Polymorphism of the *CSN3* Gene in Simmental herds and its variability during holsteinization

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ABSTRACT

Dairy farming in the Altai Republic mainly specializes in the production of hard cheeses and butter from the milk of Simmental cows, which are most adapted to mountain conditions. The breed has good health, strong bones, and hoofed horns and quickly adapts to various natural and ecological conditions. However, cows often have uneven development of the lobes, the "goat shape of the udder", and insufficient expression of the milk type. The Simmental breed is everywhere improved by the red-mottled Holstein breed to increase milk production and improve the shape of the udder and the speed of milk production, which is economically justified. The phenotypic features and characteristics of economically valuable traits of the obtained crossbreeds are described quite well, but the changes in the polymorphism of genes associated with these traits are not sufficiently studied. The purpose of our research is to compare the polymorphism of the *CSN3* gene as a marker of milk's cheese applicability in Simmental herds and to study its changes in the holsteinization. Studies have established that the ratio of genotypes is approximately the same $CSN3^{AA}$ – 55-58%, $CSN3^{AB}$ – 34-36%, and $CSN3^{BB}$ – 7-10% in herds of the Simmental breed without targeted selection for the *CSN3* gene. However, the use of animal typing by the *CSN3* gene in the breeding process increased the frequency of the desired $CSN3^{AB}$ and $CSN3^{BB}$ genotypes for the herd to 48.1 and 17.7%, respectively, and to 54.8 and 30.9% for individual lines. Holsteinization can significantly reduce the frequency of the desired $CSN3^{BB}$ genotype by 6% without taking into account the CSN3 genotypes of the gene.

Introduction

The Simmental breed, due to its strong hoofed horn, broad chest, well-developed muscles, high immunity, long productive longevity, and adaptability to grazing, has found wide distribution in the world, including in the mountainous, foothill, and steppe regions of the Altai Republic and Altai Territory (Opaleva, 2018; Shevkhuzhaev, Smakuev, 2016). Due to the high quality of milk for the production of cheeses, especially hard varieties, it occupies a leading position in the structure of dairy cattle breeds in the Altai Republic as one of the main export products (Ostroumova, Ivanov, 2004; Belkov, Panin, 2010; Karamaeva *et al.,* 2018). However, the breed needs to improve its milk production and adaptability to industrial technology while maintaining the health and strength of the backbone. To improve these qualities, the red-mottled Holstein breed is used, the efficiency of which can be increased by

using genetic markers in breeding, primarily related to cheese applicability. These genes include the *CSN3* gene, the *CSN3^B* allele of which, as studies have shown, is well associated with the qualitative composition of milk and its coagulation properties. It has been shown that cow milk with the *CSN3^{BB}* and *CSN3^{AB}* genotypes has better coagulability, a dense cluster, and a higher yield of finished products (Telnov, 2016; Mikhailova, 2019; Bigaeva *et al.,* 2019). The thermal stability of milk, as the most important indicator of milk's cheese applicability, was observed in cows with the *CSN3^{BB}* genotype (Volokhov *et al.,* 2017).

Despite the improvement in the phenotypic traits of the improved breeds (milk yield, udder shape, and milk flow rate), the holsteinization of the Simmental breed leads to a decrease in the frequency of desirable genotypes and, as a consequence, deterioration of milk's cheese applicability qualities (Dolgiev *et al.,* 2016; Danilov *et al.,* 2004; Kudrin *et al.,* 2015). At the same time, the differences in this indicator were insignificant in crossbreeds of Simmentals and Montbeliards, which the authors explained by the higher frequency of the *CSN3^{BB}* genotype in these breeds (Firsova *et al.,* 2018).

The effect of holsteinization on the phenotype of crossbreeds of different breeds and generations has been studied quite widely, but the changes in the genome and especially the polymorphism of genes associated with economically useful traits have not been studied well enough. The constant use of Holstein servicing bulls on the parent herds radically changes the allelofund of the improved breed, "displacing" its breed markers (Novikov *et al.,* 2015).
Purpose of the research: To identify *CSN3* gene polymorphism in Simmental breed herds and determine the effect of the holsteinization on the frequency of genotypes and alleles of this gene.

Methods

The studies were carried out on the Simmental breed in the farms of the Altai Republic (Altai Experimental Agriculture, Siberian Branch of the Russian Academy of Sciences, experimental production farm "Chuyskoye", SPK PKZ "Amursky", "Olenevod" JSC) and the Novosibirsk region ("Ivanovskoye" JSC).

