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Free Amino Acid Concentrations In Blood Of Lactating Ewes Of The Second Generation Hybrids Of The Romanov Sheep With Argali.

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ABSTRACT

The concentrations of free amino acids (F-AA) in the blood serum of lactating ewes (of the second generation hybrids of the Romanov sheep with Argali) on the 7th and 20th days of the animal lactation were determined in comparison with purebred sheep. On the day 7 of lactation the combined amounts of the following F-AA for purebred Romanov sheep were higher as compared to the hybrids of the second generation: a) essential amino acids (EAA) – by 15.4%, b) branched chain F-AA (BCAA) – by 16.1%, c) aromatic amino acids (AROM) – by 20.0%. On the day 20 of lactation, the combined amounts of the following F-AA for purebred Romanov sheep were higher as compared to the hybrids of the second generation: a) essential amino acids (EAA) – by 21.2%, b) branched chain F-AA (BCAA) – by 15.6%, c) aromatic amino acids (AROM) – by 40.3%. The first two parameters (EAA and BCAA) decreased with the increasing lactation time both (for purebred Romanov sheep and the hybrids of the second generation) almost in the same manner. It is important to highlight that the content of the aromatic amino acids (AROM) for purebred Romanov sheep changed slightly (in the error range) with the increasing lactation time, whereas the AROM content in the case of the hybrids of the second generation decreased drastically (in 2 time as compared the data on the 7th and 20th lactation day). The established differences are a consequence of changes in the intensity of the metabolic processes at an early stage of animal lactation and reflect the effect of interspecies hybridization of Romanov sheep with Argali.

Keywords: free amino acids, interspecific hybrids, argali, Romanov sheep breed, lactating ewes

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INTRODUCTION

Interspecific hybridization of domestic sheep (*Ovis aries*) with wild relatives, in particular with Argali (*Ovis ammon*), is a promising direction of agricultural science and practice [1]. Involving genetic resources of wild fauna in the selection process is one of the approaches to increase the productivity of livestock, nutrient diversity and product quality [1], as well as to understand of the molecular mechanisms of the animal metabolism [2]. Hybridization of closely related species makes it possible to enrich the gene pool of breeds of domestic sheep, and also can be an effective method of reconstruction and restoration of endangered fauna [1-3].

The study of physiological and biochemical features of the interspecies hybrids of Romanov sheep and Argali is given great attention during last decades. Biochemical indices of the blood of growing youngs [2] and ewes during the synchronization of estrus were studied [4].

The biological parameters of the digestive and metabolic processes of hybrid animals are considered in detail in a few recent publications [5-6]. The features of the fatty acid composition of meat [7] and the amino acid composition of milk [8] of interspecific hybrids have been studied. The biochemical and physiological characteristics of wild Argali blood have also been studied [9,10] including in comparison with domestic sheep, as well as in comparison with interspecific hybrids of sheep and first-generation Argali [9]. Our recent work has been devoted to the genetic aspects of interspecies hybrids of Romanov sheep and Argali (of the second generation) [5-6].

Amino acids are essential precursors for the synthesis of a wide array of nitrogenous substances with enormous biological importance [2]. Some of these bioactive molecules include neurotransmitters (e.g. g-aminobutyrate, dopamine, and serotonin), hormones (e.g. epinephrine, norepinephrine, triiodothyronine, and thyroxine), vasodilators, signalinggases (NO, CO, and H_2S), antioxidants(glutathione, creatine, melatonin, melanin, and taurine), methyl-group donors, as well as key regulators of general metabolism, immune response and health. Metabolism of amino acid is altered under various physiological and pathological conditions, leading to changes in whole-body homeostasis [11].

The content of free amino acids in the blood plasma of young sheep depends on the diet [12] and changes in the process of growth and development of the organism [12-13]. The content of free amino acids in blood plasma in adult sheep can vary significantly during the year seasons [14].

The content of free amino acids of sheep blood is related to the level of protein nutrition: plasma amino acids concentrations reflected dietary nitrogen content [15-16], i.e. reflected the effectiveness of amine nitrogen use by the symbiotic rumen microflora [16]. An increase in protein content in the diet causes an increase in the number of free amino acids of sheep blood plasma [15].

Plasma Amino Acids concentrations were depressed by the low protein diet, except for glycine, which was elevated, and threonine and alanine, which were not affected. Lysine is the most limiting amino acid, based upon plasma amino acids concentrations on the low protein diet compared to average plasma amino acids levels for all diets. Plasma Amino Acids levels were not involved in the regulationof voluntary intake when the diet contained sufficient protein to meet the requirements of the animal [17].

