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Association of polymorphism of κ-casein Gene and Its Relationship with Productivity and Qualities of a Cheese Production.

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ABSTRACT

The aim of this work was to study the genetic structure of the breeds of Siberia and Macedonia on the gene of κ-casein and to identify relationships of genotypes for this gene with performance of productivity and suitability of milk for cheese production. Studies were conducted on Black-and-White, Holstein (Russia, Macedonia), Red Steppe, Simmental and Yakut breeds. It was established that in all breeds the allele frequency A of κ-casein is in the several times higher compared to a frequency of allele B. A frequency of allele B was in 3 times higher in Simmental and Yakut breeds (0.385 and 0.375) than in black-and-White (0.122). According to three lactations was revealed a higher milk yield and a production of fat and protein in Black-and-White cows with genotype BB. They were on 11-12% higher in a comparison with animals with genotypes AA and AB. The milk of these cows had the best qualities for a cheese production.
Keywords: polymorphism, κ-casein, breed, cattle, productivity, cheese production qualities.

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INTRODUCTION

Thanks to the including in the breeding of farm animals the latest advances in molecular genetics and biotechnology appeared the opportunity to identify genes and polymorphic DNA variants in these genes that control quantitative traits of dairy cattle, quality milk composition and its technological parameters (Kamaldinov et al., 2010; Korotkevich et al., 2014; Yudin and Voevoda, 2015). One of these genes (with the amino acid replacement) associated with milk yield, quantity of milk fat and protein, with a percentage of fat and protein in milk, biochemical milk composition and the other traits of milk productivity can be considered as the gene of κ-casein. According to the conventional classification it belongs to the polymorphic marker based on testing one-nucleoid substitutions (SNPs) with multiple variants of sequences (alleles). Today were identified 11 alleles A, B, C, D, E, F, G, H, I, X, and A1(Erhardt , 1989; Sulimova et al., 1992; Ikonen et al., 1996; Prinzenberg et al., 1999; Kawamoto et al., 1992), among them are the most examined the first 6 alleles (Zinovjeva et al., 2004).

It was found that the most of the animals of Holstein and Black-and-White breed of cattle in general (60-90%) carry an AA genotype, while genotype BB occurs relatively rarely. (Kalashnikova et al., 1999; Altonen and Antila, 1987; Coulon, 2001; Popovski et al, 2003).

The identified polymorphisms are explained by the authors by various bloodline of studied animals of the Holstein breed, which was widely used as the improving one for many breeds (Chrenek and Plesnik, 1981; Threadgile and Womack, 1990; Kovaleva, 2008).

Long breeding that is aimed for increasing of protein content in milk determines the genetic polymorphism of milk proteins, such as κ-casein, PRL, BLG. So, for example, according to Hansen (1990) in the Jerzay breed of cattle the frequency of genotype BB of gene κ-casein is 80 %, Brown Swiss breed – 65 %, Holstein-Frisian – 20 %. In Russian breeds the highest frequency of desirable genotypes of this gene was identified in Red Gorbatov`s and Kostroma breeds (23-33 %) (Kalashnokova et al., 1999). It is interesting to mention that this gene in bulls is monomorphic on the allele B (Mitra et.al., 1998; Otaviano et al., 2005).

In the Simmental breed it was shown that the incidence of the BB genotype for the individual herds differs rather significantly. So in Simmental of Bashkortostan Republic (Russia) its frequency is at 2.5 % (Dolmatova and Valitov, 2015). In the Simmental breed of Austrian selection in Ryazan region was also noted a small number of cows with genotype BB of 4 %, and 66 % had genotype AA. A more favorable ratio of genotypes in the Simmental breed was established (Kostjunina, 2005): AA – 78.3; AB – 13.0; BB – 8.7. Konovalova et al. (2004) found that the highest frequency of desirable allele B of κ-casein gene is in the purebred animals of a domestic breeding – of 36.3 %. In preliminary research we have found that the variation of the genotype on herds of the Republic of Altai is in the range of 7-17 % (Goncharenko et al., 2013).

