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Characteristics Of Protein Components Of European Holstein Cow Milk.

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ABSTRACT

The value of the milk of the European Holstein cows kept under the conditions of the modern dairy complex with the loose systems in the adaptation period has been reported taking into consideration the comprehensive FAO/WHO requirements. A complete range and a rather high concentration of the amino acids in all the milk samples are revealed. Among the essential amino acids, the highest amounts of leucine (LEU), phenylalanine + tyrosine (PHE + TYR), and lysine (LYS) and the lowest amounts of tryptophan (TRP), methionine + cysteine (MET + CYS), and histidine (HIS) are contained in the proteins, despite the animal genotype. The lowest tryptophan (TRP) concentration is recorded in the Holland cow milk samples. With respect to the non-essential amino acids, the glutamic acid + glutamine (Glu+GLN) and arginine (Arg) were found in the high concentrations, while glycine (Gly) and alanine (Ala) were at the lowest concentrations. The total non-essential amino acid concentration in the milk samples (58.6-58.9 %) was significantly higher, than that for the essential amino acids (41.1-41.4 %). Therefore, the amino-acid index comprised only 0.69-071. The measurements of the biological value of the analyzed milk samples with the use of the DIAAS method (amino acid score of the essential amino acids with taking into consideration their absorption) can prove that the total digestible amino acid set number is higher than the number in the ideal protein for the entire breed and its genotypes. However, the amino acid score of certain amino acids varied in a rather wide range of 56 % for tryptophan (TRP) in the samples of the Holland cow milk to 190 % for phenylalanine + tyrosine (PHE + TYR) in the samples of the German cow milk, which can indicate their imbalance. The limiting amino acid in the analyzed milk was tryptophan, since its DIAAS indicator was in the range of 56 to 86 %. This is exactly tryptophan, which determines the level of the milk essential amino acids in the analyzed population. In addition, this indicator is lower in the milk samples taken from the Holland cows, while it is expressed at the highest level in the Hungarian cows. Therefore, the biological value of the Holstein cow milk proteins is caused by the animal genotype. However, the produced cow milk, which includes the complete amino acids number set present in high summarized concentration, is characterized by the low biological value, because of the tryptophan limiting amino acid, in case of keeping the high-performance animals fed with the imbalanced diets under the conditions of the modern dairy complex with the loose systems.

Keywords: European Holstein breed, amino acid, biological value, DIAAS

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INTRODUCTION

Proteins are the key essential nutrients having the basic components and structural elements represented by the amino acids. The biological activities of the other components can be exerted only in their presence. Twenty food amino acids are of great value for human nutrition. A part of them is not produced in the body; they are derived only from food. These amino acids are considered essential and vitally indispensable. The essential amino acids include tryptophan, lysine, methionine, phenylalanine, leucine, isoleucine, valine, and threonine; in addition, they are added with histidine in children. The biological value of a food product is measured with the concentration, the balance, and the level of the body absorption of the dietary protein essential amino acids [1-3].

Dairy is an important part of a balanced diet for the Russian people. The percentage of the dairy products in the components of the consumer basket (expressed in local currency) is in the range of 20 to 30 % by the regions in Russia [4]. In milk, there are all the amino acids required to form the body protein tissues. Unlike the other sources of animal and plant proteins, milk contains all the essential amino acids in the well-balanced ratio, which can be absorbed into the human body. The milk proteins have the highest biological value, yielding only to the egg whites in quality [1-3].

Therefore, the protein mass fraction is currently set up in the first place among the breeding traits such as milk quality. Despite the fact that this parameter has a strong heritage, an increase in the milk performance and production of the milk of a high biological activity can only be achieved with the properly balanced rations providing all the nutrients (including the amino acids) for cows. [8-11].

At present, the construction of the dairy complexes requires the high-tech cattle, which is usually completed with the imported Holstein cattle of the European selection. These animals having their own unique characteristics in various natural environments and under the different economic conditions can generally maintain their key properties, such as high milk production and good adaptation to the industrial technologies.

