AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 4, pp. 598-608
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

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

UDC 636.2.034:637.045:577.112

doi: 10.15389/agrobiology.2023.4.598eng
doi: 10.15389/agrobiology.2023.4.598rus

Bos taurus β-CASEIN: PROTEIN STRUCTURE, GENE POLYMORPHISM, EFFECT ON THE HUMAN GASTROINTESTINAL TRACT
(review)

T.A. TOKARCHUK, I.S. KOZHEVNIKOVA✉**, M.A. KUDRINA**

Laverov Federal Center for Integrated Arctic Research, the Ural Branch of RAS, 20, Nikolsky prosp., Arkhangelsk Province, Arkhangelsk, 163020 Russia, e-mail 1711tantan@gmail.com, kogevnikovais@yandex.ru (✉ corresponding author), sharova-marina1999@mail.ru
ORCID:
Tokarchuk T.A. orcid.org/0009-0005-1163-8856
Kudrina M.A. orcid.org/0000-0002-8255-2989
Kozhevnikova I.S. orcid.org/0000-0001-7194-9465
The authors declare no conflict of interests
Acknowledgements:
Prepared as part of the state task of the Laverov Federal Center for Integrated Arctic Research, Ural Branch of RAS "Development of a system for the production of high-quality and environmentally safe dairy products on the territory AZ RF based on the genotyped breeding animals" (FUUW-2021-0005) (registration number – 121122800216-6).
Final revision received August 3, 2022
Accepted December 14, 2022

Abstract

High-quality food products play an important role in a healthy lifestyle in the modern world. Cow's milk and milk products contain all the essential nutrients. The main components of milk are water, fat, protein, lactose, minerals, vitamins, etc. (P.C. Wynn et al., 2013). With the advent of A2 milk on the market, which reduces the symptoms of lactose intolerance and has good digestibility, the population's demand for this product began to grow. A2 milk is obtained from cows of the A2 genotype for β-casein. Cow's milk is 3.5 % protein (P. Feng et al., 2020). Casein is the most important protein component of milk and makes up about 80 % of the total protein composition of cow's milk. Casein consists of four fractions, the αs1, αs2, β and κ. β-Casein is a protein that is one of the main ones in cow's milk and makes up most of all casein. β-Casein consists of 209 amino acid residues, of which 16.7 % is proline, evenly distributed over the polypeptide, which limits the formation of the α-helix (S. Pattanayak, 2013). More than 95 % of casein in milk is in micellar form. The β-casein gene has 13 allelic variants, among which types *A1* and *A2* are the most studied, differing in the sequence of amino acids in the primary structure. Since the primary structure of the protein is different, A1 and A2 β-caseins broken down in the human gastrointestinal tract form different bioactive peptides. For *A1* allele, β-casomorphin-7, a peptide consisting of seven amino acid residues is formed. The level of this peptide is 4 times higher in A1 milk than in A2 milk. When using milk containing β-casein type A1, 12 h after consumption, people may experience bloating, abdominal pain, flatulence, heaviness in the stomach, changes in the frequency and consistency of stools, in some cases, symptoms of celiac disease. Consumption of milk containing type A1 β-casein leads to a significantly longer transit time through the gastrointestinal tract (6.3 hours longer), inflammation of the small intestine and inflammation of the gastric mucosa compared to drinking milk containing A1 type of β-casein. People with lactose intolerance have adverse gastrointestinal symptoms after drinking milk, which may be associated with the presence of β-casein A1 in milk, and not with lactose itself (H. Brüssow, 2013; D. Hu et al., 2014). Unlike variant A1, β-casein A2 increases 2-gold the natural production of glutathione, one of the most important antioxidants of the human body.

Keywords: casein, β-casein, A1 milk, A2 milk, cow's milk, milk proteins

Cow's milk and dairy products are an important part of the traditional diet of people in many countries around the world. The variety of dairy products is due to the chemical composition of milk, the main components of which are water, fat, protein, lactose, minerals, and vitamins [1].

In the modern world, disease prevention and a healthy lifestyle play an important role, so agricultural and food companies are interested in supplying high-quality products to the market [2-4]. With the advent of A2 milk on the US market in 2015, which reduces the symptoms of lactose intolerance and has good digestibility, public demand for this product began to grow. A2 milk is produced by cows of the A2 genotype for β-casein [5, 6].

Currently, molecular genetics methods identify genes that control certain useful traits of farm animals [7-9]. The study of polymorphism of milk protein genes, in particular β-casein is of significant interest for population genetic research.

The purpose of the presented review is to summarize new data on β-casein of cow's milk which have been obtained over the past few years due to a more in-depth study of both the biochemical properties of milk and dairy products, and the genetic characteristics of animals.

The digestibility of milk depends on its protein composition. Cow's milk is 3.5% protein on average [10, 11]. The protein composition significantly influences the nutritional value of milk and, in combination with all its constituent components, determines its technological properties [10]. The milk protein composition is complex and varies in structure, physicochemical properties and biological functions. There are three groups of milk proteins, the caseins, whey proteins and fat globule membrane proteins [11].

Casein is a group of phosphoproteins and makes up approximately 80% of the protein in cow's milk. The molecular weight of the casein fraction is 19000-25000 Da [12-14]. The bovine casein genes are located on chromosome 6q31-33 [15-17]. There are four main casein fractions, the αs1 (18% of total caseins), αs2 (8-11%), β (25-35%) and κ (18-15%) [18-20]. That is, the most common is β-casein [21].

β-Casein and its structure. Beta casein is a protein that accounts for 30 to 35% of all milk proteins [15]. Its molecules are the most hydrophobic and contain a large amount of proline. The β-casein molecule has a negatively charged hydrophilic region from 1 to 43 a.a. and a hydrophobic segment with a high proline content in the region from 44 to 209 a.a. This determines the similarity of β-casein with the structure of surfactants. The solubility of β-casein in water is better than that of other caseins, especially at low temperatures [22-24].