Gene of κ-casein, according to some scientists, is associated with signs of the protein content in milk and technological properties of milk (Kalashnokova, Barshinova, 2005; Ikonen et al., 1996; Marziali and Ng-Kwai-Hang, 1986).

Its use in the breeding of dairy breeds, especially in the areas of production of solid high-quality cheeses, will be aimed at meeting of the increasing market demands for quality of dairy products.

Taking this into a consideration and also a wide area of distribution of the Simmental breed, due to the high adaptive ability of animals to various climatic and forage conditions, this breed of a great scientific and practical interest worldwide for efficient use of tribal cattle resources. The areal of breeds covers the whole of Europe and Siberia, and the share of breeds in the number of cattle ranges from 5 to 80 %.

The aim of this work was to study the genetic structure of populations according to the polymorphic gene of κ-casein in dairy cattle breeds and to identify the association of the genotypes of this gene with the productive qualities and suitability of milk for a cheese production.

MATERIAL AND METHODS

Gene polymorphism of κ-casein was studied in dairy breeds in different ecological zones of Siberia, Sakha (Yakutia) Republic and Macedonia: Black-and-White, Holstein, Red Steppe, Simmental and Yakut

(Petukhov et al., 2016; Miller et. al., 2013; Narozhnyh et. al., 2013; Petukhova, 2013).

Identification of genotypes of κ-casein was performed using PCR-RFLP according to the method of Center of biotechnology and molecular diagnostics of the all-Russian scientific research Institute of livestock (Gladyr et al., 2001).

The reaction was performed in a final volume of 25 µl with 10x PCR with buffer (16.6 mm $(NH_4)_2SO_4$; 67.7 mm Tris – HCl, pH = 8.8; 0.1 (v/v) Tween 20), 1.5 mm $MgCl_2$, 200 µm of deoxynucleosidesthreephosphats, 30 pmol of each primer and 1 Unit of Tad-polymerase.

For amplification of the fragment of exon IV of the gene Kappa-casein (CSN_3) were used oligonucleotide primers Var 5 and Var 3 with the following sequence:

Var 5 5' – ATA GCC AAA TAT ATC CCA ATT CAG T – 3'
Var 3 5' – TTT ATT AAT AAG TCC ATG AAT CTT G – 3'

Primers that were synthesized on the base of the nucleotide sequence of the DNA of the gene of κ-casein in cattle are homologous to highly conserved areas of the gene.

Amplification was performed in a standard PCR analysis in a thermal cycler. After initial denaturation (95ºC, 5 min) were performed 35 cycles of amplification in the following temperature-time mode: 94ºC, 1 min for denaturation, 57ºC, 1 min for primer annealing and 72ºC, 1 min for polymerization. The mixture of amplification was treated with a restriction enzyme Hind III with a recognition sequence 5'...A↓AGCTT...3' for 16 h at t 37°. Electrophoretic separation was carried out in 2% agarose gel in buffer TAE (130V) with the addition of ethidium bromide to a final concentration of 30 ng/ml and were visualizing the fragments of PCR-RFLP under UV light.

To characterize the technological properties of milk in the preparation of soft cheese in the laboratory were selected milk samples and determined their physico-chemical and chemical-technological parameters (acidity, density, temperature, fat content, protein ratio, protein: fat, thermostability and rennet test).

Thermostability of milk was determined by the alcoholic sample (State standard - GOST 25228-82) for suitability of milk for the cheese production by the rennet sample (GOST 9225-84) (Kogenev, Barabanshikov, 1988).

From the milk of cows with different genotypes of κ-casein were made two kinds of cheese "Vityaz" and "Domashniy", according to the existing instructions for making hard and soft cheeses. In the study of a cheese quality were used the following standards: GOST 3626-73, GOST 5867-90.

The quality of fermented milk products was determined with the use of dry starter cultures with the composition: *Lactobacillus bulgaricus, Lactobacillus acidophilus and Lactic acid streptococcus; Lactobacillales: Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium longum and Streptococcus thermophilius*.

