake of essential amino acids due to the hydrolysis of exo- and endogenous proteins, secondly, by the interorgan redistribution of free amino acids and, thirdly, by the reactions of the intermediary metabolism of these compounds (Garaeva et al., 2009).

Previously, it was shown that it is free amino acids that determine the metabolism and functions of the spermatozoa (Garaeva et al., 2009), which is essential for the creation of technologies for the reproduction of farm animals. Therefore, the absence of reference intervals for the content of free amino acids in

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the semen of animals makes it difficult to interpret laboratory data. In this regard, further work on the problem should be aimed at determining the reference intervals of their concentrations, which will provide additional opportunities for diagnosing reproductive pathologies and prescribing drugs for metabolic correction (Zavyalov et al., 2022).

Based on the above, we set the task to analyze the comparative content of free amino acids in the blood and sperm of roosters.

MATERIAL AND METHODS

To carry out these researches in experiments were used 10 rabbits, which were divided into two groups of five animals in each group, a control group and experimental group. Roosters from the experimental group were administered orally 1 ml of hydroalcoholic extract from green walnuts, with a total polyphenol content of 548.37 mg/100g gallic acid equivalent (GAE). The extract was diluted in a ratio of 1/4 with distilled water and administered with the automatic device for administering drugs to animals.

Analyses of the amino acid content in blood serum, seminal fluid and spermatozoa were performed on the T339 M amino acid analyzer by ion exchange chromatography on ionites, which includes the determination of about 40 free amino acids and their derivatives.

Blood sampling from roosters was carried out from the underwing vein in the morning on an empty stomach (before feeding).

Blood and semen were centrifuged at 6000 rpm for 15 minutes. The obtained blood serum and seminal fluid were subjected to deproteinization with a 6% solution of sulfosalicylic acid in a ratio of 1:1.

The sperm was ground in a mortar with the addition of 6% sulfosalicylic acid. For better protein denaturation, the mass was frozen and thawed three times, and then centrifuged at 6000 rpm for 15 minutes.

Before analysis, the acid was evaporated from the obtained samples of blood serum, seminal plasma and spermatozoa in a vacuum rotary evaporator at 40ºC, washed with distilled water to dryness, re-dissolved with a starting buffer pH 2.2 and applied to a chromatographic column (Garaeva et al., 2009).

The obtained data were statistically processed by the Student's method.

RESULTS AND DISCUSSIONS

The results obtained are reflected in tables 1, 2 and figures 1, 2 and 3.

Previously, it was shown that in humans, seminal plasma contains two orders of magnitude more free amino acids than in spermatozoa (Mereuța et al., 2008). The same trend was noted by us in the roosters.

The total content of free amino acids in the blood serum of roosters is 437.14±45.23 μm/100 ml. The highest proportion is that of proline (31.34%), glycine (10.63%), alanine (7.73%), threonine (7.62%), glutamine (7.37%) of the total content of all amino acids.

The seminal plasma is characterized by a high content of free amino acids - 1885.42±130.43 μm/100 ml, which is more than 3 times higher than in blood serum. This physiological fluid is characterized by the highest content of glutamine (40.16%), asparagine (16.3% of all amino acids), glutamic acid (9.64%).

The total content of free amino acids in the spermatozoa of roosters is: 110.69±3.28 μm/100 g in roosters. Here, of all free amino acids, the proportion of taurine (37.28%), arginine (17.68%), glycine (15.19%), alanine (6.55%), proline (6.43%) and glutamine (5.80%).

Arginine is an integral part of tissue proteins and body fluids of animals and humans. This amino acid performs various biological functions, participates in a number of metabolic processes: synthesis of protein, nitric oxide, ornithine, creatine, polyamines (Dmitrenko et al., 2008; Unzhakov et al., 2021). Acting as a precursor for the synthesis of nitric oxide, polyamines and other molecules of biological importance, it plays a key role in pregnancy and fetal development (Hsu et al., 2019). After taking this amino acid in animals, in the seminal plasma the number of spermatozoa increases by 18% (Unzhakov et al., 2021). The beneficial effect of arginine on animal fertility is achieved through an increase in the synthesis of polyamines in sperm cells, and through the regulatory role of nitric oxide in increasing sperm motility (Balercia et al., 2004). Based on the special

role of arginine in the processes of reproduction, we made an analysis of the proportion of its content in blood serum, seminal plasma and spermatozoa. It turned out that this proportion is the same in the blood (1.92%) and seminal plasma (1.76%); but, at the same time, at least 5 times higher in spermatozoa (10.71%).