β-Casein is an amphiphilic phosphoprotein with an isoelectric point pH 4.8-5.1. It has a highly hydrophilic negatively charged N-terminal region and a hydrophobic C-terminal region [25-27]. It differs from α-casein in its pronounced dependence on temperature and the temperature dependence of its solubility in the presence of calcium ions. β-Casein is more hydrophobic than α- or κ -casein. It contains five phosphoserine residues in the hydrophilic region which give it a net negative charge under the neutral pH of milk [26, 28].

β-Casein is a chain of 209 amino acid (a.a.) residues of which 16.7% is proline evenly distributed throughout the polypeptide, which limits the formation of an α-helix.

More than 95% of the casein in milk is in micellar form. Casein micelles are nearly spherical particles ranging in size from 30 to 300 nm. Each micelle contains from several thousand to hundreds of thousands of molecules of all types of caseins. The properties and chemical composition of micelles ensure their high stability and stability in the absence of tertiary protein structure [29, 30].

The structure of the casein micelle has not been fully established. Two models based on submicelles and internal structure have been proposed. The internal structure model corresponds to a continuous three-dimensional network of folded αs1-casein polypeptide chains, to which æ-casein binds via micellar calcium phosphate; β-casein is held on the carcass by weak hydrophobic bonds and can easily leave the micelle and re-enter it. According to the submicelle model, micelles are composed of hundreds of discrete particles, the submicelles with a diameter of 10-20 nm in which casein molecules are hydrophobically bonded to each other. There are two types of submicelles, the F2 and F3. F2 submicelles consist of αs1- and æ-caseins, F3 of α- and β-caseins. Submicelles are retained within micelles mainly with the participation of colloidal calcium phosphate (Fig. 1).

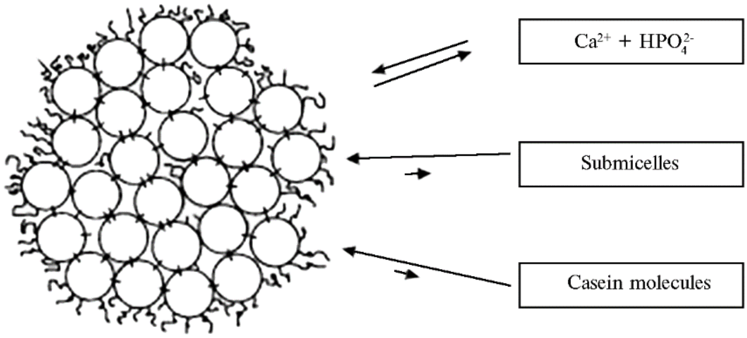

Fig. 1. Model of β-casein micelle from cow's milk. Consists of spherical submicelles bound to each other by calcium phosphate (29, 30).

The of β-casein submicelle formation is reversible. Their stability is affected by temperature, salt concentration and pH. β-Casein submicelles are more stable than low molecular weight surfactant micelles, similar to diblock copolymer micelles [29-31].

β-Casein molecules tend to self-associate in solution due to the large hydrophobic part in their structure. They remain as monomers at low temperatures (up to 5 °C) at concentrations above the critical micelle concentration, and with increasing temperature they self-associate due to hydrophobic interactions in the core of the spherical aggregate, leaving a hydrophilic outer coating [32]. The degree of polymerization of the β-casein molecule directly depends on temperature [28, 33]. Regarding the hydrophilic region, which gives a high negative charge density, β-caseins are able to bind strongly by ionic interactions the divalent ions, especially Ca^{2+}, through phosphoserine, aspartic acid, glutamic acid and free carboxyl groups. The addition of $CaCl_2$ and an increase in temperature lead to the formation of large β-casein aggregates linked by divalent bridges established with residual phosphoserine groups, which is a reversible reaction when the temperature is decreased [31, 34].

Studies carried out using various methods (circular dichroism spectroscopy and Fourier-transform infrared spectroscopy) have shown a conformational change in the structure of β-casein which creates a more hydrophobic environment. C-terminal amino acids have been shown to provide the hydrophobic environment necessary for protein self-association and the formation of micellar aggregates [30-32].

The self-association of β-casein is micelle-like. Both ionic strength and temperature increase the amount of polymer present, that is, they increase the association constant and the degree of association [31]. The number of monomeric protein constituents in these micelle-like structures — almost spherical polymers ranges from 15 at an ionic strength of 0.1 mol/l (pH 7 and 20 °C) to 52 at 0.110 mol/l (pH 6.7 and 37 °C). In this case, structures containing β-casein directly are in a molten globule-like state [29, 32]. Submicelles with these properties have been used to prepare encapsulates of therapeutic hydrophobic molecules.

β-Casein is the most active of all milk proteins, it is the first of the caseins to position itself at the oil-water interface, the presence of phosphoserine in the structure gives thickness and steric stability to the adsorbed layer surrounding the milk protein. β-Casein serves as a precursor to peptides with various biological activities, small peptides with antihypertensive properties [31, 35].

Polymorphism of β-casein. In cow's milk, 13 different forms of β-casein have been identified, and genetic variants for this protein are A1, A2, A3, B, C, D, E, F, H1, H2, I, and G [36, 37]. Genetic variants of β-casein can be detected by electrophoretic separation under acidic or alkaline conditions [38-40]. Variants A can be distinguished from B, C and D under alkaline conditions and from each other only under acidic conditions [34, 41, 42]. In breeding, it is important to identify connections between genotypes of milk protein genes and traits of cow productivity. Thus, genetic variant B of β-casein is associated with increased fat and casein content in milk, variant A is associated with increased milk yield [43-45].