However, the high performance cows having the increased metabolic rates require the high quality dietary feeds and their components, which are not always provided in the livestock enterprises, because of the deficit in the moisture rich and raw feeds or, sometimes, the low quality forages. The carbohydrates including the starches of great dietary portions are poorly absorbed in the ruminants, which can finally result in decreasing both the milk yields and the milk quality. In addition, the process of adaptation of the imported animals is conjugated with the increased load on the organism [9-11].

Therefore, it is required to assess the quality of the produced milk and especially its protein component. The literature data on the amino acid variability depending on the various factors (the lactation period, the age, the breed properties, and the trophic level and adequacy) are scanty and poorly compared with each other, because of the different methods for the assessment and the expression of the amino-acid quantity.

The classical method to measure the biological value of milk is calculation of its amino acid score. The formula for the etalon protein and the methods for the food product and diet biological value measurements are periodically revised and improved by the FAO/WHO experts. At present, the method called DIAAS to measure the amino acid value of the food products and diets (as the base methods) with taking into consideration the biological accessibility of the certain essential amino acids is recommended; in addition, the improved formula for the etalon protein is proposed [12, 13].

Therefore, the measurement of the biological value of the milk of the European Holstein cows in the adaptation period is curently in the focus on improving the quality of its protein component.

MATERIALS AND METHODS

The surveys were conducted in the EcoNiva Agro Holding, LLC, Voronezh Region in May 2018. The dairy herd formed by the European Holstein animals in the agriculture enterprise is kept with the loose system of the modern dairy complex. The milk yield per cow in the herd, the fat mass fraction, and the protein mass fraction comprised 8700 kg, 3.8 %, and 3.35 %, respectively.

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Deficit and poor quality of the bulky (raw and moisture rich) feeds caused the use of the concentrate-type feeds for dairy feeding in the test period; the mass fractions of the raw feeds, the moisture rich feeds, and the cincentrate feeds were 26 %, 23 %, and 51 %. The diet for the lactating cows contained digestible protein of 95 g per one EFU. Insufficient dietary sugar contents caused the low value for the ratio of sugar to protein (0.6), which resulted in the low absorption of the feed proteins in the ruminants. The mineral substances were adequately supplied for the animals.

The samples of milk collelcted from the mature Holstein cows of Hungarian, German and Holland selection at 5-6 months of lactation serves as the objects of the survey.

The milk amino acid composition (n=9) was determined with the high-performance liquid chromatography method using the Shimadzu LC-20 Prominance liquid chromatograph, Japan [14-15].

The amino acid index was calculated with the formula:

$$I = \frac{\sum A}{\sum A_1}, \quad (1)$$

Where A is the essential amino acid; A_1 is non-essential amino acid.

The biological value of the protein component of the analyzed population cow milk was measured with the use of the improved method for calculating the digestible indispensible amino acid score (DIAAS) with the formula adopted by FAO for calculating the etalon protein [12]:

$$DIAAS = \frac{A_2}{A_3}, \quad (2)$$

Where A_2 is a digestible amount of the amino acid, g/100g protein; A_3 is the content of the same amino acid in the etalon protein, g/100 g protein.

$$A_2 = A \bullet K, \quad (3)$$

Where K is the actual amount of the individual amino acid absorbed, % [12, 16].

The DIAAS method to measure the biological value of the protein component in the milk of the European Holstein cows was required by the up-to-date research approaches (first, the use of the stable isotopes and the capillary electrophoresis), which made it possible to ascertain that no amino acid in the milk or the other many food products is fully absorbed. This method considers the amino acids as the isolated nutrients. This method provides the records of the values of biological accessibility for each amino acid, using the nitrogen balance within the site from the oral cavity to the end of the small intestine. This method is equivalent to the diagnostic tests in evaluating the accuracy [17-19].

