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# Amino-Acid Composition Of A Cow Milk From The Farms That Are Adjacent To Zones Of A Falling Of Rocket Carrier "Proton-M".

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**ABSTRACT**

Article shows the results of amino-acid composition of protein in cow's milk which were selected from farms adjoining to the territory of a falling of the RC "Proton-M" of the Ulytau area, Karaganda region of the Republic of Kazakhstan. The identification of amino acids has been carried out on a liquid chromatography SHIMADZU LC-20 Prominence (Japan) with a fluorometric and a spectrophotometric detector. There were also applied the chemical-analytical studies on the liquid chromatograph "Zvet Yauza" with the amperometric detection. According to the research it was established that there were reliable differences comparing with the normal vales in the content of all irreplaceable and replaceable amino acids in tests of a milk protein. The sum of amino acids of milk protein was 12,2% lower in comparison with the normal values. The limiting amino acids in the studied milk were methionine+ cystine and phenylalanine + tyrosine.

**Keywords:** amino acids, cow's milk, carrier rocket "Proton-M"

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## INTRODUCTION

In connection with a difficult ecological situation, environmental pollution, there is an actual problem of quality of raw materials and food in a case of the contamination of foreign substances [1]. Agricultural production, espessially milk, is considered as one of the main sources of supply of the population with the food. Due to this, the products of an animal origin can be the main suppliers of heavy metals, radioactive nuclides and other foreign substances in a human body [2].

The actuality of an environmental problem is closely connected with a difficult system of the intake of toxicants in the human body: the soil – a plant (a forage, a diet) – an animal – a livestock product – the person. The milk received from animals in territories of unsuccessful zones has the increased acidity due to formation of sour caseinate, therefore it has the low resistance to heating [3]. Considering various relationship of toxic elements with milk components, we can say that that the degree of their transition to products of processing correlates with the amount of solids of milk and with the concentration of separate components and first of all with a protein fraction [4,6].

We didn't find the corresponding researches in a domestic and in a foreign literature on the influence of rocket 1,1- dimethylhydrazine fuel on the quality of milk. However, according to the researches and some sourses of a scientifical literature, 71 country farms of the Ulytau area are in the adjacent territory with areas of falling of CR "Proton-M" , where a large number of dairy cattle is grazed. We determined the existing farms with the essential deviations according to the organoleptic and physical and chemical indicators: wintering "Togyzbay", "Zharyk", "Almenbet", "Bayten" ,"Orynbay - ata", "Karsakbay".

1,1 dimethylhydrazine possesses the teratogenic, mutagenic, cancerogenic and other actions. In nature it cumulates in the soil, vegetation, live organisms, in any other subjects, and in the depth of soil it can remain for years [7]. From the official sources, it is known that areas of falling of the separating particles of the Proton-M rocket carrier are in the Ulytau area of the Karaganda region where there were more than 300 fallings to the present time [8].

It is established that the hydrazine and its derivatives are extremely toxic for animals and for a human, and it can cause an intoxication with a defeat of a central nervous system, a liver, blood, immune and endocrine systems, its mutagenic and cancerogenic properties are established. The hydrazine and its derivatives make a negative impact on reproductive function. There is a data about their teratogenic and embryotoxic action on the experimental animals [5].

A direct dependence of composition of milk and the forages received in a zone of ecological trouble was proved by K.Verman, A.M. Kolodkin, etc., and the content of some chemical elements in cow's milk from these zones is in tens times more, than in milk of cows of other zones [9].

According to our preliminary researches it was established that the samples of the milk which are selected from the territories adjacent to areas of falling of CR "Proton-M" had deviations according to the organoleptic and physical and chemical indicators that tell us about the influence of a different factors, or pathologies [10].

In this regard, we decided to study the full value of protein of milk of the above mentioned territories in detail. Proteins are the most valuable component of a product predetermining its food full value. Criterion of an assessment of a nutrition value of products is a definition of the amount of amino acids in a qualitative ratio. It is known that protein of natural milk is practically contains all replaceable amino acids which is acquired by a human body as well as the protein of eggs, for 96%.

## MATERIAL AND METHODS

The material for a research was presented by 18 milk samples from 6 farms adjacent to the territories of the area of falling of CR "Proton-M" of the Ulytau area of Karaganda region of the Republic of Kazakhstan. The research was conducted in the analytical laboratory "Garysh-Ecology" of Almaty and Zhezkazgan, in laboratory of veterinary-sanitary examination of "The Kazakh Agrotechnical University named after S.