1 10 20
H-Arg-Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-***SeP***-Leu-***SeP***-***SeP***-***SeP***-Glu-
21 30 40
Glu-Ser-Ile-Thr-Arg-Ile-Asn-Lys-Lys-Ile-Glu-Lys-Phe-Gln-***SeP***-Glu-Glu-Gln-Gln-Gln-
41 50 60
Thr-Glu-Asp-Glu-Leu-Gln-Asp-Lys-Ile-His-Pro-Phe-Ala-Gln-Thr-Gln-Ser-Leu-Val-Tyr-
61 70 80
Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser-Leu-Pro-Gln-Asn-Ile-Pro-Pro-Leu-Thr-Gln-Thr-
81 90 100
Pro-Val-Val-Val-Pro-Pro-Phe-Leu-Gln-Pro-Glu-Val-Met-Gly-Val-Ser-Lys-Val-Lys-Glu-
101 110 120
Ala-Met-Ala-Pro-Lys-His-Lys-Glu-Met-Pro-Phe-Pro-Lys-Tyr-Pro-Val-Glu-Pro-Phe-Thr-
121 130 140
Glu-Ser-Gln-Ser-Leu-Thr-Leu-Thr-Asp-Val-Glu-Asn-Leu-His-Leu-Pro-Leu-Pro-Leu-Leu-
141 150 160
Gln-Ser-Trp-Met-His-Gln-Pro-His-Gln-Pro-Leu-Pro-Pro-Thr-Val-Met-Phe-Pro-Pro-Gln-
161 170 180
Ser-Val-Leu-Ser-Leu-Ser-Gln-Ser-Lys-Val-Leu-Pro-Val-Pro-Gln-Lys-Ala-Val-Pro-Tyr-
181 190 200
Pro-Gln-Arg-Asp-Met-Pro-Ile-Gln-Ala-Phe-Leu-Leu-Tyr-Gln-Glu-Pro-Val-Leu-Gly-Pro-
201 209
Val-Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val-OH

Fig. 2. The primary sequence for the most common genetic variant of β-casein A2, the β-CN A2-5P (35, 61, 62). SeP residues identified as phosphorylated are in italics and bold.

Among the genetic variants of β-casein, A1 and A2 are the most studied [46-48]. A1 differs from A2 in only one amino acid, at position 67, the replacement of cytosine with adenine led to the replacement of proline with histidine [49-51]. Detection and quantitation of A2 β-casein is mandatory for A2 dairy products [14, 25, 52]. β-Casein A2 is believed to be the original β-casein protein because a point mutation caused the emergence of β-casein A1 in European cattle several thousand years ago [53-55]. β-Casein A1 is the most common type found in cow's milk produced in Europe (except France), USA, Australia and New Zealand [56-58]. The primary sequence for the most common A2 variant, the β-CN A2-5P [34, 59, 60], is shown in Figure 2.

Caseins and whey proteins (β-lactoglobulin and β-lactalbumin) are the two main groups of milk proteins [61-63]. Casein genes are candidate genes for milk protein biosynthesis [41, 64, 65]. A common source of bioactive peptides are proteins found in cow's milk. Bioactive peptides are released through the enzymatic hydrolysis of caseins and whey proteins by gastrointestinal enzymes. In vitro, the bioactive peptide β-casomorphin-7 is produced by digestion of β-casein A1, but not β-casein A2 [66-68].

Effect of β-casomorphin-7 on the function of the gastrointestinal tract. Since β-casomorphine-7 has morphine-like activity and is believed to cause diseases such as type 2 diabetes mellitus [69], sudden neonatal death syndrome [70], coronary heart disease and cardiovascular diseases [71, 72], increases the risk of cancer diseases [73], may contribute to the development of autism in children [74], studying the frequency of genetic variants A1 and A2 is of interest.

In 12 h after consuming milk containing type A1 β-casein, people experience bloating, abdominal pain, flatulence, heaviness in the stomach, changes in stool frequency and consistency, and in some cases symptoms of celiac disease [75-78]. Consumption of such milk is associated with a significant increase in colon transit time (6.6 h longer) and gastrointestinal transit time (6.3 h longer) compared to milk of A1 type β-casein and causes inflammation in the small intestine and gastric mucosa [79-81].

Milk containing A1 β-casein causes greater worsening of gastrointestinal symptoms and increased gastrointestinal transit time in lactose-intolerant individuals than in lactose-tolerant individuals, whereas milk containing A2 β-casein does not worsen symptoms of lactose intolerance [82-84]. This suggests that in some people with lactose intolerance, adverse gastrointestinal symptoms after consuming milk may be related to the presence of β-casein A1 in milk rather than lactose itself [76, 85, 86].

Milk containing A2 β-casein reduces acute gastrointestinal symptoms compared to milk containing A1 β-casein [76, 87]. In the human body, β-casein A2 approximately doubles the natural production of glutathione, an antioxidant that is involved in detoxification processes [88-90].

In β-casein A2, the presence of proline instead of histidine prevents hydrolysis of the peptide bond between amino acid residues 66 and 67 and inhibits the production of β-casomorphin-7 [91, 92]. During sequential gastrointestinal digestion of milk containing β-casein A1, the amount of β-casomorphin-7 formed is 4-fold higher than during the digestion of A2 milk [93-95]. Unlike the A1 allele, which can be a risk factor for human health, the A2 allele has breeding value [96-98].

Thus, cow's milk contains all the nutrients necessary for humans, including an average of 3.5% protein most of which is casein. The most common is β-casein. More than 95% of the casein in milk is in micellar form. At least 13 genetic variants of β-casein are described. Types A1 and A2 are the most studied and differ from each other by one amino acid at position 67 of the amino acid chain, histidine in A1 vs. proline in A2. The presence of histidine causes the release of the bioactive peptide β-casomorphin-7 during proteolysis of β-casein A1 in the intestine, while proline in protein A2 prevents rupture of the polypeptide sequence at this critical site. The bioactive peptide β-casomorphine-7 has morphine-like activity and affects the gastrointestinal tract and central nervous system of humans. It is believed that there is a correlation between the consumption of milk containing A1 β-casein and the incidence of human diseases such as type 1 diabetes, coronary heart disease and gastrointestinal digestive discomfort. Further study of the β-casein gene and the proteins it encodes will provide a more complete knowledge on metabolism of proteins of cow's milk and dairy products containing β-casein A1 and A2 and their impact on human health.