The essential amino acid requirement of a 2 to 5 year-old child, which is considered highest for this age group compared to the other age groups, is adopted as the standard (etalon protein) (Table 1.).

Table 1: Improved formula for etalon protein (FAO, 2011) [12, 15, 20]

Item	Essential amino acid								
	ILEU	LEU	LYS	MET + CYS	PHE + TYR	THR	TRP	VAL	HIS
Recommended content (as % of crude protein)	3	6.1	4.8	2.3	4.1	2.5	0.66	4.0	1.6
Aspects of calculation	Rate of amino acid absorption								

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The experimental data were processed on the computer with the Microsoft 2007 Office Excel system using the standard methods [21].

RESULTS AND DISCUSSION

Proteins are the most valuable components of milk. Their total concentration in milk comprises 3.0-3.5 %. Proteins play a principle role in human consumption. In addition, they have the effects on the technology of dairy processing, the output, and the quality of the manufactured dairy products. Therefore, the crude protein content control is provided during the raw milk aacceptance at the dairy industry enterprises [2]. The milk proteins contain ninteen amino acids. Nine of them, such as threonine, leucine, phenylalanine, isoleucine, lysine, tryptophan, histidine, valine, and methionine are essential in the human and animal bodies. The milk proteins possess the high biological value due to the well-balanced content of the essential amino acids and their high digestibility in the gastrointestinal tract. In addition, each amino acid has its own function. Thus, tryptophan is necessary for the nitrogen balance, the body growth, and the biosynthesis of the blood serum proteins, the hemoglobin, and the vitamin PP. Lysin is known to be a coordinator of the growth process, the skeleton defelopment, and the absorption of calcium. Methionine is involved in lipid metabolism and syntheses of choline and adrenalin; it stimulates the activities of some hormones, vitamins, and enzymes; moreover, it is a lipotropic substance. Pphenylalanine is involved in neurotransmission. Leucine is known to normalyze the blood glucose level, to stimulate the growth hormone, and to participate in the regeneration of the injured tissues of bones, skin, and muscles. Isoleucine supports the nitrogen balance, etc. Valine regulates the nitrogen metabolism, the coordination of movements, etc. Threonine contributes to the tissue formation, controls the growth processes, etc. [1].

Lack of availability of any essential amino acid causes the extreme limit of using the other amino acids in the protein biosynthesis, whereas the excess amino acids are determined to form the toxic metabolic products [1-3].

The studies in the amino acid composition of the proteins in the milk of the Hungarian, German, and Holland Holstein cows have found the complete set and a rather high concentration of many amino acids, which can prove their adequacy; the results are summarized and present in Table 2.

Table 2: Amino acid composition of European Holstein cow milk (as percentage of crude protein)

Amino acid	German (n=3)	Hungarian (n=3)	Holland (n=3)	Totals for entire Holstein breed (n=9)
Essential amino acid (A)				
Isoleucine (ILEU)	4.15±0.028	4.13±0.020	4.20±0.016	4.16±0.015
Leucine (LEU)	8.09±0.052	8.07±0.025	8,04±0.019	8,07±0.019
Лизин (LYS)	6.84±0.046	6.85±0.008	6.86±0.015	6.8±0.014
Methionine+cysteine (MET + CYS)	2.60±0.062	2.54±0.66	2.45±0.080	2.5±0.041
Phenylalanine + tyrosine (PHE + TYR)	7.98±0.025	8.32±0.190	8.08±0.019	8.1±0.077
Threonine (THR)	3.69±0.016	3.67±0.017	3.67±0.015	3.6±0.009
Tryptophan (TRP)	0.60±0.024	0.61±0.028	0.40±0.157	0.54±0.058
Valine (VAL)	5.05±0.047	5.06±0.024	5.10±0.014	5.1±0.018
Histidine (HIS)	2.39±0.240	2.15±0.005	2.15±0.004	2.23±0.080
∑A	41.4	41.4	41.0	41.1
Non-essential amino acids (A_1)				
Aspartic acid + asparagine (Asp+ASN)	6.56±0.029	6.5±0.011	6.40±0.009	6.51+0.015
Серин (SER)	4.76±0.033	4.7±0.026	4.68±0.014	4.73+0.017
Glutamic acid + glutamine (Glu+GLN)	18.45±0.163	18.45±0.071	18.20±0.030	18.39+0.060
Alanine (Ala)	2.71±0.018	2.70±0.022	2.71±0.005	2.71+0.009

