Milk productivity and milk quality of Ukrainian Black-and-White dairy cows with different genotypes of kappa-casein

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Citation: Polieva, I., & Korh, I. (2021). Milk productivity and milk quality of Ukrainian Black-and-White dairy cows with different genotypes of kappa-casein. *Zhivotnovadni Nauki*, *58*(3), 3-10 (Bg).

Abstract

The results of the investigation of milk production and milk quality of the Ukrainian Black-and-White dairy cows with different genotypes of kappa-casein have been presented in the article. It has been found that homozygotes of kappa-casein with genotype AA that are the most common were more often detected in the actual genetic structure of the experimental herd of cows – 63.1%. The number of the heterozygotes of kappa-casein with genotype AB was slightly lower – 31.6%. However, the proportion of the homozygotes that had a rare homozygous genotype BB was 5.3% of the total number of the array size. The expected genetic structure of the experimental herd of cows approached the actual values of the array distribution and it was 62.4; 33.2 and 4.4% that indicates the increase in the role of further selection work in the direction of the wide use of breeding bulls in the custom mating, the bulls that are primarily the carriers of genotype AB and BB and the consolidation of the herd by the quality of milk – the cheese suitability of milk. Allele A in the experimental array was detected with the frequency of 0.79; however, a much lower distribution frequency was represented by a technologically desirable allelic variant B – 0.21. For 305 days of lactation the amount of milk obtained from the cows that were the carriers of the genotype AA was more by 204.5 kg or 3.3% than the amount of milk obtained from the cows with the genotype AB. While the productive potential of the cows with the genotype BB was significantly lower than the potential of the cows with genotype AA by 422.8 kg or 6.6% with a statistically significant difference between them in favor of the latter ($p < 0.05$). The milk of better quality with higher mass fraction of fat by 0.21% ($p < 0.05$) was produced by the cows with genotype BB as compared to the cows that were the carriers of genotype AA, although the statistical significance of the intergroup difference as for the mass fraction of protein was significantly higher by 0.42% ($p < 0.01$) in favor of the former. The effect of kappa-casein genotype on the mass fraction of protein in milk in the animals with genotype AB against AA was less pronounced than fat (0.09%), according to which the difference between them of 0.16% was revealed that was also statistically significant in favor of the former ($p < 0.05$).

Key words: kappa-casein, genotype, allelle, PCR-RFLP, dairy productivity, milk quality.

Introduction

Milk and the products of milk processing are an integral part of the consumer demand in the national market and the raw materials for many sectors of the food industry. The growing significance of the above food requires the need to increase the milk productivity of cows and to improve the cheese suitability of milk as the priority issues that are traditionally the fundamental tasks of the livestock industry and are solved not only by the scientists of Ukraine but also around the world (Hristova, 2015). The problem of milk quality improvement occupies an important place in the system of its rational production and use. The theoretical provisions to assess milk protein, their genetic variants and the experimental aspects to reveal the dependence between them and the parameters of lactation course, milk composition, the yield and quality of cheese have been covered (Çardak, 2005; Oner and Elmaci, 2006; Kučerova et al., 2006; Comin et al., 2008; Legarová et al., 2010; Shahlla et al., 2014). However, it should be borne in mind that to make cheese it is necessary to produce milk with high technological characteristics, which are due to the genotype of cows (Mukhametgaliev, 2006; Khabibrakhmanova, 2009; Tsiaras et al., 2005, Dicheva et al., 2021). Numerous scientific and economic experiments have been conducted to reveal the relationship between the genotype of dairy cattle by the kappa-casein locus and its milk productivity and milk quality (Barbosa et al., 2019). However, there is an ambiguity of opinions as to which of the alleles A or B is the best.

In particular, by the results of the monitoring of individual sources of literature it has been stated that the $CSN3^{AA}$ genotype influences the increase in milk yield, fat and protein levels in milk, and the above genotype occurrence, in particular in black-and-white dairy breed, is 68% (Kudrin, 2013; Mysik, 2015; Khaizaran et al., 2014).

A number of data have been published in the scientific periodicals by other scientists (Smolyar and Kolomiets, 2006) on the presence of higher milk productivity of the cows of black-and-white breed with genotype $CSN3^{AA}$ as compared with the cows with genotype $CSN3^{BB}$ by 227.8 kg and $CSN3^{AB}$ genotype – by 114.8 kg. As milk yield increased, the differences in the mass fraction of fat in milk in each of the studied groups were insignificant, as for the mass fraction of protein the cows with $CSN3^{BB}$ genotype were better (3.68), they exceeded the cows with genotype $CSN3^{AB}$ by 0.15% and the peers with $CSN3^{AA}$ genotype – by 0.14%.

Comparing the influence of the genotype on milk production in the cows of Belarusian black-and-white breed (Epishko et al., 2014) it has been stated that the greatest contribution to the stimulation of lactogenic activity, to the increase in the content of fat and protein in milk was made by the animals with heterozygous $CSN3^{AB}$ genotype as compared to the cows with $CSN3^{AA}$ genotypes.

By the results of the generalized analysis (Kostyunina, 2005), that was carried out using the array of five studied breeds of cattle, it has been found that within the Red Horbatov breed the cows with genotype $CSN3^{AB}$ exceeded the animals with genotype $CSN3^{AA}$ by 0.12% in terms of fat content, although the cows with the genotype of kappa-casein BB dominated by the yield of milk by 40.0 kg. The representatives of the Yaroslavl breed, that are characterized by genotype $CSN3^{BB}$ exceeded the cows with genotype $CSN3^{AB}$ in terms of protein content in milk by 0.16% and with genotype $CSN3^{AA}$ – by 0.12%.