REFERENCES

1. Wynn P.C., Sheehy P.A. Minor proteins, including growth factors. In: *Advanced Dairy Chemistry*. P. McSweeney, P. Fox (eds.). Springer, Boston, MA, 2013, 1A: 317-335 (doi: 10.1007/978-1-4614-4714-6).
2. Kovalyuk N.V., Satsuk V.F., Machul'skaya E.V., Shakhnazarova Yu.Yu. *Effektivnoe zhivotnovodstvo*, 2018, 5(144): 22-23 (in Russ.).
3. Chaitskiy A.A., Baranova N.S. *Vestnik APK Verkhnevolzh'ya*, 2021, 2(54): 22-28 (doi: 10.35694/YARCX.2021.54.2.004) (in Russ.).
4. Valitov F.R. *Izvestiya Orenburgskogo gosudarstvennogo agrarnogo universiteta*, 2017, 1(63): 207-209 (in Russ.).
5. Sheyko I.P., Klimets N.V., Pesotskiy N.I., Shemetovets Zh.I. *Aktual'nye problemy intensivnogo razvitiya zhivotnovodstva*, 2022, 25-1: 40-46 (in Russ.).

6. Paramonova M.A., Valitov F.R., Ganieva I.N. *Sbornik nauchnykh statey po itogam mezhdunarodnoy nauchno-prakticheskoy konferentsii «Sovremennye nauchnye gipotezy i prognozy: ot teorii k praktike»* [Proc. Int. Conf. «Modern scientific hypotheses and forecasts: from theory to practice»]. St. Petersburg, 2021: 84-86 (in Russ.).
7. Epishko O.A., Peshko V.V., Tanana L.A., Peshko N.N., Mazurek B.G. *Materialy Mezhdunarodnoy nauchno-prakticheskoy konferentsii «Vklad nauki i praktiki v obespechenie prodovol'stvennoy bezopasnosti strany pri tekhnogennom ee razvitii»* [Proc. Int. Conf. «The contribution of science and practice to ensuring the country's food security during its technogenic development»]. Bryansk, 2021: 114-117 (in Russ.).
8. Tyul'kin S.V. *Pishchevye sistemy*, 2018, 1(3): 38-43 (in Russ.).
9. Giglioti R., Gutmanis G., Katiki L.M., Okino C.H., de Sena Oliveira M.C., Vercesi Filho A.E. New high-sensitive rhAmp method for A1 allele detection in A2 milk samples. *Food Chemistry*, 2020, 313: 126167 (doi: 10.1016/j.foodchem.2020.126167).
10. Fox P.F., Uniacke-Lowe T., McSweeney P.L.H., O'Mahony J.A. Milk Proteins. In: *Dairy chemistry and biochemistry*. Springer, Cham, 2015: 145-239 (doi: 10.1007/978-3-319-14892-2_4).
11. Gorbatova K.K., Gun'kova P.I. *Biokhimiya moloka i molochnykh produktov* [Biochemistry of milk and dairy products]. St. Petersburg, 2010 (in Russ.).
12. Ristanic M., Glavinic U., Vejnovic B., Maletic M., Kirovski D., Teodorovic V., Stanimirovic Z. Beta-casein gene polymorphism in Serbian holstein-friesian cows and its relationship with milk production traits. *Acta Veterinaria*, 2021, 70(4): 497-510 (doi: 10.2478/acve-2020-0037).
13. Sarode A.R., Sawale P.D., Khedkar C.D., Kalyankar S.D., Pawshe R.D. Casein and caseinate: methods of manufacture. In: *Encyclopedia of food and health*. A.R. Sarode (ed.). Academic Press, 2016: 676-682 (doi: 10.1016/B978-0-12-384947-2.00122-7).
14. Jaiswal K., De S., Sarsavan A. Detection of single nucleotide polymorphism by T-ARMS PCR of cross bred cattle Karan Fries for A1, A2 beta casein types. *International Journal of Scientific Research in Biological Science*, 2014, vol. 1: 18-22.
15. Haq M.R.U., Kaplia R., Sharma R., Saliganti V., Kapila S. Comparative evaluation of cow β-casein variants (A1/A2) consumption on Th2-mediated inflammatory response in mouse gut. *European Journal of Nutrition*, 2013, 53(4): 1039-1049 (doi: 10.1007/s00394-013-0606-7).
16. Bhat M.Y., Dar T.A., Singh L.R. *Casein proteins: structural and functional aspects. In:Gigli I, ed. Milk Proteins — From Structure to Biological Properties and Health Aspects*. InTechOpen, UK, 2016: 1-18 (doi: 10.5772/64187).
17. Pattanayak S. Human health impact of bovine betacasomorphin-7 of milk — a question for milk products or our breeding policy? *Exploratory Animal and Medical Research*, 2013, 3(2): 93-94.
18. Rangel A.H.N., Zaros L.G., Lima T.C., Borba L.H.F., Novaes L.P., Mota L.F.M., Silva M.S. Polymorphism in the Beta Casein Gene and analysis of milk characteristicsin Gir and Guzera dairy cattle. *Genetics and Molecular Research*, 2017, 16(2): 16029592 (doi: 10.4238/gmr16029592).
19. de Vitte K., Kerziene S., Klementavičiūtė J., de Vitte M., Mišeikienė R., Kudlinskienė I., Čepaitė J., Dilbiene V., Stankevičius R. Relationship of β-casein genotypes (A1A1, A1A2 and A2A2) to the physicochemical composition and sensory characteristics of cows' milk. *Journal of Applied Animal Research*, 2022, 50(1): 161-166 (doi: 10.1080/09712119.2022.2046005).
20. Andiç S., Ayaz R.M., Oğuz Ş. A1 milk and beta-casomorphin-7. *Food Health*, 2021, 7(2): 128-137 (doi: 10.3153/FH21014).
21. Miluchová M., Gábor M., Trakovická A. Analysis of beta-casein gene (CSN2) polymorphism in different breeds of cattle. *Scientific Papers Animal Science and Biotechnologies*, 2014, 47: 56-59.
22. Tepel A. *Khimiya i fizika moloka* [Chemistry and physics of milk]. St. Petersburg, 2021 (in Russ.).
23. Singh L.V., Jayakumar S., Sharma A., Gupta S.K., Dixit S.P., Gupta N., Gupta S.C. Comparative screening of single nucleotide polymorphisms in β-casein and κ-casein gene in different livestock breeds of India. *Meta Gene*, 2015, 4: 85-91 (doi: 10.1016/j.mgene.2015.03.005).
24. Feng P., Ding X., Gao T., Du R., Chen H. Determination of A2 β-casein and total β-casein in cow milk and milk powder by capillary zone electrophoresis. *Chinese Journal of Chromatography (Se Pu)*, 2020, 38(6): 722-729.
25. Kay S.-I.S., Delgado S., Mittal J., Eshraghi R.S., Mittal R., Eshraghi A.A. Beneficial Effects of Milk Having A2 β-Casein Protein: Myth or Reality? *The Journal of Nutrition*, 2021, 151(5): 1061-1072 (doi: 10.1093/jn/nxaa454).
26. Kovalyuk N.V., Satsuk V.F., Kovalyuk M.A., Machul'skaya E.V. *Genetika i razvedenie zhivotnykh*, 2019, 1: 22-26 (in Russ.).
27. Mayer H.K., Lenz K., Halbauer E.-M. "A2 milk" authentication using isoelectric focusing and different PCR techniques. *Food Research International*, 2021, 147: 110523 (doi: 10.1016/j.foodres.2021.110523).
28. Roin N.R., Larsen L.B., Comi I., Devold T.G., Eliassen T.I., Inglingstad R.A., Vegarud G.E., Poulsen N.A. Identification of rare genetic variants of the S-caseins in milk from native Norwegian dairy breeds and comparison of protein composition with milk from high-yielding Norwegian Red cows. *Journal of Dairy Science*, 2022, 105(2): 1014-1027 (doi: 10.3168/jds.2021-20455).
29. Bielecka M., Cichosz G., Czeczot H. Antioxidant, antimicrobial and anticarcinogenic activities of bovine milk proteins and their hydrolysates. *International Dairy Journal*, 2022, 127: 105208 (doi: 10.1016/j.idairyj.2021.105208).

