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KAPPA-CASEIN GENE POLYMORPHISM AND ITS EFFECT ON MILK OF RED AND WHITE COWS FOR CHEESEMAKING

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Abstract

The genotyping of red and white cattle by kappa-casein genes revealed the presence of three genotypes namely AA, AB, and BB. In first-calf heifers, the frequency of the AA genotype of the kappa-casein gene occurrence was 33.10%, AB was 56.69%, and BB was 10.21%. Being assessed the level of milk yield of red and white heifers with different genotypes of kappa-casein, the superiority of heifers with BB genotype compared to the same age animals with homozygous AA and heterozygous AB genotype of kappa-casein gene was revealed namely of 644 kg ($P < 0.01$) and 153 kg. The yield of milk fat and protein showed a significant difference between the genotypes of BB and AA ($P \leq 0.05$). For cheesemaking milk of 4-5 months lactation first-calf heifers was used. In terms of fat content in milk, heifers with genotype BB were better than the same age animals with genotypes AB and AA by 0.03% and 0.23% ($P < 0.01$) respectively. The protein content in milk of first-calf heifers with the BB genotype of the kappa-casein gene was higher than in the same age animals having the AA and AB genotypes by 0.17% ($P < 0.01$) and 0.06% ($P < 0.01$). In casein content, superiority also remained with first-calf animals having the genotype BB of the kappa-casein gene. They exceeded the same age animals with the AB and AA genotype by 0.04% and 0.09% respectively. The advantage of cows with the genotype BB was also noted in calcium and phosphorus content of milk. The fat content in silids was higher in cheese made of milk of cows with genotype BB (45.8%). It is 0.09–1.7% more than in cows with the genotype AB and AA. Cheese made of milk of cows with genotype AA was characterized by a reduced degree of ripening and titratable acidity compared with cheese made of milk of cows with genotype BB and AB. Lower maturation and titratable acidity indicates a slowdown in cheese affinage process.
Keywords: genotype, protein, kappa-casein, fat, milk, cheese, rennet coagulability, allele

Introduction

The widespread use of Holstein in breeding worsened the quality indicators of milk, especially in natural cheesemaking. A lot of researchers note a decrease of B allele of the kappa-casein gene in crossing offspring with the increase of Holstein breed heredity. The experience of European countries shows that in order to increase the protein content in cow's milk, it is necessary to control the presence of the B alley in the animal genotype (Aboneeva, 2009; Artemyev, 2007; Akhmetov *et al.*, 2007; Barabanshchikov, 1990; Zinnatova, 2010; Konovalova *et al.*, 2004). According to many researchers, the kappa-casein B allele positively affects the protein content in milk and the technological properties of milk in cheese production (Zinoviev *et al.*, 2004; Kalashnikova, 2003; Kalashnikova *et al*, 2006; Kostyunina, 2005; Chizhova *et al*, 2008). The dairy industry of Mordovia Republic is at a high level of technology and labor organization development. Nevertheless, the relevance of the genetic potential of animals that allow to produce large quantities of high-quality milk (as raw materials for processing at specialized enterprises in the region) is still a point of improving. The Republic of Mordovia is the largest producer of natural cheeses in Russia. The capacity of cheese making factories allows daily processing of 800 tons of milk. There red and white cows are bred in Mordovia Republic. It was developed by using the gene pool of the Simmental and red and white Holstein breeds. At present, breeding is carried out to develop a new intra-breed type of animals with a high heredity of Holstein, requiring optimization of genotypes determining quality indicators of milk (Velmatov *et al*, 2018). The aim of the work is to study milk suitability of red and white breed for cheese production, depending on the genes of kappa-casein in the genomes of cows.

Materials and Methods

Studies were carried out from 2016 to 2019 in red-and-white herd of cows of the Federal State Unitary Enterprise "The 1st of May" of FASR (Federal Agency for Scientific Research) of the Republic of Mordovia. To assess the polymorphism of the analyzed loci, we used cattle DNA samples based on the polymerase chain reaction (PCR). According to the results of the research, experimental groups of cows with the genotypes AA, AB, and BB were formed.

