AGRICULTURAL BIOLOGY,
2019, V. 54, № 2, pp. 386-394
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.2:619:57.088.1

doi: 10.15389/agrobiology.2019.2.386eng
doi: 10.15389/agrobiology.2019.2.386rus

DYNAMICS OF AMINO ACID PROFILE IN MILK OF BIV- AND BLV-BIV-INFECTED COWS DURING STORAGE

E.S. KRASNIKOVA, O.S. LARIONOVA, A.V. BANNIKOVA, A.V. EVTEEV, G.Kh. UTANOVA

Vavilov Saratov State Agrarian University, Department of Microbiology, Biotechnology and Chemistry, 1, Teatralnaya pl., Saratov, 410012 Russia, e-mail krasnikovaes77@yandex.ru (✉ corresponding author), larionova1@mail.ru, annbannikova@gmail.com, ewteew@gmail.com, sgau.microb@list.ru
ORCID:
Krasnikova E.S. orcid.org/0000-0003-4395-5862
Larionova O.S. orcid.org/0000-0001-5457-0306
Bannikova A.V. orcid.org/0000-0002-5582-0394
Evteev A.V. orcid.org/0000-0002-6155-3851
Utanova G.Kh. orcid.org/0000-0002-6659-0483
The authors declare no conflict of interests
Received December 20, 2018

Abstract

Leukemia and viral immunodeficiency of cattle rank to the number of difficult diagnosed infections of farm animals which increases the probability of virus carrying. At present time there are no legislative approved standards for bovine immunodeficiency diagnosis so the milk from sick cows can infect bulk milk obtained from healthy animals. Frequently bovine immunodeficiency virus (BIV) is detected in animals infected by bovine leukemia virus (BLV). The present work reveals that milk of cows infected by retroviruses has an altered amino acid balance of the casein fraction, as well as lower protein stability when stored in a refrigerator. The aim of our research was to assay the amino acid composition and evaluate its stability in milk of BIV-infected and BLV-BIV-co-infected cows, as compared to milk of healthy animals. A total of 6 samples of pure cow milk obtained from 2.8-6.7-year old black-and-white dairy cows infected by bovine immunodeficiency virus (n = 3) and cows with BLV-BIV coinfection (n = 3) were tested. BIV and BLV-BIV infection was approved by PCR tests. Analysis of amino acid composition of the protein fraction was performed using a capillary electrophoresis system Capel 105M (LLC "Lumex-Marketing", Russia). Milk was tested on day 1, day 3 and day 6 of storage at 4 °C. The standard data on the amino acid balance of the casein fraction of healthy cows' milk was used for the comparative analysis. Our results indicate that milk from BIV and BLV-BIV infected cows differs significantly in amino acid composition from healthy cows' milk. We consider the milk casein fraction to be more illustrative because casein is the main milk protein and its amino acid profile in not changed even under hydrolysis. The weight percentage of the essential and conditionally essential amino acids such as methionine, glutamine, histidine and glycine in infected milk reduces 3-4-fold. The content of arginine, lysine, proline and asparagine are similar to normal, tyrosine is passed into the limiting position under a deficient phenylalanine which content is 6 times lower than normal. There was a comparative excess amount of threonine and serine. The weight percentage of lysine, valine, leucine-isoleucine and glutamine is 9, 13, 17.5 and 22 times lower that in healthy cows' milk. The leucine-isoleucine and valine ratio of infected milk is approximately 1:1 whereas it amounts to 2.5:1 in healthy cows' milk. It was observed that milk of BIV and BLV-BIV infected cows is characterized by expressed dynamic of amino acid profiles during storage in a refrigerator. The essential amino acids arginine, histidine and methionine were not detected in the milk of infected cows on the sixth day of the storage in the refrigerator. The weight percentage of tyrosine increased on day 3 and decreased on day 6 of storage. Moreover, these parameters were significantly changed in BIV infected milk. The weight percentage of phenylalanine, leucine-isoleucine, valine, serine and glycine in BIV and BLV-BIV infected milk increased on day 6 of storage by 27.1 and 2.4 %; 22.3 and 8.9 %; 43.9 and 37.2 %; 25.0 and 27.3 %, and 0 and 60 %. The weight percentage of proline and threonine reduced on day 6 of storage by 10.6 and 13.7 %; 5.3 and 0 %. The weight percentage of alanine on day 6 in BIV infected milk increased by 37.5 % but in BLV-BIV infected milk reduced by 17.8 %. The imbalance and instability of amino acid profile of BLV and BLV-BIV infected milk prove are indicative of development of uncontrolled processes.