Arginine (Arg)	17.1±0.091	16.97±0.037	18.12±0.193	17.36+0.198
Proline (PRO)	7,80±0,089	7,78±0,101	7,70±0,033	7,79+0,040
Glicine (Gly)	1.54±0.005	1.53±0.012	1.56±0.016	1.54+0.007
∑ A_1	58.6	58.6	59.0	58.9
I	0.71	0.71	0.69	0.70

With respect to the essential amino acids, the proteins in the milk of the animals, despite their genotype, are characterized by the highest concentrations of leucine (LEU), phenylalanine + tyrosine (PHE + TYR), and lysine (LYS) and the lowest concentrations of tryptophan (TRP), methionine + cysteine (MET + CYS), and histidine (HIS). It should be noted that the lowest content of tryptophan (TRP) is recorded in the milk of the Holland cows.

The non-essential amino acids generally comprise more than a half of the total amount of all the amino acids in the milk. Similar to the essential amino acids, they play an important role in the human body. It is ascertained that the non-essential amino acids are produced in the human body, since the insignificant part of them is utilized for the endogenous synthesis, meeting the demands of the body. In addition, if the requirements for the essential amino acids are fully met with the insignificant costs of protein, the non-essential amino acids can also become limiting. Therefore, the non-essential amino acids should be compulsory for the dietary food, since they are not the limiting factor in the protein nutrition [1-3].

The glutamic acid + glutamine (Glu+GLN) and arginine (Arg) were found in the high concentrations, while glycine (Gly) and alanine (Ala) were in the lowest concentrations in the milk of the Holstein cows of different eco-genetics groups.

The percent complete for the non-essential amino acids (58.6-58.9 %) in the milk proteins was higher than that for the essential amino acids (41.0-41.4 %). Therefore, their amino-acid index comprised only 0.69-0.71, while the same indicator for the domestic breeds was higher and ranged from 0.83 to 1.18 [22-226].

The biological value of the food product reflects its capability to satisfy the body needs for the essential amino acids. The proteins of the high biological value are the organic substances containing all the essential amino acids of the sufficient score and the adequate balance, which are characterized with easy digestibility and a high absorption level (more than 95 %).

The calculation of the essential amino acid contents in the Holstein cow milk with regard to their actual absorption is present in Table 3.

Table 3: Amino acids absorbed in European Holstein cow milk

Item	Essential amino acid of milk										
	THR	VAL	ILEU	LEU	TYR	PHE	HIS	LYS	CYS	MET	TRP
Actual absorbtion of milk amino acids, % [12]	92	89	87	95	96	96	95	91	92	95	93
German											
Content of amino acids, %	3.69	5.05	4.15	8.09	4.00	4.03	2.39	6.84	2.16	0.38	0.60
Amount of amino acids absorbed, %	3.39	4.49	3.61	7.69	3.84	3.87	2.27	6.22	1.99	0.36	0.56
Hungarian											
Content of amino acids, %	3.67	5.06	4.13	8.07	4.05	4.07	2.15	6.85	2.12	0.37	0.61
Amount of amino acids absorbed, %	3.38	4.50	3.59	7.67	3.89	3.91	2.04	6.23	1.95	0.35	0.57
Holland											
Content of	3.67	5.10	4.20	8.04	4.03	4.05	2.15	6.85	2.11	0.34	0.40