30. Khitsenko A.V., Rogozinnikova I.V. *Molodezh' i nauka*, 2019, 3: 96-101 (in Russ.).
31. Rocha-Mendoza D., Jiménez-Flores R. Casein nomenclature, structure, and association. In: *Encyclopedia of dairy sciences (Third Edition)*. P.L.H. McSweeney, J.P. McNamara (eds.). Academic Press, 2022 (doi: 10.1016/B978-0-12-818766-1.00277-4).
32. Faĭzullin D.A., Konnova T.A., Haertle T., Zuev I. Self-association and secondary structure of beta-casein. *Russian Journal of Bioorganic Chemistry*, 2013, 39(4): 366-372 (doi: 10.1134/s1068162013040067).
33. Yang T., Liu D., Zhou P. Temperature-dependent dissociation of human micellar β-casein: Implications of its phosphorylation degrees and casein micelle structures. *Food Chemistry*, 2022, 376: 131935 (doi: 10.1016/j.foodchem.2021.131935).
34. Hernell O. Human milk vs. cow's milk and the evolution of infant formulas. *Nestlé Nutrition Institute Workshop Series: Pediatric Program*, 2011, 67: 17-28 (doi: 10.1159/000325572).
35. Gallinat J.L., Qanbari S., Drögemüller C., Pimentel E.C.G., Thaller G., Tetens J. DNA-based identification of novel bovine casein gene variants. *Journal of Dairy Science*, 2013, 96(1): 699-709 (doi: 10.3168/jds.2012-5908).
36. Massella E., Piva S., Giacometti F., Liuzzo G., Zambrini A.V., Serraino A. Evaluation of bovine beta casein polymorphism in two dairy farms located in northern Italy. *Italian Journal of Food Safety*, 2017, 6(3): 131-133 (doi: 10.4081/ijfs.2017.6904).
37. Sodhi M., Kataria R.S., Niranjan S.K., Parvesh K., Verma P., Swami S.K., Sharma A., Bharti V.K., Kumar B., Iqbal M., Rabgais S., Kumar P., Giri A., Kalia S., Gagoi D., Sarangi P.P., Mukesh M. Sequence characterisation and genotyping of allelic variants of beta casein gene establishes native cattle of Ladakh to be a natural resource for A2 milk. *Life Science Journal*, 2018, 3(2): 177-181.
38. Il'ina A.V., Abramova M.V., Zyryanova S.V. *Vestnik Buryatskoy gosudarstvennoy sel'skokhozyaystvennoy akademii im. V.R. Filippova*, 2018, 4(53): 55-62 (in Russ.).
39. Ganguly I., Kumar S., Gaur G., Singh U., Kumar A., Kumar S., Mann S., Sharma A. Status of β-casein, CSN2, polymorphism in frieswal, HF X Sahiwal cross bred cattle. *Int. J. Biotechnol. Bioeng. Res.*, 2013, 4: 249-256.
40. Valitov F.R. *Materialy Mezhdunarodnoy nauchno-prakticheskoy konferentsii v ramkakh XXVI Mezhdunarodnoy spetsializirovannoy vystavki «Agrokom-pleks-2016»* [Proc. Int. Conf. within the framework of the XXVI International specialized exhibition Agrokom-plex-2016]. Ufa, 2016, chast' II: 2730 (in Russ.).
41. Amalfitano N., Macedo Mota L.F., Rosa G.J.M., Cecchinato A., Bittante G. Role of *CSN2*, *CSN3*, and *BLG* genes and the polygenic background in the cattle milk protein profile. *Journal of Dairy Science*, 2022, 105(7): 6001-6020 (doi: 10.3168/jds.2021-21421).
42. Marzanov N.S., Abylkasymov D.A., Devrishov D.A., Marzanova S.N., Libet I.S. *Aktual'nye voprosy molochnoy promyshlennosti, mezhotraslevye tekhnologii i sistemy upravleniya kachestvom*, 2020, 1, 1(1): 368-376 (in Russ.).
43. Kostyunina O.V., Konovalova E.N., Dolmatova I.Yu., Rakina Yu.A., Gladyr' E.A. *Dostizheniya nauki i tekhniki APK*, 2013, 3: 64-67 (in Russ.).
44. Pandey A., Thakur M.S., Pandey Y. Association study of polymorphic varients of beta (b) casein gene with milk production traits (lactose, snf and density) in Malvi, Nimari, Sahiwal and H.F. cross breeds cow. *Indian Journal of Animal Research*, 2021, 55(1): 1-5 (doi: 10.18805/IJAR.B-3896).
45. Paramonova M.A., Valitov F.R., Kononenko T.V. *Materialy Mezhdunarodnoy nauchno-prakticheskoy konferentsii, posvyashchennoy 120-letiyu so dnya rozhdeniya professora O.A. Ivanovoy «Dostizheniya i aktual'nye problemy genetiki, biotekhnologii i selektsii zhivotnykh»* [Proc. Int. Conf. «Achievements and current problems of genetics, biotechnology and animal breeding»]. Vitebsk, 2021: 38-42 (in Russ.).
46. Pal S., Woodford K., Kukuljan S., Ho S. Milk intolerance, beta-casein and lactose. *Nutrients*, 2015, 7(9): 7285-7297 (doi: 10.3390/nu7095339).
47. ul Haq M.R., Kapila R., Shandilya U.K., Kapila S. Impact of milk derived β-casomorphins on physiological functions and trends in research: a review. *International Journal of Food Propertie*s, 2014, 17(8): 1726-1741 (doi: 10.1080/10942912.2012.712077).
48. Khalid R.B., Nadeem A., Javed M., Shabbir M.Z., Babar M.E. Characterization of β-casein gene sequence variants in cholistani cattle. *Pakistan Journal of Zoology*, 2021, 53(5): 1929-1937 (doi: 10.17582/journal.pjz/20200610120622).
49. Poulsen N.A., Glantz M., Rosengaard A.K., Paulsson M., Larsen L.B. Comparison of milk protein composition and rennet coagulation properties in native Swedish dairy cow breeds and high-yielding Swedish Red cows. *Journal of Dairy Science*, 2017, 100(11): 8722-8734 (doi: 10.3168/jds.2017-12920).
50. Li X., Spencer G.W.K., Ong L., Gras S.L. Beta casein proteins — a comparison between caprine and bovine milk. *Trends in Food Science and Technology*, 2022, 121: 30-43 (doi: 10.1016/j.tifs.2022.01.023).
51. Geeta Chauhan L.G., Kumar K. α-Glucosidase and α-amylase inhibitory properties of A1 and

