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TECHNOLOGICAL PROPERTIES OF MILK OF COWS WITH DIFFERENT GENOTYPES OF KAPPA-CASEIN AND BETA-LACTOGLOBULIN

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Abstract: The presence of the desirable alleles and genotypes of casein and whey protein genes in the genome of cows affects the milk protein content, quality and technological properties of their milk. Two important properties of milk its producibility is judged on are cheeseability and heat resistance. The present studies aimed at estimating the technological properties of milk of black-motley × Holstein and Kholmogorskaya breeds cows of the Tatarstan type with different kappa-casein (*CSN3*) and beta-lactoglobulin (*BLG*) genotypes. The study was carried out using a sampling of the first-calf cows of 5 cattle-breeding farms of the Republic of Tatarstan. In animals, the *CSN3* and *BLG* genotypes have been determined by a PCR-RFLP analysis. The cheeseability, heat resistance and thermostability of milk have been estimated using standard methods. The studies have established that the *CSN3* and *BLG* genotypes of cows affected the condition of a casein clot and duration of milk clotting time. The best cheese-making properties of milk were inherent in the animals with the *BB* and *AB* genotypes of the *CSN3* and *BLG* genes. They were superior to the coevals with the *AA* genotype in terms of the highest yield of the desired dense casein clot and the shortest duration of milk clotting time. The first-calf cows, which are the carriers of an *A* allele of the *CSN3* gene, were superior to the animals with the *BB* genotype of the *CSN3* gene on the thermostability of milk including that on the proportion of animals with this milk characteristic. The *BLG* genotype of the studied animals did not significantly affect the thermostability of milk. Moreover, the highest thermostability of milk was characteristic of black-motley × Holstein cows with the *AA* genotype.

Keywords: Cow, milk, cheeseability, thermostability, allele, genotype, *CSN3*, *BLG*, PCR, RFLP

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INTRODUCTION

The manufacture of dairy products is impossible if dairy raw materials do not meet the requirements for their development. In this context, attention should be paid to two important properties of milk its producibility, namely, the cheeseability and heat resistance are judged on.

The cheeseability of milk is a set of indicators of technological, physical and chemical and hygienic properties, as well as the chemical composition of milk [1]. To produce cheese and cottage cheese, only milk, which can coagulate with the formation of a dense casein clot, can be used when affected by a rennet enzyme [2, 3].

The heat resistance of milk is the technological property of milk to resist high temperatures without protein coagulation [4]. This property of milk is an important condition for the development of sterilized products that are in high consumer demand due to their long shelf life. To manufacture such products, milk is treated at high temperatures (110–160°C) [2, 3].

Therefore, high requirements are imposed to milk as the raw materials used for the manufacture of such dairy products as cottage cheese, cheese, yogurt,

canned food, including gerodietic and functional foods [5–11].

The studies on the technological properties of milk with the involvement of the modern molecular genetic methods of diagnostics in cattle breeding are of particular interest. A lot of countries currently use genetic markers that are related to the qualitative features of dairy productivity [12].

The evidence has been presented that the presence of the "desirable" alleles and genotypes of casein (alpha-casein [13], beta-casein [14] and kappa-casein [15–17]) [18] and whey (beta-lactoglobulin [15, 19] and alpha-lactalbumin [20]) milk proteins in the genome of cows have an effect on milk protein content, quality and technological properties of their milk [3, 21, 22].

In this regard, the present studies aimed at estimating the technological properties of milk of cows of black-motley × Holstein and Kholmogorskaya breeds of the Tatarstan type with different kappa-casein (*CSN3*) and beta-lactoglobulin (*BLG*) genotypes.

In accordance with the aim of the study the following tasks were being solved:

– to genotype the studied sampling of first-calf cows in several farms of the Republic of Tatarstan on the *A* and *B* alleles of the *CSN3* and *BLG* genes by a PCR-RFLP analysis;

– to determine the cheeseability and thermostability of the milk of the studied sampling of first-calf cows depending on their genotype of the *CSN3* and *BLG* genes.