Free AA pool in blood is the major precursor for milk protein synthesis: plasma AA concentrations are valid measures of the immediate free amino acid pool available for uptake by the mammary gland [18].

Therefore, the differences in the content of free blood amino acids for sheep with diverse genotypes (under identical conditions of animal keeping and the same level of protein nutrition) may reflect genetically determined features of protein metabolism and amino acid sequences associated with both digestibility and assimilation of diet protein, as well as with the intensity of the anabolic processes (in which amino acids that form the amino acid pool of blood are involved).

This research is devoted to determining the concentration of free amino acids in the blood serum of the second generation hybrids of the Romanov sheep with Argali at the early stage of their lactation.

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MATERIALS AND METHODS

Serum blood samples were collected from jugular vein before feeding from 10 healthy ewes on 7th and 20th days of lactation: group 1) purebred Romanov sheep (n=5), group 2) second generation (n=5) interspecific hybrids of Argali and Romanov sheep. Animals of all groups were kept at the same conditions and had the same diet (tab. 1).

Table 1. Diet parameters

Feed	Daily intake, kg
Hay	1.0
Senage	2.0
Mixed feed	0.8
Chemical parameters of diet	
Dry matter, %	73.2
Energy, MJ	14.4
Crude protein, g	43.8
Crude fat, g	15.6
Crude fiber, g	79.4
Phosphorus, g	1.47
Sulfur, g	3.19

Serum blood samples were deproteinized by 6% sulfosalicylic acid, centrifuged at 13000 rpm and deluted by lithium-citrate sample delution buffer (Sevko&Co, Russia). Amino acid analyze was performed by ion-exchange chromatography with the post-column ninhydrine derivatisation on HPLC system LC-20 Prominence (Shimadzu, Japan) with ion-exchange column 4,6 x 150 mm (Sevko&Co, Russia) for separation of amino acids, automatic reaction module ARM-1000 (Sevko&Co, Russia) for post-column derivatisation and buffer solutions (Sevko&Co, Russia) for elution. Physiology amino acids standard solution (Sykam, Germany) was used for quality control. Results presented in the form of the mean data (± standard error). U-test was used for statistical analyses.

RESULTS AND DISCUSSION

The concentrations of the major free amino acids (F-AA) in the serum of lactating ewes were determined: threonine, serine, glycine, the sum of alanine and citrulline, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, the sum of the histidine-based F-AA (histidine, 1-methylhistidine and 3-methylhistidine), lysine, arginine, proline, taurine. A comparative analysis of the total content of the major essential amino acids (EAA: threonine, valine, isoleucine, leucine, methionine, phenylalanine, lysine, histidine, tyrosine, tryptophan), branched chain amino acids (BCAA: leucine, isoleucine, valine) and aromatic amino acids (AROM: phenylalanine , tyrosine, tryptophan) for purebred Romanov sheep and the hybrids of the second generation (Table 2) was performed for the first time.

Table 2. The total content of the major essential amino acids (EAA), branched chain amino acids (BCAA) and aromatic amino acids (AROM) for purebred Romanov sheep and the hybrids of the second generation, nmol/ml.

F-AA/ Group	7 day of lactation			20 day of lactation		
	Rom	F2	p-level	Rom	F2	p-level
EAA	706.1±56.5	611.9±43.9		628.6±80.0	518.5±17.8	
BCAA	351.2±41.5	302.4±32.21		304.6±55.3	263.5±11.6	
AROM	167.9±7.65	139.9±10.7		165.6±11.5	118.0±3.94	≤0.05

↓- significantly dynamic from 7 to 20 day (≤0.05)

It is interesting that on the day 7 of lactation the combined amounts of the following F-AA for purebred Romanov sheep were higher as compared to the hybrids of the second generation: a) essential amino acids (EAA) – by 15.4%, b) branched chain F-AA (BCAA) – by 16.1%, c) aromatic amino acids (AROM) – by

20.0% (Table 2). On the day 20 of lactation, the combined amounts of the following F-AA for purebred Romanov sheep were higher as compared to the hybrids of the second generation: a) essential amino acids (EAA) – by 21.2%, b) branched chain F-AA (BCAA) – by 15.6%, c) aromatic amino acids (AROM) – by 40.3% (Table 2). The first two parameters (EAA and BCAA) decreased with the increasing lactation time both (for purebred Romanov sheep and the hybrids of the second generation) almost in the same manner (Table 2). It is important to highlight that the content of the aromatic amino acids (AROM) for purebred Romanov sheep changed slightly (in the error range) with the increasing lactation time (Table 2), whereas the AROM content in the case of the hybrids of the second generation decreased drastically (in 2 time as compared the data on the 7th and 20th lactation day). The established differences (Table 2) are a consequence of changes in the intensity of the metabolic processes at an early stage of animal lactation and reflect the effect of interspecies hybridization of Romanov sheep with Argali.