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amino acids, %											
Amount of amino acids absorbed, %	3.38	4.54	3.65	7.64	3.87	3.89	2.04	6.23	1.94	0.32	0.37
Totals for Holstein breed											
Content of amino acids, %	3.69	5.07	4.16	8.07	4.03	4.05	2.23	6.85	2.13	0.36	0.54
Amount of amino acids absorbed, %	3.39	4.51	3.62	7.67	3.87	3.89	2.11	6.23	1.96	0.34	0.50

The analysis of Table 3 proves that the essential amino acids of the proteins in the European Holstein cow milk are absorbed in the range of 87 % to 96 %, while the sum of the essential amino acids is higher in them, when compared to the ideal protein (Table 4). However, the amino acid scores of the certain amino acids, which are present in Table 4 and in Fig. 1, vary in a rather wide range of 56 % for tryptophan (TRP) in the Holland cow milk samples to 190 % for phenylalanine + tyrosine (PHE + TYR) in the milk samples of the German analogs, proving their imbalance.

The limiting amino acid in the cow milk samples in the context of the entire Holstein breed and its analyzed genotypes is tryptophan, since its DIAAS value was in the range of 56% to 86 %. This is exactly the amino acid that determines the current level of the other essential amino acids of the analyzed population milk. The lowest and highest DIAAS values are recorded in the Holland cow and Hungarian cow milk samples, respectively.

It should be noted that a law amino acid score (98 %) for methionine + cysteine (MET + CYS) was estimated. Thus, it has the lowest biological value in the European Holstein animals of the analyzed genotypes.

Therefore, the survey results can prove that the biological value for the proteins of the Holstein cow milk is caused by the genotype of the animals. The milk produced from these cows fed with the imbalanced diets and kept under the conditions of the modern dairy complex with the loose systems is characterized by a low biological value, despite there is a complete set and a high concentration of the amino acids.

The literature data shows that in order to produce the milk of high biological value, the animals should be supplied with the high protein diets including all the required amino acids, since their bacterial synthesis can cover only 30-40 % of the high-performance cow demand [9, 10].

Table 4: Calculation of DIAAS value for European Holstein cow milk

Item	Essential amino acid									Σ
	ILEU	LEU	LYS	MET + CYS	PHE + TYR	THR	TRP	VAL	HIS	
Etalon protein (FAO, 2011r.)										
Amount of amino acid absorbed, %	3	6.1	4.8	2.3	4.1	2.5	0.66	4.0	1.6	29.1
DIAAS value for milk, based on limiting amino acid, %	100	100	100	100	100	100	100	100	100	–
German										
Amount of amino acid absorbed, %	3.61	7.69	6.22	2.35	7.71	3.39	0.56	4.49	2.27	38.3
DIAAS value for milk, based on limiting amino acid, %	120	126	130	102	188	136	**85***	112	142	–
Hungarian										
Amount of amino acid absorbed, %	3.59	7.67	6.23	2.3	7.8	3.38	0.57	4.5	2.04	38.1

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DIAAS value for milk, based on limiting amino acid, %	120	126	130	100	190	135	**86***	113	128	–
Holland										
Amount of amino acid absorbed, %	3.65	7.64	5.97	2.26	7.76	3.38	0.37	4.54	2.04	37.6
DIAAS value for milk, based on limiting amino acid, %	122	125	124	98	189	135	**56***	114	128	–
Totals for entire Holstein breed										
Amount of amino acid absorbed, %	3.62	7.67	6.23	2.30	7.77	3.39	0.50	4.51	2.11	38.0
DIAAS value for milk, based on limiting amino acid, %	121	126	130	100	189	136	**76***	113	132	–

* Limiting acid

The main parameter characterizing the quality of cow feed protein involved in the ruminant nitrogen metabolism is protein splitting in the rumen. The feed proteins are broken down with the enzymes of microbial to amino acid origin, which are involved in proteolysis and subsequently in dezamination with occurrence of ammonia. Thereafter, the microorganisms are used for biosynthesis of a certain protein. However, the microbial biomass synthesis in the rumen is caused by the level of the available energy supplied by the fermentable carbohydrates and the other compounds of the organic substances. The high-performance cows have increasing demand for the highly digestible carbohydrates: sugars and starch. The sugar to protein ratio in their diets should be increased to 2:1. Carbohydrates are required in an adequate amount as the energy sources and the substances for both the metabolic processes and the functions of the normal microflora in the animal rumen [9, 10, 11].

