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Original Paper

Evaluation of cow genotypes by kappa-casein of dairy breeds

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The polymorphism of the kappa-casein gene (CSN3) has been studied in cows of the Lebedyn, Simmental, Ukrainian Black-and-White dairy breeds using a real-time polymerase chain reaction. The TaqMan® SNP Genotyping Assays use TaqMan® 5´-nuclease chemistry for amplifying and detecting specific polymorphisms in purified genomic DNA samples. All assays are developed using Life Technologies robust bioinformatics assay design process relying on a pipeline using heuristic rules deduced from both manufacturing and assay performance data. The objective of our study is to evaluate the cattle of Ukrainian domestic dairy breeds according to the kappa-casein genotype. It has been shown that the distribution of genotype frequency differs significantly in different breeds: Lebedyn – 19% A/A, 50% A/B and 31% B/B; Ukrainian Brown dairy – 30% A/A, 41% A/B and 30% B/B; Simmental – 44% A/A, 46% A/B and 10% B/B; Ukrainian Black-and-White dairy – 58% A/A, 27% A/B and 15% B/B. The results obtained indicate the prospects of breeding work towards creating herds with the BB genotype in the Brown cattle. The accelerated formation of the desired genetic combinations in micropopulations (herds), with the needs of the processing industry, primarily cheesemakers, is possible in the population of the Brown and Lebedyn breed. Such work with dairy herds of the Ukrainian Black-and-White and Simmental breeds requires more time for selection due to the low frequency of BB genotypes in cattle of these breeds.

Keywords: genetic polymorphism, kappa-casein, genotype, breeds

1 Introduction

Milk proteins are important from an economical point-of-view as the polymorphism correlates with different protein genes, and milk and cheese properties have been wildly de-scribed (Kolenda & Sitkowska, 2021). Kappa-casein is the only fraction of casein containing the amino acids cysteine and methionine, which make up approximately 13% of milk casein. Milk protein genes, especially kappa-casein (CSN3), are essential in milk quality (Matějíček et al., 2008). This fraction strongly affects the process of coagulating, curd firming, and cheese ripening (Nicolò et al., 2018). The effect of kappa-casein genotypes on milk quality has been studied by many scientists (Olanrewaju et al., 2020). The kappa-casein gene polymorphism has been known since 1964. In 1988, the bovine kappa-casein gene was isolated, and the structure of the gene was described. As of today, the thirteen genetic variants of bovine kappa-casein have been described: A, B, C, D, E, F, H, J, I, X, Az, A1. The genetic variants A and B are the most common, while other alleles are quite rare (Pazzola et al., 2020). The B allele of kappa-casein is associated with the production of milk with the more favorable chemical composition and technological properties, such as heat resistance, coagulation time reduced by 10–30%, coagulation strength increased by 20–100%, and 5–8% higher efficiency in the production of fresh and ripened cheese compared to the A allele. Therefore, the milk of animals with the BB kappa-casein genotype (homozygous) is characterized by better cheese suitability, to a lesser extent – the milk of cows with the AA and AB genotypes (Miluchová et al., 2018; Dell'Eva et al., 2020). At the same time, cattle with the BB genotype take precedence by the total protein content in milk over the AA genotype (Yaser & Hamad, 2019). Therefore, the indicator of cattle genotype by kappa-casein is used for successful breeding based on cheese suitability. Different breeds of dairy cattle differ significantly in the frequency of genotypes and alleles by kappa-casein.