For processing the obtained results were used standard methods of variation statistics with use of Excel software Microsoft Office 2003.

RESEARCH RESULTS AND DISCUSSION

In the studied breeds of cattle of milk and combined directions of productivity in Western Siberia, including Novosibirsk and Tyumen regions, the Altai Krai, Republic of Altai, Khakassia and Macedonia was identified a gene polymorphism of κ-casein (tab. 1).

Table 1– Polymorphism of κ-casein cows of different breeds

Breed	n	The frequency of genotypes, %		
		AA	AB	BB
* Breeding bulls	94	56.4±5.1	33.0±4,8	10.6±3.2

** Breeding bulls	53	58.5±2.0	35.8±7,0	5.7±3.0
Black-and-White	400	77.0±2.1	21.50±2,1	1.50±0.6
Holstein	150	66.0±3.9	29.3±3,7	4.7±3.0
Holstein (Macedonia)	227	55.8±3.3	37.8±3,2	6.4±1.6
Simmental	870	38.3±1.6	46.4±1,7	15.3±1.2
Red Steppe	414	41.3±2.4	48.3±2,5	10.4±1.5
Yakut	96	39.6±5.0	45.8±5,1	14.6±3.6

*Bulls of a Black-and-White breed with Novosibirskagroplem.

** The bulls of a Black-and-White breed of a breeding company Barnaulskoye.

It was shown that the highest frequency of the desired genotype BB of κ-casein is in cows population of Simmental breed cattle and Yakut, whereas such animals in a herd of a Black-and-White breed are less on 13.1-13.8% ($p<0.001$).

However, according to genetic examination that was conducted over the last years, 9% of bulls of the breeding companies of Western Siberia, Russia and Macedonia (Tanaskovska et al., 2006).

Black-and-White and Holstein breeds, which selection was carried out mainly on the increasing of yield, have a high frequency of AA genotype of the gene of κ-casein. About a half of the animals of Simmental, Red Steppe, Yakut breeds are carriers of the heterozygous genotype for this gene. The same frequency of genotypes is found in a population of Simmental cattle and autochthonous Busha cattle in Serbia (Djedovic et al., 2015).

In all the breeds are observed a predominance of allele A over allele B (table. 2).

Table 2 – Frequency of alleles of the gene of κ-casein in cows of different dairy breeds of cattle of Siberia and Macedonia

Breed	n	The frequency of alleles, %	
		A	B
Black-and-White	400	0.878±0.016	0.122±0.016
Holstein (Macedonia)	227	0.747±0.028	0.253±0.028
Holstein	150	0.807±0.066	0.193±0.066
Red Steppe	414	0.652±0.038	0.348±0.038
Simmental	870	0.615±0.016	0.385±0.016
Yakut	96	0.625±0.035	0.375±0.035

A ranked ratio of number of alleles A:B decreases in breeds – Black-and-White > Holstein > Red Steppe > Yakut > Simmental. However, it should be noted that genetic equilibrium in herds for this gene is not broken.

Associative links of genotypes of κ-casein with signs of milk production were studied in cows of Black-and-White, Holstein and Simmental breeds.

In cows of Black-and-White breed of different genotypes of κ-casein was revealed a higher level of milk yield during the first lactation in the group of animals with BB genotype at 490 and 747 kg compare to a group with genotype AA ($P<0.05$) and AB ($P<0.01$) respectively (table. 3).