STUDY OBJECTS AND METHODS

The studies were carried out in Agricultural production cooperative named after Lenin and Dusym, LLC of Atninsky District, the LLC named after Tukay of Baltasinsky District, Biryulinskiy Stud Farm, OJSC and Hammer and sickle, LLC in Vysokogorsky District of the Republic of Tatarstan with 608 first-calf cows of the black-motley × Holstein breed and 265 first-calf cows of the Kholmogorskaya breed of the Tatarstan type, respectively.

To carry out molecular genetic studies in animal were collected blood samples from the jugular vein. DNA was extracted from the samples of whole preserved (10 mM of EDTA) blood using a combined alkaline method. *DNA extraction procedure.* 100 µl of blood is mixed with 1 ml of dH_2O and centrifuged at 10,000 rpm for 10 minutes. The resulting supernatant is discarded, and 50 µl of 0.2 M NaOH is added to the precipitate and the mixture is thoroughly vortexed until the suspension is completely clarified. The resulting homogenate is thermostated at 60°C for 10 minutes. A proportional volume of 1 M Tris-HCl (pH 8.0) was added to the lysate followed by the careful vortexing of the mixture. 500 µl of 96% ethanol are added to the resulting homogenate followed by holding the mixture in a freezer (–20°C) for 30 minutes. The nucleoprotein complex is precipitated by centrifugation at 12,000 rpm for 10 minutes. The supernatant is discarded, and the residue is dried at 60°C for 12 minutes by opening the lid of the tube. 100 µl of 10% ammonia are added to the dried precipitate, the mixture is vortexed carefully and thermostated at 60°C for 10 minutes, then vortexed again and held in a thermostat at 60°C for 10 minutes. The resulting homogenate is held in a thermostat at 95°C for 15 minutes with the lid of the tube open.

In animals, the *CSN3* and *BLG* genotypes have been determined by a PCR-RFLP analysis.

The *CSN3* gene was amplified using a Tertzik thermocycler (Russia) in volumes of reaction mixtures (20 µl) containing the appropriate buffer (60 mM Tris-HCl (pH 8.5), 1.5 mM $MgCl_2$, 25 mM KCl, 10 mM 2-Mercaptoethanol and 0.1 mM Triton X-100) 0.2 mM dNTPs, 1 U Taq DNA polymerase (SibEnzyme, Russia), 0.5 mkM of the oligonucleotide primers AB1 and AB2 [23] and 1 µl of a DNA sample as follows:

× 1 : 94°C – 4 min;
× 40 : 94°C – 10 sec, 63°C – 10 sec, 72°C – 10 sec;
× 1 : 72°C – 5 min; storage: 4°C [24].

The RFLP-identification of genotypes on the allelic variants *A* and *B* of the *CSN3* gene was performed by treating 20 µl of a PCR sample of 10 U of the restriction enzyme *Hinf*I in the 1 × buffer "O" (SibEnzyme, Russia) at 37°C overnight.

The *BLG* gene was amplified using a Tertzik thermocycler (Russia) in volumes of reaction mixtures (20 µl) containing the appropriate buffer (60 mM Tris-HCl (pH 8.5), 1.5 mM $MgCl_2$, 25 mM KCl, 10 mM 2-Mercaptoethanol; 0.1 mM Triton X-100), 0.2 mM dNTPs, 1 U of Taq DNA polymerase (SibEnzyme, Russia), 0.5 mkM of the oligonucleotide primers BLGP3 and BLGP4 [25] and 1 µl of a DNA sample as follows:

× 1 : 94°C – 4 min;
× 38 : 94°C – 10 sec, 60°C – 10 sec, 72°C – 10 sec;
× 1 : 72°C – 5 min; storage: 4°C [15].

The RFLP-identification of genotypes on the allelic variants *A* and *B* of the *BLG* gene was performed by treating 20 µl of a PCR sample of 5 U of the restriction enzyme *Hae*III in the 1 × buffer "C" (SibEnzyme, Russia) at 37°C overnight.

Table 1 presents the spectrum of the genotype-specific RFLP fragments generated during the reaction.