Keywords: cow's milk, milk protein, amino acids, immunodeficiency virus, leukemia

According to the Institute of Nutrition of RAMS, annual dietary protein deficiency is more than 1.6 mln tons in Russia. Total global protein deficiency is

10-25 mln tons per year [1]. Along with this, each person accounts for 60 g of protein daily. Nitrogen body balance studies revealed that the best daily dosage of protein consumed by a healthy adult is 0.8 g per kg body weight. The value can vary depending on mental and physical load, sex, age and physiological state. Thus, 88 g per day is considered a normal protein diet for a young healthy man experiencing the minimum physical load [2]. Along with quantity, quality of protein (including balanced amino acid ratio) is also associated with protein nutritive value for a human body. Contrary to plant proteins, animal ones contain a well-balanced ratio of all the necessary amino acids. Plant products contain substantially lower amount of essential amino acid that animal ones. Even soy protein, despite the best possible amino acid composition, is short of methionine (i.e., an essential amino acid) [3].

Typically, each person consumes cow milk regardless of age, and its proper amino acid composition is well-balanced. A human body digests 96-98% of cow milk proteins. It was found that milk proteins stabilize blood pressure in hypertonic patients. Casein fraction represents about 80% of milk proteins. Namely, its average content in cow milk is 2.1-2.9% [4].

Enzymic hydrolysis of milk protein starts during storage of raw milk. It is performed by proteolytic enzymes of milk microflora and a starter, as well as by plasmin enzyme [5, 6]. Peptides are predominant products of enzymic milk hydrolysis. In this view, free amino acid fraction is comparatively small. Milk peptides stimulate secretion of insulin and growth hormone. Also, they have a positive impact on digestion of essential micronutrients (including calcium ions) [7]. Resulted from milk hydrolysis, peptides, apart from high digestibility, inhibit desquamation of intestinal mucosa cells and stimulate production of endogenous enzymes [8]. Low-molecular milk peptides penetrate into muscles rapidly causing a strong insulinotropic effect. Replenishing energy reserves of a human body, they nourish muscles with amino acids leading to promoted synthesis of muscle proteins. Microfractions of low-molecular serum proteins (i.e., glycomacropeptides) reduce risk of viral diseases, have positive influence on digestion and calcium/protein assimilation, and induce development of normal intestinal microflora [9].

Uncontrolled and unpredictable proteolysis induced by proteolytic enzymes of secondary microflora (including putrefactive one) can result in formation of several compounds with foreign odor and flavor in raw milk. Along with this, free amino acids undergo fermentation that causes their interamination, deamination and decarboxylation leading to formation of keto acids, oxy acids, carboxylic acids and aldehydes [10]. Deep degradation of milk proteins induced by foreign microorganisms leads to formation of free amino acids that, in turn, affect organoleptic properties of milk. For example, alanine, glycine, proline and serine are sweet; asparaginic and glutamic acids are sour; arginine, leucine, histidine and tryptophan are bitter; methionine and cystine are sulphuric; threonine, valine and phenylalanine are bittersweet [11]. Moreover, free amino acids are in the running to use amino acid channels. In turn, a great amount of free amino acids induces gastrointestinal disorders [12].

Quality, biological and nutritive values of cow milk depend on a breed, management conditions, feeding and health status of an animal. Most of diseases (including chronic infections) can lead to a persistent decrease in cow milk yields and lower quality of dairy products. It has been established that enzootic bovine leukosis virus (EBLV, bovine leukemia virus, BLV) in dairy cows results in somatic cell content in milk $(4.9\text{-}5.2)\times10^5/cm^3$; milk yields decrease by 13.3-15.5% [13] that, in turn, results in a significant economic damage to livestock production [14]. BLV-infected cows demonstrate decreased ferritin and iron levels in milk. Nevertheless, serum ferritin level increases being a marker of inflammation and malignant processes [15].