Seyfullin", experimental regional laboratory of the engineering profile "Scientific Center of Radio Ecological Researches" of GU of Shakarim, Semey city.

The assessment of a food value of protein of milk was made according to a total amount of amino acids, to the amount of separate replaceable and irreplaceable acids, to the relation of the sum of the irreplaceable amino acids to the replaceable, and to a method of the amino-acid score.

**Determination of amino acids in products of an animal origin.**

Determination of amino acids was carried out on the liquid SHIMADZU LC-20 Prominence chromatograph, (Japan) with the fluorometric and spectrophotometric detector. The chromatographic column with the size of 25cm*4,6mm SUPELCO C18, 5mkm (USA) with a precolumn was used for the protection of the main column from impurity. The chromatographic analysis was carried out in the gradient mode at a consumption of eluent of 1,2ml/min and a temperature of the thermostat of a column $40^0$C. Measurement was carried out by method of a highly effective liquid chromatography on a column with the turned phase with spectrophotometric and fluorometric detectors on lengths of waves of 246 nm and 260 nm with use of acid hydrolysis and a modification of amino acids with a solution of a fenilizotiotsionat (FITS) in isopropyl alcohol with receiving of feniltiogidantoins. The mixture of 6.0mm sodium acetate solution with pH 5.5 (component A), 1% solution of isopropyl alcohol in an acetonitrile (component B) and 6.0 mm of sodium acetate solution with pH 4.05 (component C) were used as a mobile phase. The standard samples of amino acids of SigmaAldrich production, acetonitrile of a high purity, isopropyl alcohol of a high purity for a liquid chromatography, FITS of SigmaAldrich, phosphoric acid and sodium acetate were used. The sample preparation: for carrying out of hydrolysis, 100 mg of sample was placed into glass ampoules with the delayed end. Further we added 10 ml. 6M of a solution of hydrochloric acid. A mixture was mixed carefully and blown by the current of nitrogen within 2 min. Glass ampoules were soldered and placed into the thermostat. Hydrolysis was carried out at a temperature of $110^0$C during 24 h. After cooling, the hydrolyzates were filtered through the membrane filters with the diameter of a pore of 0,45mkm, and were selected aliquots of 0,5ml. Aliquots were dried up at $65^0$C in an air current. A solution of NaOH (0,10 ml.) 0,15M was added to the dried-up aliquots and was carefully mixed.

Then 0,35ml of a solution of a fenilizotiotsionat in isopropyl alcohol was flowed, and 0,05ml of a distilled water was mixed and added, dried up on a water bath at t-$60^0$C, and filtered through the membrane filter with a diameter of pore 0,45mkm. The resulting solutions were subjected to chromatographic analysis. Concentration of amino acids in samples was calculated in mg on 100gr.of the product.

The measurement of mass concentration of 1,1 dimethylhydrazine in the biological environment (milk) was carried out by method of an ionic chromatography with amperometric detecting. The technique is based on the preliminary separation of proteins and fats of milk from the sour environment, alkalifying of a filtrate, distillation of about the 1,1-dimethylhydrazinene into the solution of acid and the subsequent analysis of stripping by the method of the ionic chromatography with amperometric detecting. The area of peak of the asymmetric dimethylhydrazine is proportional to its concentration. The coefficient of proportionality is established at graduation of the chromatograph. Equipments: liquid chromatograph, ionic analytical small-sized “Zvet Yauza” (MEQU. 414538.001 PS) with the electrochemical detector completed with the PCIBMAT personal computer and the corresponding software.

**RESULTS&DISCUSSION**

It is established that the low level of all irreplaceable and replaceable amino acids is observed in protein of milk. So, in a protein of the studied milk there were reliable differences according to the content of all irreplaceable amino acids in comparison with a normal values and the fluctuations were from 4,7% to 26,5%.(Fig.1,2)

The results received during the calculation are presented in table 1 and figure 1,2. Apparently from table 1, irreplaceable amino acids: valine– 182,1±1,05 mg/100g. (4,7% less than the norm), a leucine - 266,3±0,79 mg/100g. (5,9% less than the norm), an isoleucine - 178,6±1,06mg/100g (5,5% less than the norm), lysin -248±0,83 mg/100g (5% less than the norm), methionine-77,1±0,7mg/100g. (7% less than the norm),

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threonine- 147,5±0,11 mg/100g (5% less than the norm), tryptophane - 40,6±0,74mg/100g (18,8% less than the norm), phenylalanine-164±1,04 mg/100g. ( 6,3% less than the norm).