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Thus, in the Mexican population of the Jersey breed, the B allele had the highest frequency of occurrence – 0.69. The frequency of the A allele was 0.26, and E 0.05, respectively. The BB genotype was the most frequent amounting to 0.45 (Zepeda-Batista et al., 2018). The study was conducted to determine the genetic variants of κ-casein and β-lactoglobulin genes in native cattle. In case of κ-casein gene, higher frequency was observed for AA genotype (0.73) followed by AB (0.23) and BB (0.04) genotype. The A allele (0.84) was found to dominate over B allele (0.16) (Akter et al.,2020). In the population of the tropical milking Criollo, the B allele had the highest frequency of occurrence – 0.61. The frequency of the A allele was 0.39. The AB genotype was most frequent – amounting to 0.69 (Becerril-Pérez et al., 2020). The relative distribution of CSN3 alleles and genotypes in different Holstein cattle populations differed slightly in different countries. Thus, the desired B allele frequency was the highest in the Serbian population – 49%, and in the Iraqi population – 41%. The Chinese population had the lowest frequency: 14%, Slovak – 16.7%, Russian and Polish – 17%, North Macedonia –33.6%. The desired BB genotype was observed more often in the Serbian population of the Holstein breed – 23%. The lowest frequency of this genotype was characteristic of the Russian (2.9%), Iranian (2.9%), Slovak (2.8%), and Canadian (2.7%) populations. The homozygous AA genotype was observed more often in the Egyptian and Chinese populations, respectively 85 and 74%. Heterozygotes were common in the Iranian (82%) and Indonesian populations (65%) (Adamov et al., 2020; Adamov et al., 2020). Among the studied Holstein cattle, cows with the AA genotype accounted for the majority – 57.0%, heterozygous cows with the AB genotype – 34.5%. Other genotypes were less common: AE – 5.8%; BB – 0.9%; BE – 1.8% (Molee et al., 2015). It was found that the AA genotype characterized cattle of the Holstein breed with the frequency of 69.5%, AB – 27.6%, and BB – 2.9%. The frequency of allele A was 0.83, and allele B was 0.17, respectively. A statistically significant difference was found in the effect of CSN3 genotypes on the average protein content in milk. The statistical analysis confirmed that the AA genotype significantly reduced the average protein content in milk (0.09% on average) compared to the BB genotype (Miluchová et al., 2018). The genotypes such as AA (4.8%), AB (36.2%), and BB (59.0%) were found in the Italian Brown cattle by the CSN3 gene (Amalfitano et al., 2018). In animals of the Simmental breed, the AB genotype was more common (49%) than others – AA (39%), BB (9%), BE (2%), and AE (1%), respectively. The frequency of the desired B allele was 35%, A allele – 64%, E – 1%. The AA genotype characterized the Holstein cattle with a higher frequency (55%), and the desired BB genotype was not found (Bezdíček, 2007). According to other data, for kappa-casein, variant B was the predominant one in the Simmental cattle (69.6%), while the frequency of k-CN A was higher in the Swedish Red-and-White cattle (62.5%). The k-CN E allele was not identified in the Simmental cows, whereas k-CN E had a relatively high frequency in the Swedish Red breed. In cattle of the Swedish Red breed, a higher frequency of k-CN is characteristic of the A (65.5%) and E (17.2%) alleles (Poulsen et al., 2017).

The objective of our study was evaluate the cattle of Ukrainian domestic dairy breeds according to the kappa-casein genotype.