PCR was carried out on a programmable thermal cycler "Tertsik" (Russia) with volume of 20 µl, with primers Forward: 5′-ATSATTTATGGCCATTCCACCAAAG-3′ and Reverse: 5′-GC CCATTTCGCCTTCTCTGTAACAGA-3′, designed by J.F. Medrano and E. Aguilar-Cordova, (Medrano *et al.*, 1990) for amplification of a kappa-casein gene fragment of 350 bp.

To determine the allelic polymorphism of the kappa-casein gene, 20 µl of PCR sample was processed with 10 units of restriction endonucleases Hinf I in 1×buffer "O" by SibEnzyme (Russia) and in 1×buffer "W" by the same enterprise at 37 °C overnight. Restriction products were separated by electrophoresis using polyacrylamide gel. The frequency of occurrence of kappa-casein genotypes was determined by the formula:

$$p = \frac{n}{N}$$

Where

p is the frequency of the genotype,

n is the number of animals having a certain genotype,

N is the number of animals;

the frequency of individual alleles was determined by the formula:

$$P_A = \frac{2nAA + nAB}{2N} \text{ и } q_B = \frac{2nBB + nAB}{2N} \text{, where}$$

P_A is the frequency of A allele,

q_B is the frequency of the B allele,

N is the total number of alleles.

The expected results of the frequencies of genotypes in red-and-white cattle population were calculated according to Hardy-Weinberg law [16]. Milk productivity was determined by conducting control milking during the first lactation. Qualitative indicators of milk were determined in accordance with State All-union Standard 26809-86 “Milk and dairy products”.

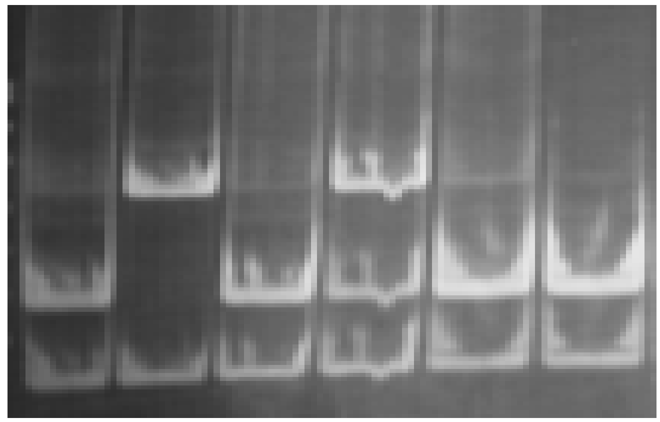
Rennet coagulability and rennet fermentation samples were determined according to generally accepted methods in accordance with State All-union Standard 9225-84. The heat resistance of milk was determined by alcohol test with State All-union Standard 25228-82. The cheese rating was determined in accordance with State All-union Standard 7615-55 "Solid rennet cheese".

To process the data researched, we used the method programs by Merkuryeva E.K. (Merkuryeva, 1970) and Plokhinsky N.A. (Plokhinsky, 1969), computer programs “Microsoft Excel” and “Statistics ver. 2.6”.

Results

Studies carried out with 284 animals of red-and-white first-calf cows made it possible to analyze the kappa casein genotype.

After the action of the HinfI restrictase, depending on the genotype of the animal, fragments of 265, 134, 85 bp in length are formed according to the kappa-casein gene. Fragments of 134 and 131 bp in length corresponds to A allele, and the fragment of 265 bp long to B allele (Fig. 1). A fragment of 85 bp length is common to both alleles and does not depend on animals’ genotype.



265 bp

Fig. 1 : Electrophoregram of the result of PCR-RFLP for cattle gene of kappa-casein with endonuclease cleavage by the HinfI enzyme. 1, 3, 5, 6 – genotype AA, 2 – genotype BB, 4 – genotype AB

Table 1 : The frequency of occurrence of A allele was 0.61, and B allele was 0.39. The frequency of AA genotype occurrence was 33.10%, AB was 56.69% and BB was 10.29%.