The established genealogical structure of breeding stock in farms was studied using data from breeding records. Molecular genetic studies were carried out in the biotechnology laboratory of Siberian Research and Design Technological Institute of Livestock. Blood DNA analysis of the studied animals was performed by the method of DNA isolation on glass developed in the Medigen laboratory (Novosibirsk) using tissue lysis with guanidine isothiocyanate followed by DNA sorption on a glass carrier. PCR-RFLP analysis was performed according to the methodological recommendations of L.A. Kalashnikova and others (Kalashnikova *et al.,* 1999; Kalashnikova *et al.,* 2015). The results were identified using the geldocumentation system E-Box-CX5. TS-20. The χ^2 method was used to assess the degree of compliance of the actual distribution with the theoretically expected one.

The accounting of productive indicators of animals was carried out according to the zootechnical data of primary accounting. The results of experimental studies were processed using the method of mathematical statistics in Microsoft Excel.

Results

The *CSN3* gene polymorphism was analyzed in four Simmental heards of the Altai Republic. In general, for the studied Simmental herds, the frequency of the desired *CSN3^{BB}* genotype was 14.3%; the frequency of the homozygous *CSN3^{AA}* genotype and the heterozygous *CSN3^{AB}* genotype was at the same level (42.5-43.2%) (Table 1).

It should be noted that the frequency of genotypes in three of them (No. 1, No. 2, and No. 3) was

Table 1. Polymorphism of the *CSN3* gene in herds of the Simmental breed (Altai Republic)

Herd	n	Frequency of the genotype, %			Allele frequency	
		AA	AB	BB	A	B
No. 1 (FY)	124	55.6 ± 4.5	34.7 ± 4.3	9.7 ± 2.7	0.73 ± 0.03	0.27 ± 0.03
No. 2 (PI)	112	58.0 ± 4.7	34.8 ± 4.5	7.2 ± 2.4	0.754 ± 0.03	0.246 ± 0.03
No. 3 (AM)	50	56.0 ± 7.0	36.0 ± 6.8	8.0 ± 3.8	0.740 ± 0.04	0.260 ± 0.04
No. 4 (OL)	491	34.2 ± 2.1	48.1 ± 2.3	17.7 ± 1.7	0.580 ± 0.02	0.420 ± 0.02
POPULATION	777	42.5 ± 2.7	43.2 ± 2.7	14.3 ± 3.3	0.641 ± 0.01	0.359 ± 0.01

approximately the same and had significant differences from herd No. 4. Thus, the $CSN3^{BB}$ genotype in these herds was 8-10.5% less than in herd No. 4 ($p < 0.01$, $p < 0.001$) and, accordingly, the frequency of the other two genotypes and alleles also differed from herd No. 4. If the CSN3A allele was prevalent over the $CSN3^B$ allele 2.7-3.1 times in the first three herds, then it was 1.38 in herd No. 4. The gene balance in the herds was not disturbed; χ^2 was in the range of 0.09-1.8.

The observed high frequency of the $CSN3^{BB}$ genotype and $CSN3^B$ allele in herd No. 4, despite holsteinization, was due to purposeful work on the selection of servicing bulls from the mid-90s, taking into account the genotypes of the $CSN3$ gene.

To determine priority lines with a higher specific weight of cows with desirable genotypes, their comparative assessment of polymorphism for the $CSN3$ gene was carried out (Table 2).

Analysis of the table shows that the highest frequency of $CSN3^{BB}$ genotype was detected in the Zabavnogo line (30.9%) and the lowest – in the Litt, Ruhtinas, and Vis Back Ideal lines. The difference was 26.1 and 1.7% (p < 0.01). Accordingly, the Zabavnogo line had the lowest frequency of $CSN3^{AA}$

genotype, only 14.3%, which was less than in the Litt. Ruhtinas, and Vis Back Ideal lines by 23.8-31.1%. At the same time, it should be noted that there was no significant difference in the frequency of the heterozygous genotype. The remaining lines in the frequency of $CSN3$ gene genotypes occupy the middle position.

In this regard, it is interesting to analyze the daughters of individual servicing bulls (Table 3).