The cheeseability of milk was determined with the help of a rennet and rennet fermentation sample. *Preparation of a rennet enzyme solution.* 1 g of rennet powder with an activity of 100 thousand units is dissolved in a mixture of distilled water and glycerol of an equal volume. After 24 hours, the solution is well mixed, filtered through a paper filter, poured into dark dishes and stored in a fridge for no more than 5 days. Immediately before use, the solution is diluted 25 times with distilled water. Then, 10 ml of the same sample of the mixed milk is added into each of three tubes. The tubes with milk are put in a water bath at 35°C, a thermometer is placed in one tube to monitor the water temperature. The milk temperature is brought to 35°C, then 1 ml of the diluted rennet enzyme solution of the same temperature is added into two tubes. The content of the two tubes is quickly mixed and placed in the water bath fixing the time. The temperature is maintained at 35°C. The duration of milk clotting time is determined in minutes, taking into account the time interval from the addition of the rennet to the formation of a dense clot.

Table 1. Primers for genotyping *Bostaurus* on the allelic variants *A* and *B* of the *CSN3* and *BLG* genes, generated PCR products and RFLP fragments

Oligonucleotide primers	PCR-product (bp)	Genotype-specific RFLP fragments (bp)		
		AA	*BB*	*AB*
AB1: 5′-TGTGCTGAGTAGGTATCCTAGTTATGG-3′ AB2: 5′-GCGTTGTCTTCTTTGATGTCTCCTTAG-3	453	*Hinf*I		
		326 100 27	426 27	426 326 100 27
BLGP3: 5′-GTCCTTGTGCTGGACACCGACTACA-3′ BLGP4: 5′-CAGGACACCGGCTCCCGGTATATGA-3′	262	*Hae*III		
		153 109	109 79 74	153 109 79 74

The heat resistance of milk was determined with the help of a thermal (crucible) sample. *Setting a crucible sample.* 2 ml of milk is added into each of molybdenum glass tubes. The tubes with milk are put in an ultrathermostat and heated to a temperature of 135°C fixing the time. If the consistency of milk does not change within 5 minutes, then it is considered heat-resistant.

The thermostability of milk was also determined taking into account the time interval from the moment the tubes were placed in the ultrathermostat until the first signs of protein coagulation.

The variational statistical analysis of the results of the studies was carried out using the biometric method [26]. The reliability of the obtained results of the studies was confirmed by the tabular data of Student's criterion.

RESULTS AND DISCUSSION

The results of cattle genotyping on the *A* and *B* alleles of the *CSN3* and *BLG* genes with the used sets of primers and restriction endonucleases for a PCR-RFLP analysis are satisfactory in terms of the reproducibility and identification of genotypes.

Thus, the primers AB1 and AB2 initiate the amplification of the *CSN3* gene locus of cattle with a length of 453 bp, and the *Hinf*I-RFLP analysis of the generated genotype-specific fragments (*AA* = 326/100/27 bp, *BB* = 426/27 bp and *AB* = 426/326/100/27) provides a correct genotyping procedure (Fig. 1).

The primers BLGP3 and BLGP4 initiate the amplification of the *BLG* gene locus of cattle with a length of 262 bp, and the *Hae*III-RFLP analysis of the generated genotype-specific fragments (*AA* = 153/109 bp, *BB* = 109/79/74 bp and *AB* = 153/109/79/74 bp) provides a correct genotyping procedure (Fig. 2).

The rationality of the use of whole milk for manufacturing protein-milk products, including cheese, is affected by its technological properties, such as coagulability under the influence of a rennet enzyme, the density of the formed casein clot and duration of milk clotting time.

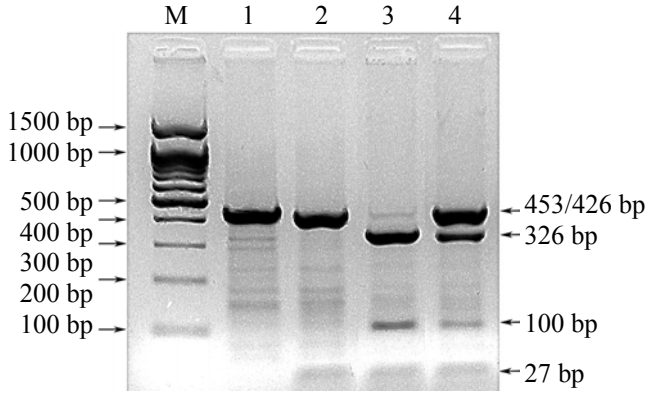

Fig. 1. Electrophoregram of the result of a PCR-RFLP analysis for genotyping *Bos taurus* on the allelic variants *A* and *B* of the *CSN3* gene with the primers AB1 + AB2 and endonuclease digestion with *Hinf*I