BLV-infection is associated with reduction of total body resistance and breast immunity inhibition. This results in less efficient phagocytosis followed by a substantial bacterial count (up to $2\times10^7\pm4\times10^2$ CFU/ml) in milk of an infected cow [16]. A BLV-infected cow shows a significant decrease in apoptotic B cell fraction and CD44 neutrophilic expression (especially, in an animal with persistent lymphocytosis) [17]. Also, levels of total protein and amino acids (including 5 essential ones, i.e., isoleucine, tryptophan, methionine, leucine and phenylalanine) decline. Electrophoresis assay of milk protein demonstrates increased fractions of proteose peptones, lactalbumins and immunoglobulins associated with decreased serum albumin fraction [18].

In general, leukosis is accompanied by another chronic bovine infection (i.e., viral immunodeficiency (BVI)). Similarly to leukosis virus, bovine immunodeficiency virus (BIV) is tropic to lymphocytes and induces persistent immune disorders. Although BVI is a relatively new disease, it is even more incident in a sick flock than EBL [19, 20].

According to our prior studies, milk of BIV- and BLV-infected cows differs significantly from healthy cows' milk in protein composition [16]. Profile of milk proteins and peptides, as well as ratio of free and bound amino acids can vary in cows with immune deficiency. Nowadays, there are no available data on alteration in amino acid composition of refrigerated milk collected from BIV- and BLV-co-infected and BIV-infected cows. Being of great academic interest, the data have a practical significance because this can justify milk biochemical transformations and draw a conclusion about a biological value of the product. From our point of view, data on milk casein fraction are the most meaningful since even hydrolysis cannot change amino acid composition of the protein.

In the work presented here we newly detected that milk of cows infected with retroviruses has altered amino acid balance of a casein fraction; also, the protein demonstrates lower stability when milk is stored in a refrigerator at 4 °C.

Our purpose was to compare and to evaluate stability of amino acid composition of casein fraction in milk of cows infected with bovine immunodeficiency virus, cows co-infected with bovine leukosis and bovine immunodeficiency viruses, and healthy cows' milk.

Techniques. We tested 6 samples of whole milk collected from BLV- and BIV-co-infected (n = 3) and BIV-infected (n = 3) black-and-white dairy cows (*Bos taurus taurus*) aged 2.8-6.7 years.

Nucleic acids were extracted and purified with a DNK Sorb B kit (InterLabServis, Russia). Presence/absence of BLV and BIV proviruses in whole blood samples was established by a conventional PCR method with LEIKOZ kit (InterLabServis, Russia) and by our own multiplex PCR (Patent of the Russian Federation no. 2615465) with specific primers synthesized by Syntol ZAO (Russia), PCR-Mix kit and application buffer (Lytech Research and Production Company, Russia). Amplification was performed in a T100 thermocycler (Bio-Rad, USA). Amplification products were detected by a horizontal electrophoresis in 2% agarose gel with 0.5 μg/l of ethidium bromide with an EP kit (InterLabServis, Russia) under standard conditions with photographic recording. The results were recorded on GelDoc XP PLUS equipment (Bio-Rad, USA). Commercial kits and equipment were used as per guidelines of their manufacturers and developers.

Clinically evident mastitis was not mentioned in cows. Also, milk organoleptic properties remained normal.

During sample preparation whole milk (10 ml) was centrifuged at 5 °C and 10,000 rpm for 30 min. Then, we separated precipitate and upper lipid fraction. In constant stirring, we added hydrochloride acid dropwise at 37 °C to adjust pH at 4.5. Further, we continued stirring for 30 min. Resultant substance was centrifuged at 25 °C and 5,000 rpm for 10 min, and supernatant was separated. The precipitate pH value was adjusted at 6.5 with 1 M sodium hydroxide solution.