Breed	n	Allocation	Genotype frequency,%			Allele frequency,%		χ^2
			AA	AB	BB	A	B	
Red-and-white	284	kappa-casein						
		H	33,10	56,69	10,21	0,61	0,39	3,83
		O	37,21	47,58	15,21			

H is the observed distribution of genotypes,
O is the expected distribution of genotypes.

In general, the expected frequency of the genotypes AA and BB occurrence is higher than the observed values by 11.0% and 32.87% respectively. The expected occurrence frequency of the AB genotype in this group of red-and-white heifers is 16% lower than the observed distribution of genotypes.

Calculations were done by Hardy-Weinberg methods and χ^2 showed a lack of genetic balance in the studied cattle population. This indicates the absence of artificial selection affecting animal genotypes for the kappa-casein gene.

Table 2 : Milk productivity of cows with different genotypes of kappa-casein

Indicators	Genotype			Difference	
	AA	AB	BB	BB-AB	BB-AA
n	94	161	29	-	-
milk yield, kg	6299,6±129,3	6790,5±267	6943,3±145,8	152,8	643,7**
fat, %	3,99±0,02	4,20±0,02	4,28±0,03	0,08	0,29***
milk fat, kg	251,3±11,7	285,2±11,2	297,6±13,3	12,4	46,3**
protein, %	3,35±0,03	3,44±0,03	3,57±0,04	0,13**	0,22***
milk protein, kg	224,9±7,1	233,6±6,7	247,9±7,9	14,3	23,0*

Note: * P<0,05; ** P<0,01; *** P<0,001

The study of the milk productivity of different genotypes cows shows that animals with the genotype BB of kappa-casein gene exceeded their counterparts with the genotype AB and AA in milk yield by 152.8 and 643.7 kg (P ≤0,001), in the content of fat mass fraction by 0.08-0.29% (P≤0,05;0,001), in protein by 0, 13-0, 22% (P≤0,05; P≤0,001). Relative difference between BB and AA genotypes was noted in the yield of milk fat and protein (P≤0,05) (Tab. 2).

Aboneeva (2009); Denicourt *et al.* (1990); Lunder and Afforselles (2000) note that the kappa-casein gene controls the quality of milk, which is subsequently used in cheese making. The authors also note that the genotypes AB and BB of the kappa-casein gene are more preferable for the production of natural cheese.

The suitability for cheese making and technological properties of cow's milk can be judged by the data in Table 3. Milk obtained from experimental animals in taste, aroma, color and texture was suitable for natural cheeses production. By the content of fat and protein milk obtained from cows with genotype BB and AB kappa-casein is most preferable. Significant differences were found out in fat mass fraction in the milk of cows of genotype BB and AA (P≤ 0,001).

In terms of protein content in milk, cows with genotype BB of kappa-casein are superior to their counterparts of genotype AA by 0.17% and AB by 0.06% (P≤ 0,01), and in casein content, the advantage of cows with BB genotypes is preserved. Similar results were obtained on the content of calcium and phosphorus in milk.

Milk of cows with the genotype BB (18.1 °T) is characterized by the highest acidity, the differences between groups are 0.01-0.03 °T. Milk quality can be judged by fermentation and rennet-fermentation tests. In our experience, milk of all groups is assigned to the first class.