2 Material and methods

The genotyping of 189 heads of cattle was carried out during the research. Genotyping of the following cattle breeds was carried out: Lebedyn (n = 78), Ukrainian Brown dairy (n = 44), Simmental (n = 41), Ukrainian Black-and-White dairy breed (n = 26). Blood samples were taken under sterile conditions into 2.7 mL monovettes containing EDTA potassium salt as an anticoagulant (Sarstedt, Germany), with the following samples' freezing and storage at -20 °C. DNA for genotyping was extracted from the samples using Monarch® Genomic DNA Purification Kit New England BioLab kits (USA) according to manufacturer's protocol. TaqMan®Custom was used to perform allelic discrimination. The TaqMan® SNP Genotyping Assays use TaqMan® 5´-nuclease chemistry for amplifying and detecting specific polymorphisms in purified genomic DNA samples. All assays are developed using Life Technologies robust bioinformatics assay design process relying on a pipeline using heuristic rules deduced from both manufacturing and assay performance data. These assays use TaqMan® minor groove-binding (MGB) probes for superior allelic discrimination, improved signal-to-noise ratios, and design flexibility. TaqMan real-time PCR Two primers were designed to amplify the 101 bp product involving SNP rs43703011 (genomic DNA: X14711 (http://www.ncbi.nih.gov); forward primer, 5´-AAG CAG TAG AGA GCA CTG TAG CTA-3´; reverse primer, 5´-TGA TCT CAG GTG GGC TCT CAA TAA-3´). Two fluorogenic TaqMan probes were designed with different fluorescent dye reporters to allow single-tube genotyping. The first probe was targeted at the wild type allele A (5´-VIC-CTTCTGGAGAAGCTTCTA-3´) and the second one at the mutated allele B (5´-FAM- CTTCTGGAGAATCTTCTA-FAM-3´) of the CSN3 gene. The NFQ quencher was linked to the 3´ end of both probes. Primers and probes were designed using Primer Express software, version 3.0 (Applied Biosystems, CA, USA) and were obtained from Applied Biosystems. The accuracy of the used sequence source was verified by comparison with sequences from the GenBank database using BLAST

(http://www.ncbi.nlm.nih.gov/BLAST/). Real-time PCR was performed in 20 µl reactions with 10 µl of TaqMan universal PCR master mix containing AmpliTaq Gold DNA Polymerase (Applied Biosystems, CA, USA), 200 nM concentration of forward and reverse primer, 100 nM of each probe and 2 µl (50–100 ng) of sample DNA. The PCR reaction was obtained using the FAST 7500 Real Time PCR System (Applied Biosystems). The time and temperature profile of the PCR reaction consisted of the following steps: 2 min at 50 °C for UNG activation, 10 min at 95 °C for starting AmpliTaq Gold activity, 40 cycles of 95 °C for 15 s and 60 °C for 1 min. As a negative control, we used a sample without a template. An allelic discrimination experiment consisted of three steps: a pre-read run, an amplification run and a post-read run. Each sample was visually verified by analysing the generated PCR curves. Analyses of amplification products were performed using SDS software, version 4.2. The allele frequency was calculated taking into account the number of homozygotes and heterozygotes found in the corresponding allele using the following formula:

$$P(A) = \frac{2N_1 + N_2}{2n}$$

where:
N_1 and N_2 – number of homozygotes and heterozygotes for the studied allele, respectively; n – sample number

In order to assess the statistical reliability of the discrepancy between the distribution of the obtained results the Pearson criterion was used:

$$\chi^2 = \frac{\sum (A-T)^2}{T}$$

where:
A – actual number of genotypes; T – theoretical number of genotypes

The actual (available) heterozygosity was determined by direct calculation using the following formula:

$$H_O = \frac{N_2}{n}$$

The expected heterozygosity was determined using the following formula:

$$H_E = 1 - \sum_{i=1}^{n} p_i^2$$

where:
$p_1, p_2 \ldots p_n$ – frequency of alleles

The fixation index was calculated using the following formula:

$$F_{is} = \frac{H_E - H_O}{H_E}$$

3 Results and discussion

The results of DNA testing of the kappa-casein locus for the availability of A and B-allelic variants in cattle of the studied breeds have shown that the Lebedyn breed cattle is characterized by the highest frequency of the desired homozygous BB genotype (Table 1).