Table 1. The content of free amino acids and end products of nitrogen metabolism in blood serum (μm/100 ml), seminal fluid (μm/100 ml) and spermatozoa (μm/100 g) of roosters

Amino acids	Blood	Seminal plasma	Spermatozoa
Cysteic acid	1.41±0.79	2.04±0.34	0.66±0.04
Taurine	19.097±7.06	62.99±9.71	3.67±0.07
Aspartic acid	6.98±1.60	74.04±0.30	4.21±0.22
Threonine	33.30±4.25	51.17±0.10	5.57±0.12
Serine	23.83±5.38	95.96±2.96	6.58±0.47
Asparagine	7.44±1.51	352.13±30.56	7.55±0.76
Glutamic acid	8.10±2.05	207.54±17.36	7.57±0.22
Glutamine	32.23±9.18	757.26±42.11	21.75±0.18
α-Aminoadipic acid	1.19±0.21	34.41±3.82	4.67±0.17
Proline	137.0±8.73	56.90±3.58	6.66±0.02
Glycine	46.47±2.27	37.56±2.01	8.88±0.08
Alanine	33.78±8.03	4.48±0.24	0.51±0.03
Citrulline	1.35±0.07	4.66±0.73	0.49±0.03
α-Aminobutyric acid	1.39±0.18	14.69±1.52	3.78±0.02
Valine	20.42±1.16	14.69±1.52	3.78±0.02
Cysteine	1.84±0.34	9.76±0.97	1.56±0.07
Methionine	1.20±0.16	4.37±1.04	0.71±0.04
Isoleucine	4.06±1.09	6.23±0.97	1.55±0.11
Leucine	6.68±2.01	10.98±1.58	2.25±0.14
Tyrosine	3.95±0.63	16.64±2.80	1.22±0.03
Phenylalanine	4.85±0.91	8.99±0.95	1.13±0.06
γ-Aminobutyric acid	0.85±0.18	3.71±0.48	0.30±0.01
Ornithine	6.11±1.02	10.76±0.50	0.67±0.01
Lysine	21.00±4.44	14.03±0.63	4.70±0.15
Histidine	4.25±1.20	10.96±0.52	1.78±0.05
Arginine	8.40±1.68	33.15±4.54	11.86±2.62
Urea	58.40±1.90	129.13±21.23	27.97±0.79
Ammonia	35.99±13.66	139.37±19.07	24.49±1.49
Σ Free amino acids	437.14±45.23	1885.42±130.43	110.69±3.28
Σ Indices of nitrogen metabolism	531.54±57.05	2153.92±170.73	163.143±5.57

Taurine also plays an important role in sperm metabolism (Ostapiv et al., 2014): due to taurine deficiency, the shape of spermatozoa flagella is disrupted, which does not allow them to fertilize an egg. Interestingly, in our experiment, there is a relatively constant proportion of its content: in blood serum 4.37%, in seminal plasma 3.31%, in spermatozoa 3.32%. This is probably due to the fact that taurine is not formed in spermatozoa, but enters them from the genital tract (Ostapiv et al., 2014).

The concept of functional amino acids allows them to be considered as homeostasis modifiers that regulate key metabolic pathways and contribute to improving health and reproduction (Sheibak, 2015). The results of the analysis of the content of functional groups of free amino acids in the blood serum, seminal plasma and spermatozoa of roosters, combined into functional groups, are shown in table. 2.