The polymorphism of the $CSN3$ gene in the daughters of servicing bulls, as well as in the lines, differed significantly. Since the daughters of Ballet 418 represent the most numerous group in the Zabavnogo line, this group had the highest frequency of the $CSN3^{BB}$ genotype and the lowest frequency of the $CSN3^{AA}$ genotype. At the same time, attention should be paid to Rusak 2257 and Mustard 1517 bulls, in daughters of which the frequency of the desired genotype $CSN3^{AA}$ was 2.6-4.5%, as at the level of the black-and-white breed. The highest frequency of $CSN3^{AA}$ genotype (61.1%) was found in the daughters of Service 1398 bulls, but they also had the lowest occurrence of heterozygotes (16.7%). Other manufacturers occupy the middle position.

It should be noted that during holsteinization,

Table 2. Polymorphism of herd lines No. 4 by *CSN3* gene

Line	n	Genotype		
		AA	AB	BB
Litt. Ruhtinas	21	38.1 ± 4.6	57.1 ± 10.8	4.8 ± 4.7
Vis Back Ideal	44	45.4 ± 7.5	45.4 ± 7.5	9.2 ± 4.4
Zabavnogo	42	14.3 ± 5.4	54.8 ± 7.6	30.9 ± 7.1
Montvic Chieftain	160	37.5 ± 3.8	46.3 ± 3.9	16.2 ± 2.9
Reflection Sovering	86	34.9 ± 5.1	48.8 ± 5.4	16.3 ± 4.0
Floriana	26	26.9 ± 8.7	53.9 ± 9.8	19.2 ± 7.7
Zippera	32	40.6 ± 8.7	40.6 ± 8.7	18.8 ± 6.9

Table 3. *CSN3* gene polymorphism in daughters of servicing bulls

Servicing bull	Line	n	Genotype		
			AA	AB	BB
Scarlet 6353	Zippera	32	40.6±8.2	40.6±8.2	18.8±6.9
Ballet 418	Zabavnogo	42	14.3±5.4	54.8±7.6	30.9±7.1
Warrior 37320	V.B. Ideal	18	55.6±11.7	44.4±11.7	-
Phosphate 2093	V.B. Ideal	26	38.5±9.5	46.2±9.8	15.3±7.1
Severe 2087	R. Sovering	32	21.9±7.3	62.5±8.6	15.6±6.4
Service 1398	R. Sovering	18	61.1±11.4	16.7±8.8	22.2±9.8
Duet 1051	M. Chieftain	20	35.0±10.6	50.0±11.2	15.0±7.9
Sheet 4103	M. Chieftain	81	35.8±5.3	46.9±5.5	17.3±4.2
Rusak 2257	M. Chieftain	38	50.0±8.1	47.4±8.1	2.6±2.5
Mustard 1517	L. Ruhtinas	21	38.1±10.6	57.4±10.7	4.5±4.5

Table 4. Polymorphism of *CSN3* genes in herds of purebred Simmentals and improved Holstein cows, %

Breed	n	Genotype		
		AA	AB	BB
Simmentals	145	40.7 ± 4.08	44.1 ± 4.12	15.2 ± 2.98
Holsteinized Simmentals	109	48.6 ± 4.79	42.2 ± 4.73	9.2 ± 2.76

which undoubtedly had an effective effect on milk yield, udder shape, and milk flow rate, without taking into account *CSN3* gene typing, the frequency of desirable genotypes decreased (Table 4).

An approximately equal ratio of the AA and AB genotypes of the k-casein gene was observed in a herd of purebred Simmental cows, while the frequency of the *CSN3^BB^* genotype was 15.2%. It decreased by 6% after holsteinization and, accordingly, the frequency of the *CSN3^AA^* genotype increased by 7.9%.

Conclusion

1. The ratio of genotypes was approximately the same in herds of the Simmental breed of the Altai Republic without targeted selection for the *CSN3* gene: *CSN3^ÀÀ^* – 55-58%; *CSN3^BB^* – 34-36%, and *CSN3^ÀÀ^* – 7-10%.

2. Involvement of marker selection for the *CSN3* gene made it possible to increase the frequency of the desired genotypes *CSN3^AB^* and *CSN3^BB^* to 48.1 and 17.7%, respectively. In some lines, the frequency of the *CSN3^AA^* genotype reached 30.9%, which can serve as a good basis for its subsequent increase in the herd as a whole. It is most appropriate to use Ballet 418, Service 1398, and Sheet 4103 servicing bulls. Their daughters had 17.3-22.2% frequency of the desired *CSN3B^BB^* genotype.

2. Selection of bulls without taking into account genotypes for the k-casein gene during the holsteinization of the Simmental breed reduced the frequency of *CSN3* genotypes by 6.0% and increased the frequency of *CSN3^AA^* genotype by 7.9%.

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