Notation: (M) DNA markers 100 bp + 1.5 Kb (SibEnzyme); (1) a PCR product (453 bp); (2–4) *Hinf*I-RFLP profiles: 2) the genotype *BB* (426/27 bp); (3) the genotype *AA* (326/100/27 bp); (4) the genotype *AB* (426/326/100/27 bp).

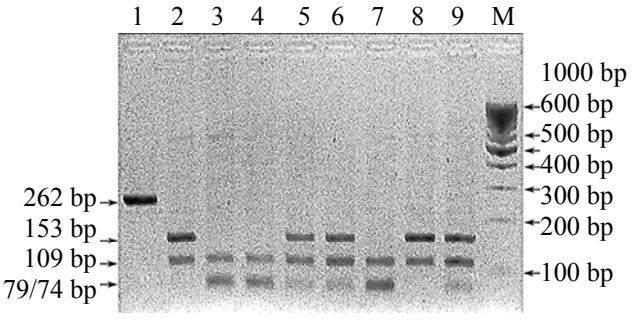

Fig. 2. Electrophoregram of the result of a PCR-RFLP analysis for genotyping *Bos taurus* on the allelic variants *A* and *B* of the *BLG* gene with the primers BLGP3 + BLGP4 and endonuclease digestion with the *Hae*III restriction enzyme

Notation: (M) DNA markers 100 bp (SibEnzyme); (1) a PCR product (262 bp); (2–9) *Hae*III-RFLP profiles: (2, 8) the genotype *AA* (153/109 bp); (3, 4, 7) the genotype *BB* (109/79/74 bp); (5, 6, 9) the genotype *AB* (153/109/79/74 bp).

The study has determined that the kappa-casein (*CSN3*) genotype of cows is associated both with the condition of a casein clot and with duration of milk clotting time. In all three samples, the milk from the cows of the Kholmogorskaya breed of the Tatarstan type with the *AA* genotype of the kappa-casein gene had the worst cheeseability properties. Both friable and flabby casein clots (Tables 2, 3, 4) were obtained from the milk of the cows (46.8–48.6%) of this genotype.

The presence of the allele *B* of the kappa-casein gene in the animal genome significantly affected the improvement of the condition of a casein clot. The proportion of milk with the condition of a casein clot characterized as dense in the cows of the homozygous genotype *BB* was 100%, and in the cows with the heterozygous genotype *AB* – 81.8–84.1%.

The most desirable in cheese-making is milk the clotting time of which when treated with a rennet enzyme is within the range of 15–40 minutes. If the milk clotting time is more than 40 minutes, there is a large loss of raw materials with a low yield of cheese due to a disruption in the manufacturing process. The best indicators on duration of milk clotting time have been noted in the first-calf cows with the genotype *BB* of the kappa-casein gene. The milk of these animals coagulated in the period with the lowest time interval – 16.9–18.2 min. The milk clotting time in the animals with the *AA* genotype turned out to be longer and was 30.4–31.3 minutes ($P < 0.001$).

The similar studies carried out using a single sampling of black-motley × Holstein cows with different genotypes of the kappa-casein gene also showed that there are intergroup differences in the cheese-making properties of milk. The groups of the cows carrying the allele *A* of the kappa-casein gene in their genotype had a higher proportion of animals with the worst condition of a casein clot. Both friable and flabby casein clots were obtained from the milk of 50.0% of the cows with the *AA* genotype (Table 5).

The presence of the allele *B* of the kappa-casein gene in the animal genome had a significant effect on the condition of a casein clot. The proportion of milk with the condition of a casein clot characterized as dense in the cows with the heterozygous genotype *AB* was 80.6%, and in the cows with the homozygous genotype *BB* was equal to 100.0% (Table 5).