Table 3 – Association of the gene of κ-casein with milk productivity of cows of Black-and-White breed

Genotype	n	Milk yield, kg *	Fat, % *	Fat, kg *	Protein, % *	Protein, kg *
1 lactation						
AA	222	4948±60.5	3.88±0.01	192.8±2.5	3.26±0.01	161.3±1.9
AB	61	4691±114.5	3.86±0.03	181.2±4.5	3.26±0.02	152.9±4.1
BB	6	5438±191.0	3.91±0.07	212.7±9.8	3.32±0.05	180.5±8.4
3 lactation						
AA	95	5652±119.7	4.00±0.02	226.3±4.9	3.28±0.01	185.4±3.0

AB	43	5466±132.6	4.06±0.04	221.7±5.4	3.29±0.01	179.3±4.6
BB	6	6396±313.2	4.18±0.12	266.8±11.1	3.37±0.02	215.5±10.9

*Mean±SE

A higher content of milk fat was also observed in cows with BB genotype of κ-casein 19.9 and 31.5 kg in comparison with other variants (P<0.01). Cows with BB genotype compared to cows with genotype AA and AB in the milk protein had the following ratio: +19.2 kg (P<0.05) and +27.6 kg (P<0.01).

In the third lactation, as well as in the first, was marked a superiority of all indicators of milk production in cows with the genotype of κ-casein BB. In an average in a third lactation a milk yield in the group of cows with genotype BB amounted to 6396 kg of milk, which is on 744 kg more than in animals with AA genotype (P<0.05) and on 930 kg more comparing to the individuals with heterozygous genotype AB (P<0.01). In the yield of a milk fat the cows with the BB genotype were superior to the peers with the compared genotypes on 40.5 and 45.1 kg, respectively (P<0.001). The content of milk protein in cows with genotype of κ-casein BB was higher on 30.1 and 36.2 kg than in animals of AA and AB genotypes, respectively (P<0.01).

In Macedonia animals with a Holstein breed with BB genotype had on 10% more of a milk fat compared with the AA genotype and on 8% higher than the heterozygotes AB. The milk of these cows had a shorter period of rennet coagulation than in animals with the other genotypes (Tanaskovska et al., 2003).

In Simmental breed in some farms, and with a lower productivity in the herd, was identified a confirmed superiority of protein content in milk from cows of a Black-and-White breed with the gene BB genotype of κ-casein.

In the population of Simmental cattle of Serbia in the opposite the heterozygous AB cows had a higher milk yield (6157 kg) and a production of milk fat (25 kg), compare to the homozygous BB and AA individuals (Diedović et al., 2015). So, the protein content in milk of cows with genotype BB of κ-casein was higher than with AA on 0,07-0,18 % ($p < 0.001$). Cows with heterozygous genotype also had the superiority over the animals with genotype AA at the first lactation by 0.05 % ($p < 0.01$) in the second and in the third – 0.08% ($p < 0.01$, $p < 0.001$). Similar data were obtained in cows of different breeds (Konovalova, 2008; Barshinova, 2005, etc.) So the protein content of different genotypes of κ-casein is reduced in a number of BB>AB>AA, and this regularity was observed in all lactations, including the fourth lactation and older.

One of the main indicators of the suitability of milk for cheese production is its ability to coagulate under the action of rennet enzyme with the formation of the normal density of the bunch. Given this, a comparative evaluation of milk of cows of different breeds with a consideration of the genotypes of κ-casein, which showed that according to the volume of the separated whey – a milk did not differ (0,90-0,92 ml). Alcoholic test had evidenced of a good quality of milk of all genotypes (77.7-78.5%).

At the same time, the phase of coagulation of milk was slightly longer than in cows with genotype AA - 3.51 against 3.18 min in animals with genotype BB.

Accordingly the total duration of coagulation of milk cows with the AA genotype lengthened on 0.57 min compared to the genotype BB.

The study of a physico-chemical composition showed that milk of cows with genotype BB of gene κ-casein contained more fat – 4.23% vs of 3.96 and 4.40 of genotypes AA and AB, respectively. Also milk has a higher content of protein – on 0.05 % in comparison with AA genotype. But the more significant differences were found in a content of sugar, which level in the milk of cows was in the range of 4.43%, which is higher on 0.07-0.11% than in cows with the genotypes AB and AA. Also this milk has a higher density on 0.58-0.68%, contains more calcium on 10.4-11.8% in a comparison with the genotypes AA and AB.