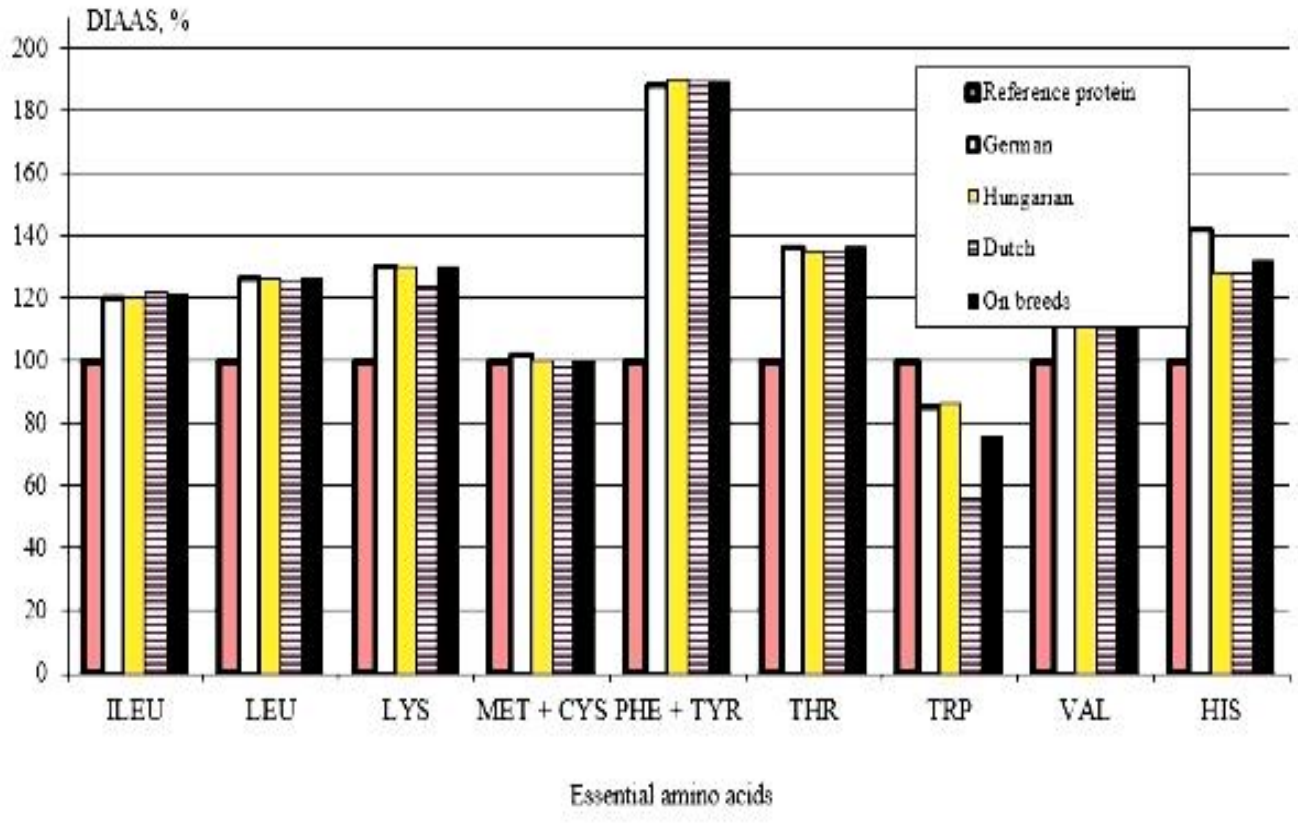

Fig.1. Amino acid chart for amino acids of European Holstein cow milk

Therefore, the diets for the high-performance cows should be optimized using the DIAAS methods for assessing the protein composition and including the amino acids being in a deficit to produce the milk of high biological value.