A2 cow milk casein hydrolysate. *International Journal of Current Microbiology and Applied Sciences*, 2021, 10(2): 2210-2217 (doi: 10.20546/ijcmas.2021.1002.263).
52. Kumar A., Kumar S., Singh R.V., Chauhan A., Kumar A., Sonwane A., Ilayakumar K., Singh R. Investigation of genetic polymorphism at β-casein A1/A2 loci and association analysis with production & reproduction traits in Vrindavani crossbred cows. *Animal Biotechnology*, 2021: 33(7): 1562-1570 (doi: 10.1080/10495398.2021.1917423).
53. Malarmathi M., Senthil K. T., Parthiban M., Muthuramalingam T., Palanisammi A. Analysis of β-casein gene for A1 and A2 genotype using allele specifi c PCR in Kangeyam and Holstein Friesian crossbred cattle in Tamil Nadu. *Indian Journal of Veterinary and Animal Sciences and Research*, 2014, 43: 310-315.
54. Hockey M., Aslam H., Berk M., Pasco J.A., Ruusunen A., Mohebbi M., Macpherson H., Chatterton M.L., Marx W., O'Neil A., Rocks T., McGuinness A.J., Young L.M., Jacka F.N. The Moo'D Study: protocol for a randomised controlled trial of A2 beta-casein only versus conventional dairy products in women with low mood. *Trials*, 2021, 22(1): 899 (doi: 10.1186/s13063-021-05812-6).
55. Mumtaz S. β Casein polymorphism in indigenous and exotic cattle breeds of Pakistan. *Pakistan Journal of Zoology*, 2022, 54(3): 1451-1454 (doi: 10.17582/jornal.pjz/20190307030303).
56. Gorlov I.F., Sycheva O.V., Kononova L.V. *Molochnoe i myasnoe skotovodstvo*, 2016, 6: 18-19 (in Russ.).
57. Marzanov N.S. Abylkasymov D.A., Libet I.S., Devrishov D.A. V sbornike: *Aktual'nye voprosy molochnoy promyshlennosti, mezhotraslevye tekhnologii i sistemy upravleniya kachestvom* [In: Current issues in the dairy industry, cross-industry technologies and quality management systems]. Moscow, 2020: 368-376 (doi: 10.37442/978-5-6043854-1-8-2020-1-368-376) (in Russ.).
58. Khavkin A.I., Vasia M.N., Novikova V.P. *Eksperimental'naya i klinicheskaya gastroenterologiya*, 2021, 196(12): 102-109. (doi: 10.31146/1682-8658-ecg-196-12-102-109) (in Russ.).
59. Khavkin A.I., Vasia M.N., Novikova V.P. *Eksperimental'naya i klinicheskaya gastroenterologiya*, 2021, 12: 110-118 (doi: 10.31146/1682-8658-ecg-196-12-110-118) (in Russ.).
60. Huppertz T., Fox P.F., Kelly A.L. The caseins: structure, stability, and functionality. In: *Proteins in food processing: Second Edition*. Woodhead Publishing, 2018: 49-92 (doi: 10.1016/B978-0-08-100722-8.00004-8).
61. Sharma V., Sharma N., Jawed B. Nautiyal S., Singh R. High resolution melts curve analysis for the detection of A1, A2 β-casein variants in Indian cows. *Journal of Microbiology and Biotechnology Research*, 2013, 3: 144-148.
62. De Gaudry D.K., Lohner S., Schmucker C., Kapp P., Motschall E., Horrlein S., Roger C., Meerpohl J.J. Milk A1 p-casein and health-related outcomes in humans: a systematic review. *Nutrition Reviews*, 2019, 77(5): 278-306 (doi: 10.1093/nutrit/nuy063).
63. Daniloski D., McCarthy N.A., Markoska T., Auldist M.J., Vasiljevic T. Conformational and physicochemical characteristics of bovine skim milk obtained from cows with different genetic variants of β-casein. *Food Hydrocolloids*, 2022, 124(Part A): 107186 (doi: 10.1016/j.foodhyd.2021.107186).
64. Yudin N.S., Voevoda M.I. Molecular genetic markers of economically important traits in dairy cattle, Russ. *Genetika*, 2015, 51(5): 506-517 (doi: 10.1134/S1022795415050087).
65. Duarte-Vázquez M.Á., García-Ugalde C., Villegas-Gutiérrez L.M., García-Almendárez B.E., Rosado J.L. Production of cow's milk free from beta-casein A1 and its application in the manufacturing of specialized foods for early infant nutrition. *Foods*, 2017, 6(7): 50 (doi: 10.3390/foods6070050).
66. Bodnár Á., Hajzser A., Egerszegi I., Póti P., Kuchtík J., Pajor F. A2 milk and its importance in dairy production and global market. *Animal Welfare, Ethology and Housing Systems*, 2018, 14(1): 1-7 (doi: 10.17205/SZIE.AWETH.2018.1.001).
67. Ho S., Woodford K., Kukuljan S., Pal S. Comparative effects of A1 versus A2 beta-casein on gastrointestinal measures: a blinded randomised cross-over pilot study. *European Journal of Clinical Nutrition*, 2014, 68(9): 994-1000 (doi: 10.1038/ejcn.2014.127).
68. Auestad N., Layman D.K. Dairy bioactive proteins and peptides: a narrative review. *Nutrition Reviews*, 2021, 79(supp_2): 36-47 (doi: 10.1093/nutrit/nuab097).
69. Aune D., Norat T., Romundstad P., Vatten L.J. Dairy products and the risk of type 2 diabetes: A systematic review and dose-response meta-analysis of cohort studies. *American Journal of Clinical Nutrition*, 2013, 98(4): 1066-1083 (doi: 10.3945/ajcn.113.059030).
70. Hu D., Huang J., Wang Y., Zhang D., Qu Y. Dairy foods and risk of stroke: A meta-analysis of prospective cohort studies. *Nutrition, Metabolism and Cardiovascular Diseases*, 2014, 24(5): 460-469 (doi: 10.1016/j.numecd.2013.12.006).
71. Kuellenberg de Gaudry D., Lohner S., Bischoff K., Schmucker C., Hoerrlein S., Roeger C., Schwingshackl L., Meerpohl J.J. A1- and A2 beta-casein on health-related outcomes: a scoping review of animal studies. *European Journal of Nutrition*, 2022, 61: 1-21 (doi: 10.1007/s00394-021-02551-x).
72. Patterson E., Larsson S.C., Wolk A., Åkesson A. Association between dairy food consumption and risk of myocardial infarction in women differs by type of dairy food. *Journal of Nutrition*,