Replaceable amino acids: alanine -89,3±0,74 mg/100g. (8,9% less than the norm), arginine-112,6±0,98 mg/100g. (7,7% less than the norm), aspartic acid - 208,5±0,94 mg/100g. (4,8% less than the norm), a histidine - 81,5±1,25 mg/100g. (4,8% less than the norm), glycine - 37,6±0,94 mg/100g. (20% less than the norm), glutamine acid - 496,5±0,94 mg/100g. (4,8% less than the norm), proline-269,5±0,98mg/100g. (3,1% less than the norm), tyrosine-172,6±1,1 mg/100g. (is 6% less than the norm), cystine - 19,1±0,78 mg/100g. (26,5% less than the norm), serine-176,1±0,97 mg/100g (5,4% less than the norm).

**Table 1: The amino-acid composition of protein of milk in mg / 100g.**

| № | Amino-acid composition of protein of milk | M±м | Normal values |
|---|---|---|---|
| | ***Irreplaceable amino acids*** | | |
| 1 | Valine | 182,1±1,05 | 191 |
| 2 | Isoleucine | 178,6±1,06 | 189 |
| 3 | Leucine | 266,3±0,79 | 283 |
| 4 | Lysine | 248±0,83 | 261 |
| 5 | Methionine | 77,1±0,7 | 83 |
| 6 | Threonine | 147,5±0,11 | 153 |
| 7 | Tryptophan | 40,6±0,74 | 50 |
| 8 | Phenylalanine | 164±1,04 | 175 |
| | **The sum** | **1,304,2** | **1,385** |
| | ***Replaceable amino acids*** | | |
| 1 | Alanine | 89,3±0,74 | 98 |
| 2 | Arginine | 112,6±0,98 | 122 |
| 3 | Aspartic acid | 208,5±0,94 | 219 |
| 4 | Histidine | 81,5±1,25 | 90 |
| 5 | Glycine | 37,6±0,94 | 47 |
| 6 | Glutamic acid | 496,5±0,94 | 509 |
| 7 | Proline | 269,5±0,98 | 278 |
| 8 | Tyrosine | 172,6±1,1 | 184 |
| 9 | Cystine | 19,1±0,78 | 26 |
| 10 | Serine | 176,1±0,97 | 186 |
| | **The sum** | **1,457,65** | **1759** |