From this milk in the laboratory were prepared kefir, yogurt and a cottage cheese, their taste quality was determined by the presence of the allele B in the gene of κ-casein. Yogurt and kefir from milk with BB and AB genotypes were more dense compare to the milk with the AA genotype.

When fermenting milk for making of a cottage cheese in the sample of genotype AA, there was a significant separation of serum, whereas a sample of milk from cows with BB genotype was distinguished by homogeneity and uniform density and a more pleasant taste, on which, perhaps, influenced a higher (on 0.32%) sugar content. Among the other parameters in a chemical study were not revealed any significant differences. The yield of cheese from milk of cows with genotype BB was higher by 10.3% than from the milk of cows with the AA genotype.

A series of experiments on the preparation of cheese from milk of cows of different genotypes had shown the benefits of AB and BB genotypes. These cheeses regardless of the method of preparation (soft type "Domashnij" and a solid type "Vitjaz") did not only differ with the best taste, but also with the higher yield on 9-11 % of finished products compare to the milk of AA genotype.

In Macedonia the milk of Holstein cows with genotype BB of κ-casein had a shorter period of rennet coagulation than those with the other genotypes (Tanaskovska et al., 2003).

The obtained results are consistent with the findings of scientists about the need for identification of functionally important DNA mutations for efficient MAS-breeding and genomic selection of different types of farm animals not only on grounds of productivity, but also of a resistance to diseases, prenatal mortality of young animals (Petukhov et al. 2012, 2015; Korotkevich et al., 2014; Zheltikov et al., 2010).

There is an information about the relationship of the genotype of the gene TNF-α (gene for tumor necrosis factor alpha) with regulation of different metabolic processes, which makes it a promising gene candidate, controlling body weight and growth performance in animals (Karolchik et al., 2014; Konnai et al., 2006; Lai et al., 2013; Lyukhanov et al., 2015).

However, the search for candidate genes, despite of the large number of already known (QTL), are continuing (Weller and Ron, 2011; Pedersen et.al., 2012; Hu et. al., 2013).

The identified advantage in the qualitative indicators of milk and its good cheese production values of Simmental cows with genotype BB of gene κ-casein was also observed in cows of a Black-and-White (Kalashnikova et al., 1999). Thus, the obtained results can be used in the breeding of dairy cattle, especially for areas of cheese making, such as the Republic of Altai with a special trace element composition of the vegetation.

INSIGHTS

- Were identified a polymorphism of gene of κ-casein in 6 breeds of Siberia, Yakutia and Macedonia and the frequency of the desirable BB genotype which is at the level: Black-and-White – 1.5; Holstein – 4.7; Simmental – 15.3; Red Steppe – 10.4; Sakha (Yakutia) Repulic – of 14.6%. In all the breeds was observed a predominance of allele A over allele B. Frequency of allele B in the populations of Black-and-White and Holstein cattle was lower than in other breeds of animals. A ranked ratio of number of alleles A:B decreases in breeds – Black-and-White > Holstein > Red Steppe > Yakut > Simmental.
- Milk productivity of cows of Black-and-White breed for the first and third lactation with BB genotype of κ-casein is higher on 11-11.7% than those with genotypes AA and AB. The yield of milk fat of cows with BB genotype was superior to peers with other genotypes by 11-12% respectively. The content of milk protein in cows with genotype of κ-casein BB was higher by 11.2% and 12%, than the animals of AA and AB genotypes, respectively ($P<0.01$).
- The protein content in milk of cows with genotype BB of κ-casein was higher than with AA on 0,07-0,18 % ($p < 0.001$). Heterozygous cows had the superiority over the animals with genotype AA at the first lactation by 0.05 % ($p < 0.01$), in the second and the third – on 0.08% ($p < 0.01$, $p<0.001$). In the fourth lactation and older the excess is also 0.05% ($p < 0.01$). The milk of the cows with genotype BB had the best cheese production qualities: phase of coagulation, the total duration of milk coagulation was shorter, and the yield was higher by 9-11% in a comparison with AA and AB genotypes.

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