Table 2. Comparative content of combined functional groups of free amino acids in blood serum (μm/100 ml), seminal plasma (μm/100 ml) and spermatozoa (μm/100 g) of roosters

Amino acids	Blood	Seminal plasma	Spermatozoa
Σ Non-essential amino acids	301.59±34.10	1642.21±106.50	70.66±0.27
Σ Essential amino acids	104.15±15.90	154.57±11.90	33.31±2.80
Σ Immunoactive amino acids	136.53±23.95	846.57±56.29	46.01±0.18
Σ Glucogenic amino acids	164.79±22.06	330.33±10.49	35.68±0.91
Σ Ketogenic amino acids	40.54±8.49	56.87±6.96	10.84±0.20
Σ Proteinogenic amino acids	405.74±48.74	1796.78±118.40	103.98±3.07
Σ Sulfur amino acids	23.54±7.43	79.16±12.07	6.74±0.23

Previous studies have revealed the involvement of immuno-endocrine aspects not only in the regulation of spermatogenesis, but also in the formation of infertility under the influence of adverse factors on the male reproductive system (Pichugova et al., 2016). In this regard, of particular interest is the detailing of the content of immunoactive amino acids in blood and semen. We found that if the proportion of immunoactive amino acids in the blood is 31.23%, then in the seminal plasma and spermatozoa it is significantly higher – respectively 44.90% and 41.57% of the total content of amino acids.

The role of SH-groups of thiols and proteins in sperm is due to their participation in complex biochemical processes. They provide germ cells with the necessary energy and create conditions for the process of sperm fertilization (Musabekov, 2012). There are data of the participation of low molecular weight SH-groups in the regulation of metabolic processes in germ cells. Thus, it was found that the species stability of rabbit, ram, boar and bull ejaculate during storage depends to a certain extent on the content of SH-groups of proteins in germ cells, and not in sperm plasma, since animals with less stable sperm have significantly more SH-groups in plasma than in spermatozoa. The level of SH-groups in the seminal plasma and germ cells of ejaculate affects the fertilization and fertility of sows (Musabekov, 2012). Violation of the oxidative balance, in the maintenance of which sulfur-containing compounds play a leading role, in the male reproductive organs and in the ejaculate of rats is the leading biochemical mechanism of the pathogenesis of reproductive pathology (Agletdinov, 2010). The results of our study demonstrate that the proportion of sulfur-containing amino acids is almost the same in blood (5.38%), seminal plasma (4.20%) and spermatozoa (6.09%).

Since SH-groups are part of the active centers of a number of enzymes, participate in their catalytic action, in the binding of substrates, coenzymes and metal ions (Pitkanen et al., 2002), the closest correlation of the status of various immune bonds was identified specifically with sulfur-containing amino acids (Ciochină et al., 2011).

The proportion of proteinogenic amino acids in the entire volume of free amino acids is 92.82% in blood serum, 95.30% in seminal plasma, and 93.94% in spermatozoa, that is, the same. Interestingly, at the same time, the share of the fund of essential amino acids in the blood is 23.83%, in the seminal plasma - 8.20% and in the spermatozoa are significantly more - 30.09%. The same trend was previously identified for people (Mereuța et al., 2008).

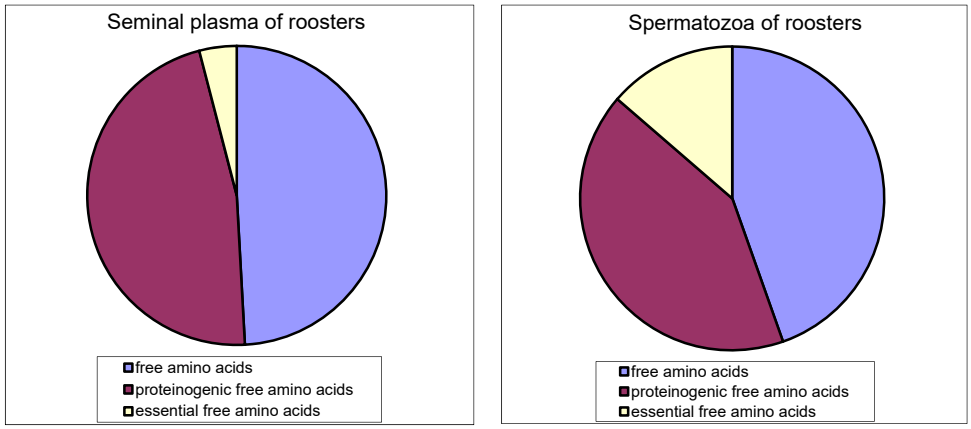

Figure 1. The proportion of proteinogenic, including essential amino acids, in the seminal plasma and spermatozoa of the rooster

In this regard, it was of interest to identify the characteristic values of the essential/non-essential amino acids ratio in the blood, seminal plasma and spermatozoa of the rooster, which are 0.34±0.02, 0.09±0.01 and 0.47±0.17, respectively. Changes of this ratio in the blood probably reflect the provision of roosters with a full nutrition with a sufficient content of essential amino acids, and in semen - the provision of these amino acids to the morphological structures of spermatozoa and their functioning.