Table 2. Cheeseability of milk of the first-calf cows of the Kholmogorskaya breed of the Tatarstan type depending on their *CSN3* genotype in Hammer and Sickle, LLC

Total of cows	Condition of a casein clot and duration of milk clotting time	Distribution of cows		Including that with a *CSN3* genotype					
				AA		*AB*		*BB*	
		n	%	n	%	n	%	n	%
n = 225	dense	141	62.7	82	52.6	53	84.1	6	100
	friable	73	32.4	66	42.3	7	11.1	–	–
	flabby	11	4.9	8	5.1	3	4.8	–	–
	time, min	28.5 ± 0.84		30.6 ± 0.99		24.6 ± 1.32***		18.2 ± 3.40***	

Note. Difference between *BB*, *AB* and *AA* genotypes: *** $P < 0.001$.

Table 3. Cheeseability of milk of the first-calf cows of the Kholmogorskaya breed of the Tatarstan type depending on their *CSN3* genotype in Agricultural Production Cooperative Society named after Lenin

Total of cows	Condition of a casein clot and duration of milk clotting time	Distribution of cows		Including that with a *CSN3* genotype					
				AA		*AB*		*BB*	
		n	%	n	%	n	%	n	%
n = 219	dense	147	67.1	57	51.4	81	81.8	9	100
	friable	56	25.6	43	38.7	13	13.1	–	–
	flabby	16	7.3	11	9.9	5	5.1	–	–
	time, min	27.2 ± 0.34		31.3 ± 0.46		23.6 ± 0.93***		16.9 ± 3.10***	

Note. Difference between *BB*, *AB* and *AA* genotypes: *** $P < 0.001$.

Table 4. Cheeseability of milk of the first-calf cows of the Kholmogorskaya breed of the Tatarstan type depending on their *CSN3* genotype in Biryulinskiy Stud Farm, OJSC

Total of cows	Condition of a casein clot and duration of milk clotting time	Distribution of cows		Including that with a *CSN3* genotype					
				AA		*AB*		*BB*	
		n	%	n	%	n	%	n	%
n = 164	dense	104	63.4	58	53.2	43	82.7	3	100
	friable	50	30.5	44	40.4	6	11.5	–	–
	flabby	10	6.1	7	6.4	3	5.8	–	–
	time, min	27.8 ± 0.59		30.4 ± 0.68		23.1 ± 0.76***		17.3 ± 2.52***	

Note. Difference between *BB*, *AB* and *AA* genotypes: *** $P < 0.001$.

Table 5. Cheeseability of milk of the black-motley × Holstein first-calf cows depending on their *CSN3* genotype in the LLC named after Tukay

Total of cows	Condition of a casein clot and duration of milk clotting time	Distribution of cows		Including that with a *CSN3* genotype					
				AA		*AB*		*BB*	
		n	%	n	%	n	%	n	%
n = 107	dense	66	61.7	34	50.0	29	80.6	3	100
	friable	33	30.8	28	41.2	5	13.9	–	–
	flabby	8	7.5	6	8.8	2	5.5	–	–
	time, min	29.2 ± 0.67		31.7 ± 0.82		25.4 ± 0.78***		18.9 ± 1.81***	

Note. Difference between *BB*, *AB* and *AA* genotypes: *** P < 0.001.

Table 6. Cheeseability of milk of the black-motley × Holstein first-calf cows depending on their *BLG* genotype in the LLC named after Tukay

Total of cows	Condition of a casein clot and the duration of milk clotting time	Distribution of cows		Including that with a *BLG* genotype					
				AA		*AB*		*BB*	
		n	%	n	%	n	%	n	%
n = 107	dense	66	61.7	6	42.8	34	56.6	26	78.8
	friable	33	30.8	6	42.8	22	36.7	5	15.1
	flabby	8	7.5	2	14.4	4	6.7	2	6.1
	time, min	29.2 ± 0.67		33.0 ± 1.23		28.8 ± 0.89**		28.2 ± 1.30**	

Note. Difference between *BB*, *AB* and *AA* genotypes: ** P < 0.01.