It was even more interesting to compare the contents of individual amino acids for purebred Romanov sheep and the hybrids of the second generation with Argali (Table 3).

Table 3. Concentrations of some individual amino acids for purebred Romanov sheep and the hybrids of the second generation with Argali, nmol/ml.

AA/ Group	7 day of lactation			20 day of lactation		
	Rom	F2	p-level	Rom	F2	p-level
TAU	78.6±7.21	136.8±18.7	≤0.05	81.1±12.8	84.5±5.06↓	
THR	59.6±9.90	49.1±8.94		57.2±5.30	29.9±7.36	≤0.05
SER	62,7±4.55	87.4±11.8		81.4±12.1	106.3±7.48	
GLY	529.5±30.9	787.6±122.6		586±106.6	764.9±52.1	
ALA+ CIT	151.1±18.7	147.0±12.7		93.5±34.2	169.7±4.01	≤0.05
VAL	161±23.1	130.8±15.2		145.1±32.2	119.4±6.87	
MET	9.01±1.84	10.4±1.82		10.6±1.74	7.70±0.80	
ILEU	101.9±12.7	78.9±9.51		80.8±9.72	64.5±5.44	

↓- significantly dynamic from 7 to 20 day (≤0.05)

In contrast, the concentrations of threonine, isoleucine or valine were higher both (on 7 and 20 days) for purebred Romanov sheep as compared to the hybrids of the second generation with Argali (Table 3). The total concentration of alanine and citrulin was higher for hybrid animals (as compared to the purebred Romanov sheep) only at 20th lactation day, whereas it was almost the same at 20th lactation day (Table 3).

The taurine concentration was higher in the serum of hybrids of the second generation than in the purebred ewes (especially on 7th lactation day). Taurine is presented in all animal tissues as one of the major free amino acids that can be synthesized in adequate amounts during the oxidation of cysteine. Taurine plays an important role in bile acids metabolism. It is known that the primary bile acids are conjugated either with taurine or glycine. In sheep, taurine conjugates of bile acids are predominating. Some of the taurine excreted in the bile is returned to the liver in the enterohepatic circulation [19]. There is no doubt that the established differences in the content and dynamics of changes in the content of taurine are a consequence of the influence of interspecific hybridization with argali on taurine metabolism. Perhaps such differences appeared to be due to the higher intensity of the restoration of taurine conjugates of bile acids in the liver and the release of free taurine. In addition, this may be due to differences in the rate of taurine synthesis or the availability of free cysteine for synthesis. The availability of cysteine, in turn, can be related to the intensity of the synthesis of milk proteins in the body of lactating ewes. We were unable to track the relationship between free cysteine and taurine in the blood serum, since free cysteine was not determined in this study.

These and further (Table 4) differences in the content of individual amino acids were established for the first time.

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Table 4. Concentrations of some individual amino acids for purebred Romanov sheep and the hybrids of the second generation with Argali, nmol/ml.

AA/ Group	7 day of lactation			20 day of lactation		
	Rom	F2	p-level	Rom	F2	p-level
LEU	88.3±7.81	93.4±9.97		78.8±14.8	79.6±0.65	
TYR	30.4±1.76	29.3±3.19		42.9±5.69	32.4±3.05	
PHE	45.9±1.81	39.3±3.18		45.1±1.47	31.5±1.72	≤0.05
HIS+ MetHIS	121.8±13.0	113.6±5.28		104.1±17.1	106.3±3.99	
TRP	91.6±4.65	71.3±7.83	≤0.05	77.6±9.02	54.0±5.84	
LYS	27.0±3.35	27.9±2.50		29.3±3.25	25.6±1.83	
ARG	151.9±13.9	138.3±1.75		125.1±12.7	116.4±8.81	
PRO	87.1±12.5	108.2±12.2		126.3±16.1	98.8±9.23	

↓- significantly dynamic from 7 to 20 day (≤0.05)

In contrast, the concentrations of arginine, phenylalanine and tryptophan were higher both (on 7 and 20 days) for purebred Romanov sheep as compared to the hybrids of the second generation with Argali (Table 4). The proline concentration was higher for pure-breed ewes on the 7th day of lactation, but for second-generation hybrids was higher on the 20th day of lactation (Table 4).

There was a small decrease in lysine concentration for second-generation hybrids only on the 20th day of lactation, whereas the lysine content was almost the same for all animals on the 7th day of lactation as well as for pure-breed ewes on the 20th day of lactation (Table 4).