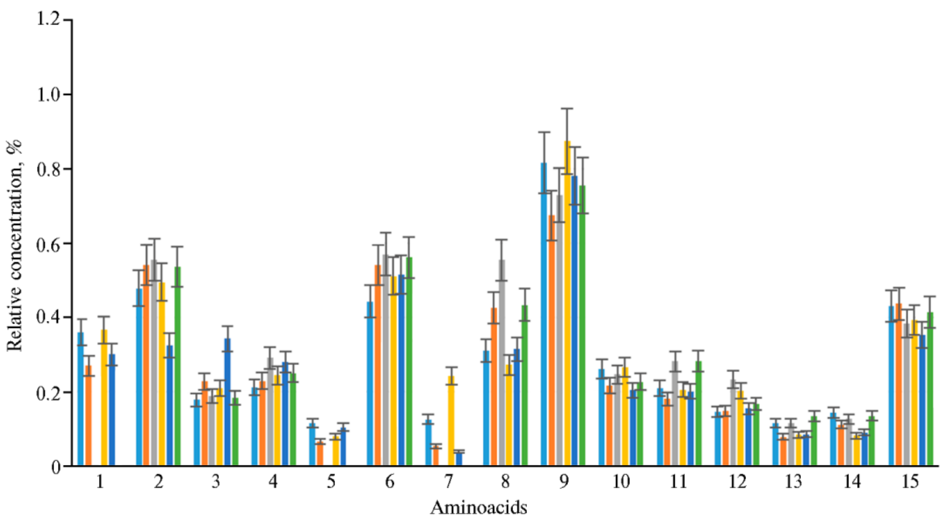
We assessed amino acid composition of protein fractions with Capel 105M capillary electrophoresis system (LLC Lumex-Marketing, Russia; procedure no. M-04-38-2009, amendment no. 1 dated 01.02.2010) as per the manufacturer's guidelines. Milk was tested on days 1, 3 and 6 of storage in a refrigerator at 4 °C.

To perform a comparative analysis, we used common standard data on amino acid balance of casein fraction in healthy cows' milk.

We processed our data in Statistica 8.0 software (StatSoft, Inc., USA). Difference significance was evaluated with Mann-Whitney U test. Statistically significant differences must comply with 95% ($p < 0.05$). The paper presents mean weight percentages of primary amino acids in a milk casein fraction (M) and standard deviations ($\pm\sigma$).

Results. According to follow-up stability studies of amino acid composition of infected cows' milk, amino acid content is very variable (Fig.). This can be caused by active biochemical processes in milk.

Arginine, methionine and histidine demonstrated the most variable weight percentages because they were below the limit of detection on the day 6 of the storage in a refrigerator. Other amino acids showed relatively stable (i.e., threonine, glutamine, asparagine, phenylalanine, proline, leucine-isoleucine) or even more variable levels (serine, glycine, alanine, lysine, valine, tyrosine and glutamine). Most of these changes were random. The content increased on day 3 (tyrosine) or the day 6 (phenylalanine, valine, serine, glycine) of the storage. Equally, it decreased eventually (i.e., threonine, proline).



Following weight percentages of amino acids were mentioned in milk of BIV (bovine immunodeficiency virus)**-infected or BLV** (bovine leukemia virus)**-co-infected black-and-white cows (*Bos taurus taurus*) when stored in a refrigerator at 4°C:** 1 — arginine, 2 — lysine, 3 — tyrosine, 4 — phenylalanine, 5 — histidine, 6 — leucine + isoleucine, 7 — methionine, 8 — valine, 9 — proline, 10 — threonine, 11 — serine, 12 — alanine, 13 — glycine, 14 — glutamine, 15 — asparagine; ■ ■ ■ ■ ■ ■ — on day 1 (BIV), day 3 (BIV), day 6 (BIV), day 1 (BLV-BIV), day 3 (BLV-BIV), and day 6 (BLV-BIV), respectively ($M\pm\sigma$). Differences are statistically significant at $p < 0.05$.

It is known that casein fraction of cow milk has the most stable amino acid composition. Considering a lactation phase, it slightly (0.01-0.15%) varies in black-and-white cows depending on amino acid concentration [21].

Relative content of amino acids (normal percentage, %) **in milk of BIV** (bovine immunodeficiency virus)**-infected or BLV** (bovine leukemia virus) **+ BIV-co-infected black-and-white cows after storage in a refrigerator at 4 °C** ($M\pm\sigma$)