2013, 143(1): 74-79 (doi: 10.3945/jn.112.166330).
73. Aune D., Navarro Rosenblatt D.A., Chan D.S.M., Vieira A.R., Vieira R., Greenwood D.C., Vatten L.J., Norat T. Dairy products, calcium, and prostate cancer risk: A systematic review and meta-analysis of cohort studies. *The American Journal of Clinical Nutrition*, 2015, 101(1): 87-117 (doi: 10.3945/ajcn.113.067157).
74. Cieślińska A., Sienkiewicz-Szłapka E., Wasilewska J., Fiedorowicz E., Chwała B., Moszyńska-Dumara M., Cieśliński T., Bukało M., Kostyra E. Influence of candidate polymorphisms on the dipeptidyl peptidase IV and μ-opioid receptor genes expression in aspect of the β-casomorphin-7 modulation functions in autism. *Peptides*, 2015, 65: 6-11 (doi: 10.1016/j.peptides.2014.11.012).
75. Ramakrishnan M., Eaton T., Sermet O., Savaiano D. A single meal of milk containing A2 β-casein causes fewer symptoms and lower gas production than milk containing both A1 and A2 β-casein among lactose intolerant individuals. *Current Developments in Nutrition*, 2020, 4(suppl 2): 772 (doi: 10.1093/cdn/nzaa052_041).
76. He M., Sun J., Jiang Z.Q., Yang Y.X. Effects of cow's milk beta-casein variants on symptoms of milk intolerance in Chinese adults: a multicentre, randomised controlled study. *Nutrition Journal*, 2017, 16(1): 72 (doi: 10.1186/s12937-017-0275-0).
77. Trivedi M.S., Shah J.S., Al-Mughairy S., Malav S., Hodgson N.W., Simms B., Trooskens G.A., Criekinge W.V., Deth R.C. Food-derived opioid peptides inhibit cysteine uptake with redox and epigenetic consequences. *The Journal of Nutritional Biochemistry*, 2014, 25(10): 1011-1018 (doi: 10.1016/j.jnutbio.2014.05.004).
78. Vivanco-Maroto S.M., Santos-Hernández M., Sanchón J., Picariello G., Recio I., Miralles B. In vitro digestion of milk proteins including intestinal brush border membrane peptidases. Transepithelial transport of resistant casein domains. *Food Research International*, 2022, 157: 111238 (doi: 10.1016/j.foodres.2022.111238).
79. Brooke-Taylor S., Dwyer K., Woodford K., Kost N. Systematic review of the gastrointestinal effects of A1 compared with A2 β-casein. *Advances in Nutrition*, 2017, 8(5): 739-748 (doi: 10.3945/an.116.013953).
80. Jianqin S., Leiming X., Lu X., Yelland G.W., Ni J., Clarke A.J. Effects of milk containing only A2 beta casein versus milk containing both A1 and A2 beta-casein proteins on gastrointestinal physiology, symptoms of discomfort, and cognitive behavior of people with self-reported intolerance to traditional cows' milk. *Nutrition Journal*, 2015, 15(1): 35 (doi: 10.1186/s12937-016-0147-z).
81. Barnett M.P., McNabb W.C., Roy N.C., Woodford K.B., Clarke A.J. Dietary A1 β-casein affects gastrointestinal transit time, dipeptidyl peptidase-4 activity, and infl ammatory status relative to A2 β-casein in Wistar rats. *International Journal of Food Sciences and Nutrition*, 2014, 65(6): 720-727 (doi: 10.3109/09637486.2014.898260).
82. Jianqin S., Leiming X., Lu X., Yelland G.W., Ni J., Clarke A.J. Effects of milk containing only A2 beta casein versus milk containing both A1 and A2 beta casein proteins on gastrointestinal physiology, symptoms of discomfort, and cognitive behavior of people with self-reported intolerance to traditional cows' milk. *Nutrition Journal*, 2016, 15: 35 (doi: 10.1186/s12937-016-0147-z).
83. Schettini G.P., Lambert S.M., da Silva Souza B.M.P., Costa R.B., de Camargo, G.M.F. Genetic potential of Sindhi cattle for A2 milk production. *Animal Production Science*, 2020, 60(7): 893-895 (doi: 10.1071/an18677).
84. Teixeira D.P., Costa R.B., de Camargo G.M.F. Guzerat indicine cattle and A2 milk production. *Animal Biotechnology*, 2023, 34(2): 467-469 (doi: 10.1080/10495398.2021.1962336).
85. Muntean I.A., Bocsan I.C., Wiest L.K., Pintea I., Dobrican C.T., Duca E., Ureche C., Buzoianu A.D., Deleanu D. Predictive factors for oral immune modulation in cow milk allergy. *Nutrients*, 2022, 14(3): 494 (doi: 10.3390/nu14030494).
86. Kamiński S. Co-occurrence of kappa-casein B and beta-casein A1 alleles in Holstein bulls. *Applied Animal Science*, 2021, 37(2): 122-125 (doi: 10.15232/aas.2020-02094).
87. Brüssow H. Nutrition, population growth and disease: a short history of lactose. *Environmental Microbiology*, 2013, 15(8): 2154-2161 (doi: 10.1111/1462-2920.12117).
88. Sebastiani C., Arcangeli C., Ciullo M., Torricelli M., Cinti G., Fisichella S., Biagetti M. Frequencies evaluation of β-casein gene polymorphisms in dairy cows reared in central Italy. *Animals*, 2020, 10(2): 252 (doi: 10.3390/ani10020252).
89. Kuz'menko N.B., Kuzina A.N. *Lechashchiy vrach*, 2016, 1: 16-19 (in Russ.).
90. Pizzorno J. Glutathione! *Integr. Med. (Encinitas)*, 2014, 13(1): 8-12.
91. Antonopoulos D. Vougiouklaki D., Laliotis G. P., Tsironi T., Valasi I., Chatzilazarou A., Halvatsiotis P., Houhoula D. Identification of polymorphisms of the *CSN2* gene encoding β-casein in greek local breeds of cattle. *Veterinary Sciences*, 2021, 8(11): 257 (doi: 10.3390/vetsci8110257).
92. Boutrou R., Gaudichon C., Dupont D., Jardin J., Airinei G., Marsset-Baglieri A., Benamouzig R., Tomé D., Leonil J. Sequential release of milk protein-derived bioactive peptides in the jejunum in healthy humans. *The American Journal of Clinical Nutrition*, 2013, 97(6): 1314-1323 (doi: 10.3945/ajcn.112.055202).
93. Chia J., McRae J.L., Enjapoori A.K., Lefèvre C.M., Kukuljan S., Dwyer K.M. Dietary Cows'