CONCLUSION

The analyses on the protein compound in the milk of the Hungarian, German, and Holland Holstein cows kept at the modern dairy complex with the loose systems have revealed almost complete sets and rather high concentrations of all the aminoacids, which can characterize its adequacy.

With respect to the essential amino acids, the proteins in the milk of the animals, despite their genotype, are characterized by the highest concentrations of leucine (LEU), phenylalanine + tyrosine (PHE + TYR), and lysine (LYS) and the lowest concentrations of tryptophan (TRP), methionine + cysteine (MET + CYS), and histidine (HIS). It should be noted that the lowest content of tryptophan (TRP) is recorded in the milk of the Holland cows.

Both the non-essential amino acids and the essential amino acids play the important roles in the human body. The non-essential amino acids are produced in the human body, since the insignificant part of them is utilized for the endogenous synthesis, meeting the demands of the body. If the requirements for the essential amino acids are fully met with the insignificant costs of protein, the non-essential amino acids can also become limiting. Therefore, the non-essential amino acids should be compulsory for the dietary food, since they are not the limiting factor in the protein nutrition. The glutamic acid + glutamine (Glu+GLN) and arginine (Arg) were found in the high concentrations, while glycine (Gly) and alanine (Ala) were at the lowest concentrations in the milk of the analyzed animal population.

The percentage complete for the non-essential amino acids (58.6-58.9%) in the milk proteins was higher than that for the essential amino acids 41.0-41.4%). Therefore, their amino-acid index comprised only 0.69-0.71, while the same indicator for the domestic breeds was higher and ranged from 0.93 to 1.16.

The DIAAS method to measure the biological value of the protein component in the milk of the European Holstein cows was required by the up-to-date research approaches (first, the use of the stable isotopes and the capillary electrophoresis), which made it possible to ascertain that no amino acid in the milk or the other many food products is fully absorbed. This method considers the amino acids as the isolated nutrients. This method provides the records of the values of biological accessibility for each amino acid, using the nitrogen balance within the site from the oral cavity to the end of the small intestine. This method is equivalent to the diagnostic tests in evaluating the accuracy.

The total digestible amino acid set number in the milk of the cows of the analyzed Holstein genotypes is higher than the number in the ideal protein in the context of the entire breed and its genotypes. However, the amino acid scores of the certain amino acids vary in a rather wide range from 56% for tryptophan (TRP) in the Holland cow milk samples to 190 % for phenylalanine + tyrosine (PHE + TYR) in the milk samples of the German analogs, proving their imbalance.

The limiting amino acid in the context of the entire Holstein breed and its analyzed genotypes is tryptophan, since its DIAAS value was in the range of 56% to 86%. This is exactly the amino acid that determines the limit of the other essential amino acids of the analyzed population milk. The low amino acid score (98%) for methionine + cysteine (MET + CYS) was estimated in the milk samples collected from the Holland cows. Therefore, it has the lowest biological value, when compared to that for the German and Hungarian cows.

The biological value for the proteins of the Holstein cow milk is caused by the genotype of the animals. The milk produced from these cows fed with the imbalanced diets and kept under the conditions of the modern dairy complex with the loose systems is characterized by a low biological value, despite there is a complete set and a high total concentration of the amino acids.

The biological value for the proteins of the Holstein cow milk is caused by the genotype of the animals. The milk produced from these cows fed with the imbalanced diets and kept under the conditions of the

modern dairy complex with the loose systems is characterized by a low biological value, despite there is a complete set and a high total concentration of the amino acids.

In order to produce the milk of high biological value, the diets for the high-performance cows should be optimized with all the nutrients, using the DIAAS methods for assessing the protein composition and including the amino acids being in a deficit.

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