Amino acid	BIV			BLV-BIV		
	day 1	day 3	day 6	day 1	day 3	day 6
Arginine	8.8±0.9	5.6±0.6*	–*	8.9±0.9	6.6±0.6*	–*
Lysine	5.8±0.6	6.6±0.6*	6.8±0.7*	6.1±0.6	6.6±0.7	6.5±0.6
Phenylalanine	4.2±0.4	4.6±0.4	5.8±0.6*	4.9±0.5	4.6±0.4*	5.0±0.5*
Tyrosine	2.1±0.2	2.8±0.3*	2.3±0.2	2.6±0.2	4.2±0.4	2.2±0.2
Histidine	3.7±0.3	2.1±0.2*	–*	2.4±0.2	2.1±0.2*	–*
Leucine + Isoleucine	2.9±0.3	3.5±0.3*	3.7±0.3*	3.3±0.3	3.5±0.3	3.6±0.3
Methionine	4.5±0.5	1.4±0.1*	–*	8.6±0.8	1.9±0.2*	–*
Valine	4.3±0.4	4.4±0.4	7.7±0.7*	3.8±0.3	5.9±0.6*	6.0±0.6*
Proline	7.2±0.7	6.9±0.7	6.5±0.6*	7.7±0.7	6.0±0.6*	6.7±0.7*
Threonine	5.3±0.5	4.2±0.4*	5.1±0.5	5.4±0.5	4.4±0.4*	4.6±0.4*
Serine	3.3±0.3	3.2±0.3	4.4±0.4*	3.3±0.3	2.9±0.3*	4.5±0.4*
Alanine	4.8±0.4	5.1±0.5	7.8±0.8*	6.7±0.6	4.9±0.5*	5.5±0.5*
Glycine	4.3±0.4	3.1±0.3*	4.3±0.4	3.1±0.3	2.9±0.3	5.0±0.5*
Glutamine	0.6±0.1	0.4±0.1*	0.6±0.1	0.4±0.1	0.5±0.1*	0.6±0.1*
Asparagine	6.1±0.6	6.2±0.6	5.4±0.5*	5.5±0.5	5.9±0.6	5.8±0.6

N o t e. Dashes stand for amino acid level below the limit of detection (arginine and histidine level < 0.50%; methionine < 0.25%).
* Differences with the values on day 1 are statistically significant at $p < 0.05$.

The table illustrates amino acid levels in milk of BLV-BIV- and BIV-infected cows as compared with reference means in healthy animals [22]. Mean amino acid levels in milk of BIV-infected cows were 5-20 times lower than in healthy ones (see the Table), and better part of essential amino acids (such as methionine, glycine, histidine, glutamine) demonstrated trace levels. Substantial changes were mentioned in amino acid balance of milk. Even though infected cows showed substantially lower levels of tyrosine, leucine and serine than healthy ones, arginine, lysine, proline and asparagine levels were closer to normal values.

As for humans, reference amino acid dietary intake, in general, is calculated considering dietary features and individual needs. While evaluating amino acid balance, content of 11 essential amino acids (i.e., lysine, methionine, tryptophan, valine, arginine, histidine, leucine, isoleucine, phenylalanine, threonine and glycine) is considered as a limitation. Other 10 amino acids (asparagine, alanine, aspartate, glutamate, glycine, glutamine, serine, proline, tyrosine and cysteine) are regarded as replaceable ones. However, this is an uncertain division [23]. In particular, milk of BIV-infected cows demonstrated deficiency of arginine and histidine. At the same time, due to lack of phenylalanine, tyrosine became a limitative factor despite a sufficient content.

Several persons need increased reference daily intake of amino acids. Concurrently, excessive amino acids can have an adverse impact because a body must spend more energy to dispose them [24]. An excessive amino acid can replace a deficient one in metabolism. In turn, this can lead to skeletal disorders, toxicosis and increased adipopexia. We detected relatively excessive serine and threonine levels in milk of BIV-infected cows. Similar trend was mentioned in cows co-infected with BIV and BLV (see the Table).

To prevent metabolic disorders, amino acid balance index (i.e., dietary amino acid ratio) should be maintained. This is caused by antagonism between amino acids with similar structure (i.e., threonine-tryptophan, arginine-lysine, leucine-isoleucine) during metabolism [25]. We revealed a balance conflict between leucine-isoleucine and valine because their weight percentage must be about 2.5:1 [22] in healthy cows' milk whereas it was 1:1 in stored milk of infected cows. Altered amino acid metabolism (i.e., their relative ratio) leads to change in whole body homeostasis. Leucine is a significant essential amino acid because it is required to stimulate protein synthesis and to inhibit cell proteolysis. Valine has less diverse functions within a body [4, 25].

According to several foreign works, to provide early diagnosis of bovine subclinical mastitis, milk amino acid composition should be assayed by high-performance liquid chromatography [26]. Moreover, indicator values associated with the most common staphylococcal mastitis are mentioned there [27]. However, since these data are rarely reported, these veterinary studies are important and promising.