The study of the features of protein metabolism in interspecific hybrids of sheep and argali has a certain scientific practical interest. For example, it is shown that in hybrid animals, the increase in energy and growth rate in comparison with pure analogs is accompanied by a decrease in the total protein content in blood and certain fractions, with an increase in the albumin-globulin ratio [2]. This may be due to the intensification of protein metabolism, in particular anabolic processes, which ensure rapid growth of animals. The hybrid rams recorded an increase in the intensity of enzymatic processes in the rumen, an increase in the mass of the symbiotic microflora and, as a consequence, an increase in the nitrogen utilization factor [5-6].

Apparently, the difference in the content of free essential amino acids in the blood serum is related to the characteristics of the organism of the animals being studied. Plasma is generally regarded as the blood compartment from which amino acids are extracted by the mammary gland for protein synthesis [18]. Most likely, the established features can be expressed in a change in the intensity of the synthesis of milk protein in the body of hybrid animals, compensation for the loss of amino acids spent on the synthesis of milk protein. The study of the amino acid composition of the milk of interspecific hybrids of the second generation in comparison with purebred sheep showed significant differences in the content of essential amino acids in milk proteins: hybrid animals had a significantly increased content of individual essential amino acids (in particular, methionine, isoleucine and lysine) on the 1 and 3 days of lactation, while at 7 and then from 7 to 20 day, the concentration of amino acids decreased to the level of purebred animals [8]. Perhaps the established differences in the content of essential amino acids - the decrease in the concentrations of individual amino acids - is a consequence of the decompensation of a high content of essential amino acids in milk proteins of the first days of lactation, most likely due to the predominance of individual protein fractions of milk protein in hybrid animals compared to purebreds or an increase in the content of free amino acids milk. This may also indicate the absence of differences in the content of essential amino acids and branched-chain amino acids on the 20th day of lactation. It is believed that many essential amino acids, for example branched chain amino acids, tryptophan and others are functional amino acids and regulate some physiological processes in the animal body [11,19]. Recent years have witnessed the discovery that amino acids (AA) are not only cell signaling molecules but are also regulators of gene expression and the protein phosphorylation cascade [19]. Perhaps the intensity of processes of decompensation of amino acids spent on the synthesis of milk proteins from the amino acid pool of blood can also be linked by the principle of feedback with the physiological role of certain essential amino acids in the animal body.

Pregnancy and lactation are factors in the change in physiological status, accompanied by changes in metabolism in animals [21]. The metabolism of nitrogenous compounds in animals is largely related to the processes of anabolism and catabolism of amino acids and proteins. In the healthy animal, an equilibrium is established between intake and synthesis of amino acids, on the one hand, and breakdown and excretion of excess nitrogenous material, in the form of urea, on the other. Excessive loss of nitro-genous material can occur in illness because of cellular breakdown, lactation with production of milk protein, and in urinary or gut losses. During growth, pregnancy, and recovery from disease, there is a positive nitrogen balance as amino acids and other nitrogenous compounds are supplied to meet the body's requirements [19].

Based on the results of the study, it is obvious that interspecific hybridization with argali significantly affects the metabolism of protein and amino acids in the organism of lactating ewes. The profiles of free amino acids in the blood serum of hybrid animals of both the second and third generation had significant differences both in amino acid content and in the dynamics of amino acid concentration changes in the amino acid pool of blood compared to purebred ewes. Apparently, the established differences are a consequence of changes in the intensity of the metabolic processes accompanying the synthesis of milk proteins at an early stage of lactation and are a consequence of the introduction of argali into the selection process.

CONCLUSION

In the study, the concentrations of free amino acids in the blood serum of lactating ewes of interspecific hybrids of Romanov sheep and Argali (second generation) were determined at an early stage of animal lactation. On the 7th day of lactation, the differences were recorded by the concentration of essential amino acids and branched chain amino acids, taurine, isoleucine, leucine, phenylalanine, tryptophan and arginine ($p \leq 0.05$). On the 20th day of lactation, the differences were established for the content of aromatic amino acids, threonine, glycine, the sum of alanine and citruline, isoleucine, phenylalanine, tryptophan and proline ($p \leq 0.05$). The dynamics of changes in the content of amino acids from 7 to 20 days of lactation was established. A decrease in the concentration of aromatic amino acids, taurine, the amount of alanine and citrulline, phenylalanine, lysine in hybrids of the third generation ($p \leq 0.05$), taurine in hybrids of the second generation ($p \leq 0.05$) has been established. Apparently, The established differences are a consequence of changes in the intensity of the metabolic processes at an early stage of animal lactation and reflect the effect of interspecies hybridization of Romanov sheep with Argali.

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