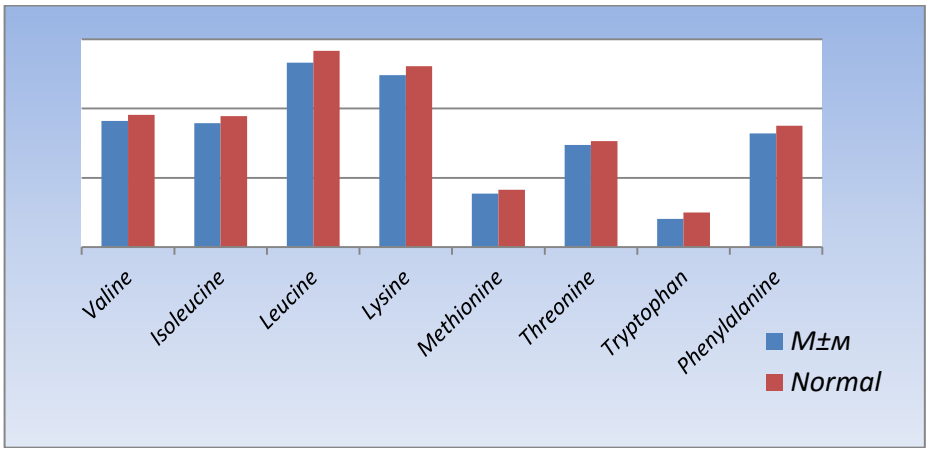


**Figure 1: A content of the irreplaceable amino acids of protein of milk**

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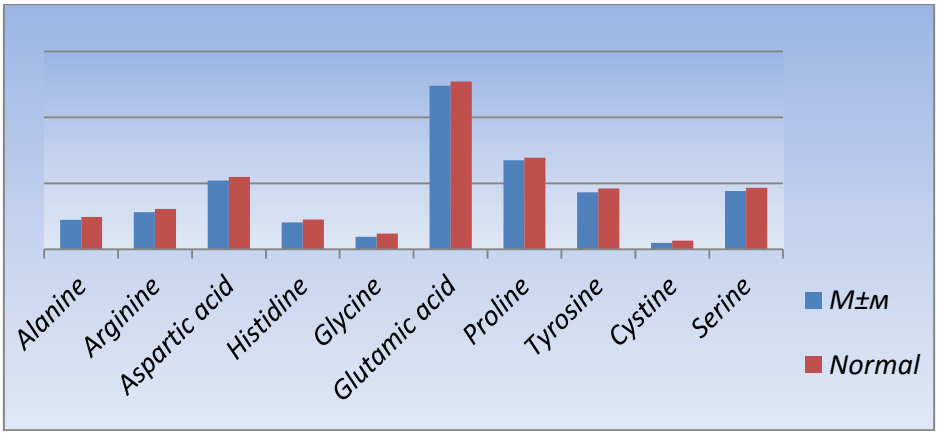


**Figure 2: A content of the replaceable amino acids of protein of milk**

The ratio of the sum of irreplaceable amino-acids to the replaceable was bigger in the samples of milk which were selected by us and consisted of 0,89 (norm 0,78).

The analysis of the obtained data shows that the studied samples differ from the normal values according to the sum of amino acids - 2,761,85 mg/100g, i.e. in 12,2% were respectively lower.

Results of an assessment of a nutrition value of protein of milk by the comparison with the standard scale of amino acids recommended by the joint committee FAO and WHO, (a method of the amino-acid score, i.e. an index) are presented in table 2 and figure 3.

**Table 2: Amino-acid score of a cow milk,%**

| ***Irreplaceable amino acids*** | Valine | Isoleucine | Leucine | Lysine | Methionine + Cysteine | Threonine | Tryptophan | Phenylalanine + tyrosine |
|---|---|---|---|---|---|---|---|---|
| Index | 113,8 | 139,5 | 118 | 141 | 68 | 115,25 | 126,8 | 85,4 |

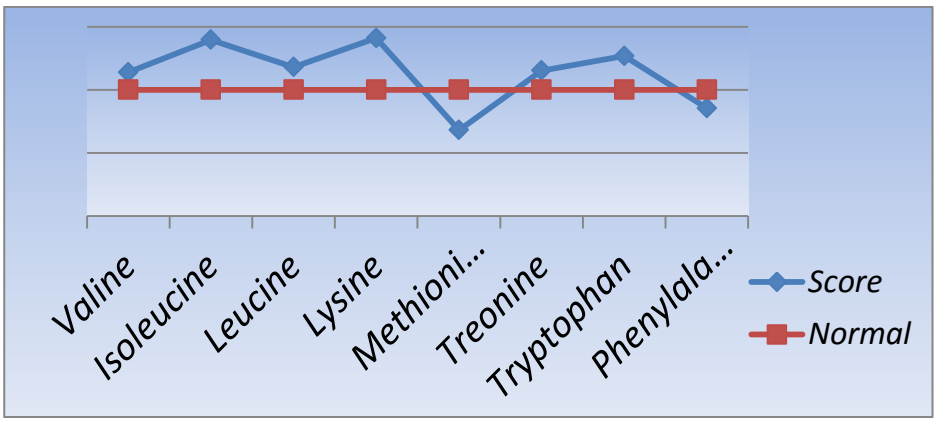


**Figure 3: The amino-acid score of a cow milk**

From the above table we can see that the limiting amino acids in milk proteins are methionine (68%) and phenylalanine (85,4%). This protein is defective.

## CONCLUSION

Thus, in the studied protein of milk, the sum of amino acids was on 12,2% lower in a comparison with the norm. Such decrease was de to the lowering of the level of all irreplaceable and replaceable amino acids.

Lowering of the level of many amino acids in milk can be caused by violation of protein formation ability of a liver, i.e. a decrease of the level of these amino acids in blood reflected for the content of a milk in a following.

The special attention should be payed for the deviation in the index of a nutrition value of a protein of milk – a metionin + cysteine (score is 68%) and phenylalanine (score is 85,4%). It is known that up to 80–89% of methionine can be transformed to cysteine , and 70–75% of phenylalanine — into tyrosine [11]. For this reason the level of the irreplaceable amino acids of methionine and phenylalanine should be more higher, because a substantial part of them is consumed for the formation of cysteine and tyrosine. Amino acids - cysteine and tyrosine according to their physiological value are close to the irreplaceable amino acids. Thus, the intake of cysteine and a tyrosine with food allows to reduce the need for irreplaceable amino acids such as methionine and phenylalanine. Also, these amino acids unlike others have a weak polarity due to the water repellency and they are poorly soluble in water.