Thus, amino acid levels in milk of cows infected with bovine immunodeficiency virus or co-infected with bovine leukemia and immunodeficiency viruses was 5-20 times lower than in healthy cows. Essential and conditionally essential amino acids (i.e., methionine, glutamine, histidine and glycine) showed more significantly decreased weight percentages. Infected cows demonstrated substantially lower levels of tyrosine, leucine and serine than healthy ones. Less significant decrease was mentioned in arginine, lysine, proline and asparagine levels whereas tyrosine became a limitative factor as a result of phenylalanine deficiency. Excessive relative concentrations of threonine and serine were detected. Disproportional weight percentage of leucine-isoleucine and valine was seen in milk of healthy and infected cows. The storage even got this worse. BIV- and BLV-BIV-infected cow milk demonstrated an evident alteration of amino acid composition when stored in a refrigerator. Several essential amino acids (i.e., arginine, histidine and methionine) were not detected in refrigerated milk of infected cows on the day 6 of storage. Tyrosine weight percentage increased on the third day of the storage followed by further decrease. Although weight percentage of phenylalanine, leucine, isoleucine, valine, serine and glycine increased on the day 6 of the storage, proline and threonine demonstrated reduced weight percentages. Imbalanced and instable amino acid composition of milk collected in BLV-BIV- and BIV-infected cows indicates uncontrolled inner processes. Infected animals should be timely removed from a flock, and bovine health monitoring system should be improved.

REFERENCES

1. Ovsyannikova O.V., Ksenz M.V. *Sfera uslug: innovatsii i kachestvo*, 2012, 7: 30-43 (in Russ.).
2. Teunissen-Beekman K.F., Dopheide J., Geleijnse J.M., Bakker S.J., Brink E.J., de Leeuw P.W., van Baak M.A. Effect of increased protein intake on renal acid load and renal hemodynamic responses. *Physiological Reports*, 2016, 4(5): e12687 (doi: 10.14814/phy2.12687).
3. Wu G. Dietary protein intake and human health. *Food & Function*, 2016, 7: 1251-1265 (doi: 10.1039/c5fo01530h).
4. McGregor R.A., Poppitt S.D. Milk protein for improved metabolic health: a review of the evidence. *Nutrition & Metabolism*, 2013, 10: 46 (doi: 10.1186/1743-7075-10-46).
5. Saubade F., Hemery Y.M., Guyot J.P., Humblot C. Lactic acid fermentation as a tool for increasing the folate content of foods. *Critical Reviews in Food Science and Nutrition*, 2017, 57(18): 3894-3910 (doi: 10.1080/10408398.2016.1192986).
6. Savilahti E. Probiotics in the treatment and prevention of allergies in children. *Bioscience and Microflora*, 2011, 30(4): 119-128 (doi: 10.12938/bifidus.30.119).
7. Ricci-Cabello I., Herrera M.O., Artacho R. Possible role of milk-derived bioactive peptides in the treatment and prevention of metabolic syndrome. *Nutrition Reviews*, 2012, 70(4): 241-255 (doi: 10.1111/j.1753-4887.2011.00448.x).
8. Heine W.E. Protein source and microflora. In: *Probiotics, other nutritional factors, and intestinal microflora. 2nd Nestle Nutrition Workshop, Beijing, China, May 1997.* N.A. Hanson, R.H. Yolken (eds.). Philadelphia, Lippincott-Raven, 1999: 175-188.
9. Caldow M.K., Digby M.R., Cameron-Smith D. Short communication: Bovine-derived proteins activate STAT3 in human skeletal muscle in vitro. *Journal of Dairy Science*, 2015, 98(5): 3016-