Table 7. Cheeseability of milk of the black-motley × Holstein first-calf cows depending on their *BLG* genotype in Dusym, LLC

Total of cows	Condition of a casein clot and the duration of milk clotting time	Distribution of cows		Including that with a *BLG* genotype					
				AA		*AB*		*BB*	
		n	%	n	%	n	%	n	%
n = 158	dense	103	65.2	13	52.0	42	57.5	48	80
	friable	41	25.9	8	32.0	25	34.3	8	13.3
	flabby	14	6.7	4	16.0	6	8.2	4	6.7
	time, min	28.5 ± 0.59		29.8 ± 1.11		29.1 ± 0.95		27.3 ± 0.92	

The best indicators on duration of milk clotting time were characteristic of the first-calf cows with the genotype *BB* of the *CSN3* gene. The clotting time of their milk was the shortest – 18.9 min. The longest clotting time was noted for the milk of the cows with the *AA* genotype and was equal to 31.7 minutes. In this case, the milk from the animals with the heterozygous genotype *AB* was at the intermediate level of the analyzed indicator – 25.4 min. The first-calf cows carrying the allele *B* of the *CSN3* gene in their genome were favorably inferior to their coevals with the *AA* genotype by 6.3–12.8 min (Table 5).

Similar results were obtained when carrying out a rennet test of the milk of the cows with different *CSN3* genotypes in the studies of animals of the Yaroslavl breed [27], of the holsteinized Kholmogorskaya breed of the "Tsentralny" type [28], the Samara type of black-motley cattle [29], of the Ural black-motley breed [17], the red-motley breed of the created Volga type [30], the Volga type of the red-motley breed [31], the Simmental and red-motley breeds [16], the Italian Holstein breed [32], the Danish Jersey and Holstein breeds [33], the dairy breeds of different ecological zones of the Siberia, Sakha (Yakutia) and Macedonia, namely black-motley, Holstein, red steppe and Simmental [34], the Sicilian Cinisara breed [35], Estonian Holstein, red-motley Holstein, Estonian red, the Estonian native breed [36] and the Macedonian Holstein breed [37]. In their studies, the milk from the cows with the *AB* and *BB* genotypes of the *CSN3* gene compared to the milk from the animals with the *AA* genotype when affected by the enzyme had shorter coagulation periods. However, the studies of Norwegian red cattle have provided some other results. Thus, the duration of milk clotting time when affected by a rennet enzyme from the animals with different genotypes of the kappa-casein gene was in the following order: *AB*<*AA*<*BE*<*BB* [38].

It is believed that the whey protein beta-lactoglobulin, like the other protein fractions of whey, does not lend itself to rennet coagulation, and therefore they are absent in cheese mass. Nevertheless, the genetic types of this protein can affect the process of isolating whey from a casein clot and thereby improve the quality of cheese mass [3].

The study revealed that of 2 sampling of black-motley × Holstein first-calf cows with different beta-lactoglobulin (*BLG*) genotypes, the milk of the first-calf cows with the *BB* genotype had the best cheese-making properties. When affected by a rennet enzyme,

a dense casein clot was obtained from the milk of 78.8%–80.0% of cows, and a flabby clot – from only 6.1%–6.7%, respectively (Tables 6 and 7).

The ability of milk to coagulate proved to be worse in the animals with the genotypes *AB* and *AA* of the beta-lactoglobulin gene. Thus, the yield of a dense and flabby clot was 56.6%–57.5% and 6.7%–8.2% (the genotype *AB*), as well as 42.8%–52.0% and 14.4%–16.0% (the genotype *AA*), respectively.

Most of the processing lines for cheese production are designed for the duration of the process of milk clotting to 40 minutes. The increase in milk clotting time leads to an increase in the losses of raw materials and, respectively, to a low cheese yield. The best indicators on clotting time were characteristic of the cows with the genotypes *AB* and *BB* of the beta-lactoglobulin gene. In these groups of animals, the milk clotting occurred for 27.3–29.1 min. This indicator in the cows with the *AA* genotype was the worst and was 29.8–33.0 minutes, which is higher than that in the animals carrying the allele *B* of the *BLG* gene by 0.7–4.8 min.