Cattle of the Ukrainian Black-and-White dairy breed had a slightly lower frequency value of the desired genotype, and cattle of the Simmental breed were characterized by a frequency three times lower than cattle of the Lebedyn breed. Cattle of the Lebedyn breed also differed in the higher frequency of animals with a heterozygous genotype. The homozygous AA genotypes were found in cattle of the Ukrainian Black-and-White dairy breed. The use of the χ^2 criterion enabled us to determine the degree of compliance of the current genotype distribution with the expected values. The Hardy-Weinberg equation calculation showed no difference between the actual

Table 1 Frequency of alleles and genotypes by the kappa-casein gene locus

Breed	Distribution	Genotype (%)			Allele (pcs)		χ^2	Statistical significance
		AA	AB	BB	A1	A2		
Lebedyn	A	19	50	31	0.442	0.558	0.558	$P > 0.05$
	E	20	49	31				
Ukrainian Brown dairy	A	30	40	30	0.500	0.500	0.500	$P > 0.05$
	E	25	50	25				
Simmental	A	44	46	10	0.671	0.329	0.329	$P > 0.05$
	E	45	44	11				
Ukrainian Black-and-White dairy	A	58	27	15	0.712	0.288	0.288	$P > 0.05$
	E	51	41	8				

and expected genotype frequency for most breeds. This may indicate a lack of selection by this trait and the preservation of genetic balance. That is, animal breeding is carried out on the basis of traditional methods for assessing milk productivity without taking into account genetic factors that affect the qualitative composition of milk protein. The exception is cattle of the Ukrainian Black-and-White dairy breed that, in our opinion, is due to the previous use of Original Brown Cattle on the bull population. This, in our view, may cause a high frequency of homozygotes of the desired genotype. A statistically significant difference in the χ^2 criterion was found in the Ukrainian Black-and-White dairy breed. The lower than expected frequency of heterozygotes was recorded in this group of cattle.

There is a generally accepted opinion that the violation of random crossing should cause a deviation in genotype frequency from the expected Hardy-Weinberg equation. As a result of inbreeding, genotype frequency should change towards the predominance of homozygotes. This is clearly observed in cattle of the Ukrainian Black-and-White dairy breed. The expected heterozygosity in them outweighed the actual one. However, in cattle of the Lebedyn and Simmental breeds, on the contrary, the actual heterozygosity exceeded the expected one. This is also indicated by the negative value of Wright's fixation index. This indicates a slight excess of heterozygotes in these samples (Table 2).

Using the genetic and statistical methods of analysis, we have tried to assess the prospects by determining the digital values of such genetic constants as the degree of homozygosity (Ca), the degree of polymorphism (Na), we have tried to assess the prospects of creating herds with the BB genotype by kappa-casein (Table 3).

The degree of homozygosity in the studied cattle populations ranges from 50.0 to 58.9%, which indicates that the studied trait most consolidates in the Brown cattle. The level of polymorphism (number of effectively active alleles – Na) in the kappa-casein locus of the Ukrainian Brown dairy cattle and Lebedyn breeds is the highest. The former has amounted to 2. The heterozygosity test has both positive and negative values, which indicates that populations of the Ukrainian Brown, Ukrainian Black-and-White dairy breeds are characterized by an insufficient proportion of heterozygotes compared to the theoretically calculated one. The excess coefficient (D) confirms this statement. In general, it can be stated that the data of genetic and statistical analysis indicate that breeds differ significantly in the genetic structure of kappa-casein.

In addition, we have analyzed the catalogue of stud bulls of dairy and dairy-meat breeds for breeding stock (Table 4).

Table 2 Values of the main indicators of variability by the kappa-casein gene

Breed	H_O	H_e	F_{is}
Lebedyn	0.500	0.493	-0.011
Ukrainian Brown dairy	0.409	0.500	0.182
Simmental	0.463	0.442	-0.049
Ukrainian Black-and-White dairy	0.269	0.411	0.344

H_O – actual heterozygosity, H_e – expected heterozygosity, F_{is} – fixation index