Table 3 : Physical-chemical and technological properties of milk (n = 10)

Indicators	**Genotype K-Cas**		
	AA	**AB**	**BB**
Acidity, °T	17,8±0,42	18,0±0,51	18,1±0,62
Density, A	29,7±0,24	29,9±0,28	30,6±0,34
Fat content, %	3,98±0,03***	4,18±0,04	4,21±0,03
Protein content, %	3,48±0,03***	3,59±0,03	3,65±0,04
Casein, %	2,61±0,04	2,66±0,05	2,70±0,05
Milk solids, %	12,79±0,19	13,0±0,21	13,12±0,28
Lactose, %	4,61±0,05	4,50±0,05	4,51±0,06
Ash, %	0,72±0,01	0,73±0,01	0,75±0,01
Calcium, mg %	127±0,91***	130±0,88	134±0,99
Phosphorus, mg %	97±1,01***	98±1,02**	102±0,09
Duration of rennet coagulation, min.	29,8	29,1	27,3
Fermentation class of milk	I	I	I
Rennet fermentation class of milk	I	I	I
Heat resistance	II	II	II

Rennet coagulation of milk from experimental cows of different genotypes ranged from 27.3-29.8 minutes. By coagulability, milk of cows with B allele is most desirable that indicates a positive effect of this allele on this indicator (Table 3).

Using the method of A.P. Belousov (Belousov, 1933), the milk of experimental cows can be attributed to the second type, the most suitable for cheese making.

Natural cheeses makers consider that the milk belonging to the second type is most desirable, since technological modes of production have been developed according to it.

According to Table 4, one can judge the quality of Poshekhonsky cheese produced from milk of cows with different genotypes. According to the content of milk solids in cheese, significant differences between animal genotypes have not been established.

Milk solids of chees made of milk of cows with genotype BB contain 45.8% fat, which is 0.09-1.7% more than in chees made of milk of cows with genotypes AB and AA.

Cheeses made of milk of cows with genotype AA were characterized by a reduced degree of maturity and titratable acidity compared to cheese made of milk of cows with genotype BB and AB. Lower maturation and titratable acidity indicate a slowdown in cheese ripening.

Table 4 : Quality of Poshekhonsky cheese of 45% fat content

Indicators	**Genotype K-Cas**		
	AA	**AB**	**BB**
Humidity, %	41,0	40,6	40,0
Salt, %	2,2	2,3	2,4
Fat in solids, %	44,1	44,7	45,8
Solids, %	59,0	59,4	60,0
Titratable acidity, °T	207	218	225
Maturation by Shilovich, °Sh	100	116	119
Cheese score:			
Taste and smell	41	43	44
Consistency	21	22	23
Color	5	5	5
Slice	9	10	10
Appearance	8	8	8
Packaging	5	5	5
Total	89	93	95

Studies have found out that in taste and smell cheeses made of milk of cows with genotype BB had a stronger expression of taste and aroma, for which they received 44 points. The difference between the genotypes for the consistency of cheese was 1-2 points. The highest total score was gained by cheeses made of milk of cows with genotype BB.

Table 5 : Milk Production Efficiency

Indicators	Genotype		
	AA	**AB**	**BB**
Milk yield for 305 days of lactation, kg	6299	6790	6943
Fat content in milk, %.	3,98	4,18	4,21
Yield in terms of basic fat content, kg	7373	8348	8597
The selling price of 1 kg of milk, rubles	22,0	22,0	22,0
Revenue from milk sale, rubles	162206	183656	189134
Cost of 1 kg of milk, rubles	18,3	17,0	16,8
The cost of one animal maintaining, rubles	135362	142288	144357
Profit from milk sale, rubles	26844	41368	44777
Profitability of milk production, %.	19,8	29,0	31,0

When calculating the economic efficiency of milk production, it was revealed that the animals having B allele in the genotype were the most effective.

The highest profitability was obtained for the group of cows with the genotype BB of 31.0% kappa-casein, they are 2.0–11.2% superior than their counterparts with the genotype AB and AA.

Conclusion

Based on the studies, it should be noted that the kappa-casein gene affects the qualitative characteristics and technological properties of milk of red-and-white cows. B allele in this population of cows has a relatively low occurrence. Milk production practice approves that it is time to test cattle for the presence of the genotype BB of the kappa-casein gene and through selection increase its frequency of occurrence in the herd.

Studies have shown that cows with genotypes BB and AB of the kappa-casein gene have approved the economic feasibility of increasing these genotypes in the stock.

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