Similar results were obtained when carrying out a rennet test of the milk of the cows with different *BLG* genotypes in the studies of the domestic Kholmogorskaya breed [3], the Ukrainian black-motley breed [39], the Danish Jersey and Holstein breeds [33], Norwegian red cattle [38], the Russian black-motley and Bestuzhev breeds [22]. In their studies, the milk from the cows with the genotypes *AB* and *BB* of the *BLG* gene, in comparison with the milk from the animals with the genotype *AA*, had shorter coagulation periods when effected by an enzyme. However, different results were obtained in the studies of the Swedish red and Holstein breeds [40], the Estonian Holstein, red-motley Holstein, Estonian red and Estonian local breeds [36] and the French Holstein breed [41]. Thus, duration of milk clotting time when affected by a rennet enzyme from the animals with different *BLG* genotypes was in the following order: *AA*<*AB*<*BB*. The studies of individuals of the Sicilian Cinisara breed with different genotypes of the beta-lactoglobulin gene also showed that the coagulation properties when affected by a rennet enzyme corresponded to the following sequence *BB*<*AA*<*AB* in terms of duration [35].

The long-term storage of milk and dairy products is impossible without high-temperature treatment (63–150°C) which is used for pasteurization, sterilization, thickening and drying. When treated by high temperatures, the product often undergoes irreversible protein coagulation and rapid milk coagulation. Therefore, the solution to the problem related to an increase in the heat resistance of milk is of high practical importance. In this regard, we have studied the heat resistance of milk of the cows with different genotypes of the kappa-casein gene.

The study has determined that the milk of three sampling of first-calf cows of the Tatarstan type with the *BB* genotype of the *CSN3* gene had a lower thermostability (33.1–35.2 min), with the *AA* genotype – a high thermostability (60.1–65.8 min), and that with the genotype *AB* showed an intermediate value (57.5–62.9 min) (Table 8).

It has been determined in the studies of the thermostability of milk of a single sampling of black-motley × Holstein first-calf cows with different *CSN3* genotypes that the milk of cows with the *BB* genotype had a lower thermostability (39.3 min), with the *AA* genotype – increased thermostability (57.2 min), and with the genotype *AB* – an intermediate value (56.5 min). The first-calf cows with the genotype *AA* of the *CSN3* gene were superior to the coevals with the genotypes *BB* and *AB* by 17.9 min and 0.7 min, respectively (Table 9).

Similar results on the heat resistance of milk from the cows with different *CSN3* genotypes were obtained in the studies of Holstein, Ayrshire, Kholmogorskaya and Kholmogor × Holstein cross-breeds of domestic lineage [3], the red-motley breed and the created Volga type of Russian breeding [30]. In their studies, the milk from the cows with the genotypes *AA* and *AB* of the *CSN3* gene had a higher thermostability compared with the milk from the animals with the *BB* genotype. However, the studies of the Bestuzhev breed and domestic Bestuzhev × Ayrshire cross-breeds [3] gave some other results. Thus, the milk from the cows with the genotypes *AB* and *BB* of the *CSN3* gene was more heat resistance compared to the milk from the animals with the *AA* genotype.

Table 8. Thermostability of milk of the first-calf cows of the Kholmogorskaya breed of the Tatarstan type with different *CSN3* genotypes

Farm	Thermostability of milk (min) of cows (head) with different *CSN3* genotypes		
	AA	*AB*	*BB*
Hammer and Sickle, LLC	156 head	63 head	6 head
	65.8 ± 0.72 min	62.9 ± 1.27* min	33.1 ± 2.22*** min
Agricultural Production Cooperative Society named after Lenin	111 head	99 head	9 head
	63.7 ± 1.10 min	60.3 ± 1.25* min	35.2 ± 2.14*** min
Biryulinskiy Stud Farm, OJSC	109 head	52 head	3 head
	60.1 ± 1.82 min	57.5 ± 2.86 min	34.8 ± 3.35*** min

Note. Difference between *BB*, *AB* and *AA* genotypes: * P < 0.05; *** P < 0.001.