Phenylalanine and tyrosine are aromatic amino acids, their role in an organism is connected with a function of a suprarenal glands and thyroid gland, they participate in a formation of a thyroxine– the main hormone of a thyroid gland. This hormone regulates a metabolic rate, for example, accelerates "burning" of the nutrients which are exist in abundance.

Methionine and cysteine are a sulfur-containing amino acids, their role in an organism is connected with detoxicated properties. Sulfuric acid is a product of the fermentative transformations of sulfur-containing amino acids, connecting in a liver with toxic products of an exchange form of the pair essential sulfuric acids that are bringing toxins out of an organism [12].

With a lack of methionine in food,the expressed changes from a myocardium, the aorta, coronary vessels which are followed by accumulation of the lipoid substances is observed. At first, the dystrophic changes in a liver are observed which are characterized by the excess deposition of fat in a parenchyma of this organ, the concentration of RNA sharply decreases, the quantity of a glycogen in hepatocytes decreases. According to the scientist L.A. Cherkes, a long methyl insufficiency leads to the development of cirrhosis [12]. Researches of the last years specify that metabolic imbalance of amino acids, especially sulfur-containing, noticeably affects on health of the person [13, 14]. Thus, the metabolic imbalance of methionine leads to serious hereditary illnesses that is connected with its influence on epigenetic regulation of an expression of many genes, including, in mitochondrions [15]. And if earlier a metabolic imbalance of methionine was considered only as the reason of multifactorial defects of central nervous system, now it is possible to speak about its systemic influence on health and about a significant role of methylation in maintenance of health of a person.

Sulfur comes to an organism with a food, as a part of inorganic and organic compounds, and mainly - as a part of sulfur-containing amino acids: irreplaceable amino acid of methionine, and also cysteine and cystine which are actively participate in metabolic processes of an organism. So, the closest correlation of a condition of various links of immunity is revealed with sulfur-containing amino acids. Not only a cellular and a humoral immunity, but also an activation of lymphocytosis are connected with the level of their contents. It follows, that the influence of this functional group of amino acids on a condition of the immune answer, especially because of the strong antioxidant action of these amino acids, participate in synthesis of nucleic acids, collagen and other proteins [16].

In the studied milk samples the residual amounts of 1,1 dimethylhydrazine were not detected, apparently significant changes in milk samples of the organoleptic, physical and chemical parameters occurred due to the influence of transformation products of UDMH.

According to the data of a scientific literature, transformation products of 1,1 dimethylhydrazine totally have more than 50 chemical compounds. Unfortunately, there are no techniques for identification of products of its transformation in milk. Among the most ecologically dangerous products of transformation of UDMH in the selected samples were compounds like N-nitrosodimethylamine, dimethyl hydrazone, formaldehyde, tetrametiltetrazen , 1-formil-2,2-dimethylhydrazine (FDMG) and 1-metil-1H-1,2,4-(MTA) triazole.

Experiments proved that the revealed moderate dysproteinemia can be regarded as an initial phase of violation of a protein metabolism under the influence of N-nitrosodimethylamine in experimental animals. The action of the derivatives of 1,1 dimethylhydrazines on various fermental systems isn't specific. They cause the increase or oppression of activity of many enzymes. So, according to the results of biochemical blood tests, it is possible to conclude that in severe intoxication by N-nitrosodimethylamine, generally there are violations for the liver that lead to the increase of activity of aminotransferases, thymol test and bilirubin in kidneys. That is also confirmed by hypoproteinemia and by decreasing of creatinine. A moderate increase of glucose and decrease of the activity of α-amylase in severe intoxication by N-nitrosodimethylamine, perhaps, are also connected with dysfunction of a liver [18]. The maintenance of the growing animals on a semi-synthetic diet with a deficiency of protein and excess of fats within 30 days after a single intake of UDMH led to the development of nonspecific reactive hepatitis of portal type with inflammatory and destructive processes mainly in the portal tract [19].

**ABBREVIATIONS**

CR-carrier-rocket, NDMA- N-nitrosodimethylamine, RGP „Research Center" - the national state enterprise,the research center, of a special purity.

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We wish you a good health and prosperity !!!

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