Milk Protein A1 Beta-Casein Increases the Incidence of T1D in NOD Mice. *Nutrients*, 2018, 10(9): 1291 (doi: 10.3390/nu10091291).

94. Firouzamandi M., Gholami M., Toloui M., Eshghi D. Genetic variation of β-casein gene using AS-PCR and ARMS-PCR techniques in bovine populations. *Russian Journal of Genetics*, 2018, 54(11): 1352-1357 (doi: 10.1134/S1022795418110054).
95. Sorvacheva T.N., Evdokimova T.A., Safronova A.N., Chediya E.S. *Voprosy dietologii*, 2018, 8(3): 42-46 (doi: 10.20953/2224-5448-2018-3-42-46) (in Russ.).
96. Lange K.W., Hauser J., Reissmann A. Gluten-free and casein-free diets in the therapy of autism. *Current Opinion in Clinical Nutrition and Metabolic Care*, 2015, 18(6): 572-575 (doi: 10.1097/MCO.0000000000000228).
97. Bisutti V., Pegolo S., Giannuzzi D. Mota L. F. M., Vanzin A., Toscano A., Trevisi E., Ajmone Marsan P., M. Brasca, Cecchinato A. The β-casein (CSN2) A2 allelic variant alters milk protein profile and slightly worsens coagulation properties in Holstein cows. *Journal of Dairy Science*, 2022, 105(5): 3794-3809 (doi: 10.3168/jds.2021-21537).