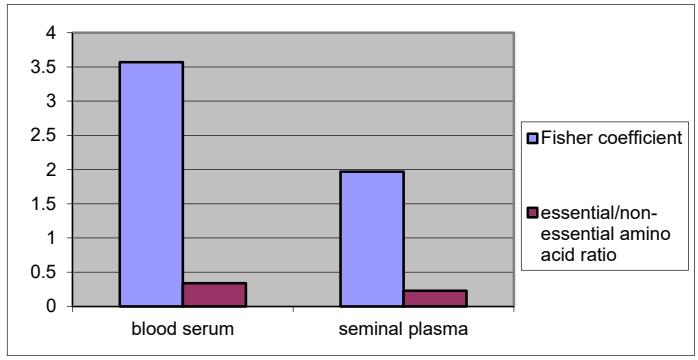

Figure 2. Characteristic values of the Fisher coefficient and essential/non-essential amino acids ratio in roosters in blood serum and seminal plasma

Previously it was shown (Boronciuc et al., 2008) that aliphatic and aromatic amino acids are of great importance in the regulation of protein metabolism in gametes. The indicator of the ratio of the sums of concentrations of free aromatic amino acids and branched chain amino acids (aliphatic amino acids) - the Fisher coefficient - is one of the objective criteria for assessing protein metabolism, including in the reproductive organs. Deviations of the Fisher coefficient can serve as indicators of the sanogenicity of metabolic processes in physiological fluids, including semen.

The analysis of the data obtained by us showed that the characteristic values of the Fisher coefficient in the blood serum of roosters are 3.57±0.24, while in the seminal plasma they are significantly less (1.25±0.02).

Next, we identified the proportion of the content of the final products of nitrogen metabolism (urea and ammonia), as an indicator of the intensity of metabolic processes in the blood, seminal plasma and spermatozoa of the rooster.

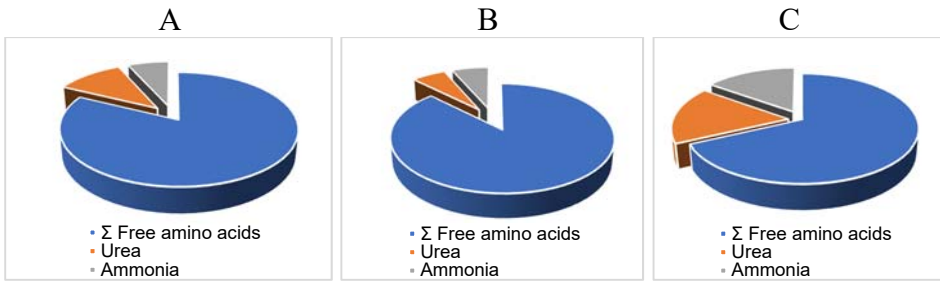

Figure 3. Comparative content of the amount of free amino acids and final products of nitrogen metabolism in the blood (A), seminal plasma (B) and spermatozoa (C) of roosters

The total share of the final products of nitrogen metabolism in roosters in blood serum, seminal plasma and spermatozoa was 17.76%, 12.47% and 32.10%, respectively. This probably indicates more intense metabolic processes in spermatozoa.

CONCLUSIONS

Thus, the fund of free amino acids has specific quantitative characteristics in blood serum, seminal plasma and spermatozoa.

The additional introduction of arginine and taurine into the diet can improve fertility in mature males of farm animals.

A spermogram supplemented with indicators of nitrogen metabolism in semen can be used to identify possible pathologies of sperm production in animals, which will increase its diagnostic value.

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