3019 (doi: 10.3168/jds.2014-9035).
10. Hagi T., Sasaki K., Aso H., Nomura M. Adhesive properties of predominant bacteria in raw cow's milk to bovine mammary gland epithelial cells. *Folia Microbiologica*, 2013, 58(6): 515-522 (doi: 10.1007/s12223-013-0240-z).
11. Gorbatova K.K., Gun'kova P.I. *Khimiya i fizika moloka i molochnykh produktov* [Chemistry and physics of milk and dairy products]. Moscow, 2012 (in Russ.).
12. Wu G. Amino acids: metabolism, functions, and nutrition. *Amino Acids*, 2009, 37(1): 1-17 (doi: 10.1007/s00726-009-0269-0).
13. Yang Y., Fan W., Mao Y., Yang Z., Lu G., Zhang R., Zhang H., Szeto C., Wang C. Bovine leukemia virus infection in cattle of China: Association with reduced milk production and increased somatic cell score. *Journal of Dairy Science*, 2016, 99(5): 3688-3697 (doi: 10.3168/jds.2015-10580).
14. Bauermann F.V., Ridpath J.F., Dargatz D.A. Bovine leukemia virus seroprevalence among cattle presented for slaughter in the United States. *Journal of Veterinary Diagnostic Investigation*, 2017, 29(5): 704-706 (doi: 10.1177/1040638717702183).
15. Schnell S.A., Ohtsuka H., Kakinuma S., Yoshikawa Y., Watanabe K., Orino K. Iron and ferritin levels in the serum and milk of bovine leukemia virus-infected dairy cows. *Front. Vet. Sci.*, 2015, 2: 12 (doi: 10.3389/fvets.2015.00012).
16. Krasnikova E.S., Utanova G.Kh., Fedosov N.A., Shcherbakov A.A. *Nauchnoe obozrenie*, 2015, 17: 10-15 (in Russ.).
17. Della Libera A.M., de Souza F.N., Batista C.F., Santos B.P., de Azevedo L.F., Sanchez E.M., Diniz S.A., Silva M.X., Haddad J.P., Blagitz M.G. Effects of bovine leukemia virus infection on milk neutrophil function and the milk lymphocyte profile. *Veterinary Research*, 2015, 46: 2 (doi: 10.1186/s13567-014-0125-4).
18. Zakrepina E.N. *Leikoz krupnogo rogatogo skota i ego vliyanie na kolichestvennye i kachestvennye pokazateli molochnoi produktivnosti korov. Avtoreferat kandidatskoi dissertatsii* [Cattle leukemia and its impact on the quantitative and qualitative indicators of cow's milk production. PhD Thesis]. Vologda-Molochnoe, 2001 (in Russ.).
19. Brujeni G.N., Poorbazargani T.T., Nadin-Davis S., Tolooie M., Barjesteh N. Bovine immunodeficiency virus and bovine leukemia virus and their mixed infection in Iranian Holstein cattle. *J. Infect. Dev. Ctries*, 2010, 4(9): 576-579 (doi: 10.3855/jidc.711).
20. Bhatia S., Patil S.S., Sood R. Bovine immunodeficiency virus: a lentiviral infection. *Indian Journal of Virology*, 2013, 24(3): 332-341 (doi: 10.1007/s13337-013-0165-9).
21. Barkova A.S., Kolchina A.F., Barashkin M.I., Loretts O.G., Tarasenko M.N. Aminokislotnyi sostav moloka korov cherno-pestroi porody. *Materialy VII Mezhdunarodnoi nauchno-prakticheskoi konferentsii «Obrazovanieto i naukata na XXI vek — 2012»* [Proc. VII Int. Conf. «Education and Science in XXI century–2012»]. Sofiya, 2012: 95-100 (in Russ.).
22. Nadtochii L.A., Orlova O.Yu. *Innovatsii v biotekhnologii. Chast'. 2. Pishchevaya kombinatorika* [Innovations in biotechnology. Part 2. Food combinatorics]. St. Petersburg, 2015: 8 (in Russ.).
23. Yu X., Long Y.C. Autophagy modulates amino acid signaling network in myotubes: differential effects on mTORC1 pathway and the integrated stress response. *FASEB J.*, 2015, 29(2): 394-407 (doi: 10.1096/fj.14-252841).
24. Wu G. Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. *Journal of Animal Science and Biotechnology*, 2014, 5(1): 34 (doi: 10.1186/2049-1891-5-34).
25. Wu G. Functional amino acids in growth, reproduction, and health. *Advances in Nutrition*, 2010, 1(1): 31-37 (doi: 10.3945/an.110.1008).
26. Ianni F., Sardella R., Lisanti A., Gioiello A., Cenci Goga B.T., Lindner W., Natalini B. Achiral-chiral two-dimensional chromatography of free amino acids in milk: a promising tool for detecting different levels of mastitis in cows. *J. Pharm. Biomed. Anal.*, 2015, 116: 40-46 (doi: 10.1016/j.jpba.2014.12.041).
27. Bochniarz M., Kocki T., D browski R., Szczubia· M., Wawron W., Turski W.A. Tryptophan, kynurenine, kynurenic acid concentrations and indoleamine 2,3-dioxygenase activity in serum and milk of dairy cows with subclinical mastitis caused by coagulase-negative staphylococci. *Reprod. Domest. Anim.*, 2018, 53(6): 1491-1497 (doi: 10.1111/rda.13299).