Table 9. Thermostability of milk of black-motley × Holstein first-calf cows with different *CSN3* genotypes in the LLC named after Tukay

Farm	Thermostability of milk (min) of cows (head) with different *CSN3* genotypes		
	AA	*AB*	*BB*
LLC named after Tukay	68 head	36 head	3 head
	57.2 ± 1.61 min	56.5 ± 2.52 min	39.3 ± 5.43** min

Note. Difference between *BB*, *AB* and *AA* genotypes: ** P < 0.01.

Table 10. Thermostability of milk of black-motley × Holstein first-calf cows with different *BLG* genotypes

Farm	Thermostability of milk (min) of cows (head) with different *BLG* genotypes		
	AA	*AB*	*BB*
LLC named after Tukay	14 head	60 head	33 head
	58.5 ± 2.87 min	56.3 ± 1.91 min	56.0 ± 2.47 min
Dusym, LLC	25 head	73 head	60 head
	58.9 ± 4.67 min	57.4 ± 2.24 min	52.7 ± 2.52 min

We also carried out a study of the thermostability of milk of two sampling of black-motley × Holstein cows with different *BLG* genotypes. The study showed that the thermostability of milk of first-calf cows with different *BLG* genotypes was within the ranges of 52.7–56.0 min (the genotype *BB*) and 58.5–58.9 min (the genotype *AA*). The animals carrying the *B* allele of the *BLG* gene were inferior in this indicator to the coevals with the genotype *AA* by 1.5–6.2 min (Table 10).

Similar results on the thermostability of milk of cows with different *BLG* genotypes were obtained in the studies of animals of the Russian black-motley breed [19], the Ukrainian black-motley breed [39] and the domestic black-motley and Bestuzhev breeds [22]. In their studies, the milk from the cows with the genotypes *AA* and *AB* of the *BLG* gene had a higher heat resistance compared to the milk from the animals with the genotype *BB*. However, the studies of the Holstein, Ayrshire and Kholmogorskaya breeds and Bestuzhev × Ayrshire cross-breeds of domestic selection [3] gave some other results. Thus, the thermostability of milk with different *BLG* genotypes was expressed in the following order: *AA*<*AB*<*BB*, while the order for the Bestuzhev breed and domestic Kholmogor × Holstein cross-breeds was *AB*<*AA*<*BB*, respectively.

CONCLUSIONS

The selected systems of cattle genotyping on the *A* and *B* alleles of the *CSN3* and *BLG* genes by a PCR-RFLP analysis allowed us to genotype correctly the sampling of first-calf cows in several cattle-breeding farms in the Republic of Tatarstan.

The study of first-calf cows of the Kholmogorskaya breed of the Tatarstan type and black-motley × Holstein cows has shown that the best cheese-making properties of milk are inherent in the animals with the genotype *BB* and *AB* of the *CSN3* gene. Their milk had the highest yield of the desired dense casein clot, as well as the shortest duration of milk clotting time, and they were significantly superior to their analogs with the *AA* genotype on these indicators. As for the *BLG* gene, the first-calf cows with the genotype *BB* and *AB* had the best cheese-making properties of milk. These animals were superior to their coevals with the *AA* genotype in terms of the highest yield of the desired dense casein clot and the shortest duration of milk clotting time.

The first-calf cows of the Kholmogorskaya breed of the Tatarstan type and black-motley × Holstein cows, which are the carriers of an *A* allele of the *CSN3* gene, were superior to the animals with the *BB* genotype of the *CSN3* gene on the thermostability of milk including that on the proportion of animals with this milk characteristic. The *BLG* genotype of the studied animals did not significantly affect the thermostability of milk. Moreover, the highest thermostability of milk was characteristic of the black-motley × Holstein cows with the *AA* genotype.

It is advisable to use the milk from the cows with the genotypes *BB* and *AB* of the *CSN3* and *BLG* genes that has the best cheese-making properties for manufacturing cheeses and products of lactic acid fermentation. When processing the milk from the cows with the *AA* and *AB* genotypes of the *CSN3* gene as the most heat-resistant, it is advisable to use it to produce drinking pasteurized and sterilized milk with a long shelf life and canned milk. It is ineffective to differentiate cow milk on heat resistance with considering of the *BLG* genotype.

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