Table 3 Genetic structure of a herd of cattle by the kappa-casein locus

Indicators	Breeds							
	Lebedyn		Ukrainian Brown dairy		Simmental		Ukrainian Black-and-White dairy	
	actual	theoretical	actual	theoretical	actual	theoretical	actual	theoretical
Heterozygotes	39	39	18	22	19	18	7	11
Homozygotes	39	39	26	22	22	23	19	15
Hetero/homozygote ratio	1.00	1.00	0.69	1	0.86	0.78	0.37	0.73
Heterozygosity test	0.026	–	-0.308	–	0.072	–	-0.328	–
Heterozygosity degree, Ca (%)	50.7	–	50.0	–	55.8	–	58.9	–
Polymorphism level, Na	1.97	–	2.00	–	1.79	–	1.69	–
Excess coefficient D	0.013	–	-0.181	–	0.049	–	-0.344	–
Proportion of homozygotes (%)	50.0	–	59.0	–	54.0	–	73.0	–

Table 4 Evaluation of stud bulls by kappa-casein genotype, heads*

Breed	Genotypes		
	AA	AB	BB
Lebedyn	6	4	2
Swiss	0	0	8
Simmental	3	6	0
Holstein (Black-and-White)	63	133	68

Among all breeds, only the Swiss breed is represented by stud bulls only with the desired homozygous BB genotypes. The majority of cattle of other breeds are heterozygous stud bulls and homozygous stud bulls with the AB genotype. Among bulls of the Simmental breed, there are no BB genotypes at all. Attention should be paid to the use of stud bulls of the Swiss breed for its conservation since among them there are stud bulls of all the studied genotypes. A negative factor may be the lack of stud bulls of the Ukrainian Black-and-White dairy breed evaluated by the kappa-casein genotype, which leads to the need to use stud bulls of the Holstein breed to create herds with the desired genotype.

Our study has found that the B allele of kappa-casein is more common in the population of the Lebedyn cattle (0.558) compared to the A allele (0.442). In the Ukrainian Black-and-White dairy cattle, the breeding of which is widely carried out with the involvement of the Holstein and Simmental stud bulls, on the contrary, the frequency of the A allele (0.712 and 0.671, respectively) is higher than the frequency of the B allele (0.288 and 0.329, respectively). These results are consistent with the previously published studies, in which the frequency of the B allele in the Brown cattle has been 0.66–0.705, and in the Simmental breed, it has been slightly lower – 0.566–0.630. Contradictory data have been obtained on cattle of the Holstein breed, according to various authors (Amalfitano et al., 2018; Molee et al., 2015; Bezdíček, 2007).

The homozygous AA and BB genotypes in total were manifested in more than half of the studied livestock (73%) of cattle of the Ukrainian Black-and-White dairy breed. No more than half of the homozygous genotypes prevailed in the studied cattle of the Simmental (54%) and exactly half of the studied breed of the Lebedyn breed. As a result, cows of the Ukrainian Black-and-White dairy breed have a significant difference between the values of H0 and He. Wright's fixation index indicates a shortage of heterozygotes in the population of the Ukrainian Black-and-White dairy breed.

4 Conclusions

The conducted work has resulted in determining the frequency of alleles and genotypes by the kappa-casein locus. It is established that the breeds of dairy cattle in Ukraine differ significantly in this trait. The genetic equilibrium found in our studies reflects the global trends in breed populations and indicates the lack of targeted breeding towards increasing BB homozygotes.

The accelerated formation of the desired genetic combinations in micropopulations (herds), with the needs of the processing industry, primarily cheesemakers, is possible in the population of the Brown and Lebedyn breed. Such work with dairy herds of the Ukrainian Black-and-White and Simmental breeds requires more time for selection due to the low frequency of BB genotypes in cattle of these breeds.

Such micropopulations with the desired homozygous BB genotype by kappa-casein create prerequisites for improving milk quality as a raw material for milk processing enterprises specialized in cheese production. The stabilization of the dairy cattle breeding industry and its development in Ukraine will be significantly facilitated by an increase in the price of raw materials (milk) that is possible subject to the sale of milk exclusively with the BB kappa-casein genotypes for cheese production.

Due to the highest frequency of the BB genotype in Brown cattle, it is possible to preserve and distribute it among farms of the region and Ukraine as a whole.

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