Instead, when testing the cows of the Red-Spotted breed, the first-borns with genotype $CSN3^{BB}$ were the most productive. For 305 days of lactation they produced more milk by 424 kg, fat – by 0.05% and milk fat – by 33.7 kg as compared to the peers with genotype $CSN3^{AA}$. In turn, the differences between the animals with kappa-casein genotypes BB and AB by the above indicators were 436 kg; 0.02% and 14.7 kg, respectively (Kalashnikova et al., 2002).

The additional evidence for these provisions is the data presented in the paper (Barshinova, 2005), in which the researchers using the comparative assessment of the milk productivity of the first – lactating cows reported that the milk yields of the mothers of the Red-Spotted bulls with genotype $CSN3^{BB}$ were higher by 1408 kg as compared to the cows with genotype $CSN3^{AA}$, although the number of animals in the herd that inherited the $CSN3^{BB}$ gene was negligible.

The issue of testing cattle for kappa-casein genotypes was not paid enough attention in Ukraine until the beginning of the 21st century. Nowadays the highest frequency of the occurrence of genotype $CSN3^{BB}$ is known to be inherent in the animals of Lebedyn, Grey and Ukrainian white-headed dairy breeds and some others (Badagueva et al., 1996; Dyman and Glazko, 1997).

At the same time, the problem of the use of cows with the desired genotype by the kappa-casein locus connected with the quality of milk and the cheese suitability of milk has been open and is far from being definitely resolved.

Materials and methods of the research

The experimental part of the study has been conducted in the conditions of the breeding plant that breeds the Ukrainian Black-and-White dairy cows, the department of «Profintern» of the state enterprise of the research farm «Hontarivka» of the Institute of Animal Science of the National Academy of Agricultural Science. The processing of the obtained data and the analytical part of the research were done on the research base of the Testing Centre of the Institute of Animal Science, NAAS.

A group of dairy cows of the Ukrainian black-and-white dairy breed in the amount of 95 heads was formed for the scientific and economic experiment. The group was formed by the principle of the analogues-pairs depending on the breed, live weight and the time of the last calving (Ibatullin and Zhukorsky, 2017).

The analysis of gene polymorphism was performed by PCR-RFLP. The genomic DNA was isolated from the individual samples of the biological material (hair follicles) taken from the experimental cows with the use of the commercial set of reagents «DNA-sorb B» (Amplisens, Russia). Oligonucleotide primers:

F: 5 'GAAATCCCTACCATCAATACC-3';

R: 5 'CCATCTACCTAGTTTAGATG-3'.

Were used to amplify the fragment of kappa-casein gene (CSN3).

The length of the amplified fragment was 273 bp.

To restrict the locus (CSN3) the endonuclease of restriction Hinfl (ThermoScientific, USA) was used according to the manufacturer's instruction.

When using the Hinfl restriction enzyme in the animals with genotype AA the fragments of the length of 113, 91, 49 bp were detected; the fragments 224, 113, 91, 49 represented the animal genotype AB and the animals with homozygous variant BB had two fragments of 224 and 49 bp length. According to the results of the DNA-testing of CSN3 the selected cows were divided into three subgroups with genotypes: AA, AB and BB.

PCR was conducted on the amplifier «Amply-4-2» (Biokom, Russia), the detection was performed with the use of a transilluminator «UVD», (Biokom). The analysis of the restriction products was done using a chamber for horizontal electrophoresis in a 2.5% agorose gel and the voltage of 15 V/cm. Genotype occurrence frequencies, individual alleles, and genetic equilibrium between the actual and theoretically expected genotype frequency distributions were calculated using GenAlex 6.5 software.

During the research the experimental cows were placed in the same conditions, the feeding was rationed.

Dairy productivity was recorded on the basis of the results of the individual monthly control milking of the cows with the subsequent calculation for each month, season of the year and for the whole lactation period.

The determination of the chemical structure of milk was conducted in the average samples monthly by the infrared spectrometry method and conductometric method on the milk analyzer «Bentley» made in the USA (DSTU 8396:2015, 2015; DSTU 7671:2014, 2014).

The experimental material of the research was processed by the methods of variational statistics. The licensed software Microsoft Office Excel 2010 was used for mathematical processing of the results. Differences between the average values of the compared indicators of the experimental groups were considered statistically insignificant at the level of their theoretical significance: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Results and discussion

As a result of studies and by comparing the actual data obtained from the data of genetic testing, it has been found that the experimental group of cows was represented by all three genotypes of kappa-casein and was polymorphic at this locus (Table 1).

When evaluating the materials of the amplification, a clear regularity has been observed on the prevalence of the carriers of the homozygous genotype AA of kappa-casein (60 heads) among the array of the cows. The revealed tendency and the consideration of the pedigree cards of the bulls, the sperm production of which has been allowed for the use in the system of artificial insemination of cows on the research farm, testify to the long-term application of the intensive selection work in the direction of the purposeful use of the breeding bulls-carriers of genotype AA, the marked indicator of which is the increase in milk yields.

30 animals of the selected number were heterozygous and had the genotype AB and only 5 cows had the most desirable homozygous genotype BB that is associated with high technological properties of milk when making cheese. It is noteworthy that the theoretically expected distribution of genotypes AA and BB had slightly lower absolute values than actually obtained ones by 0.7 and 0.9%. Whereas among the animals that carried the genotype AB, on the contrary, there was a tendency to increase the share of heterozygous animals by 1.6%, ie, in general there was a shift towards a decrease in the homozygous genotypes and a slight increase in the number of heterozygous animals that, at the same time, determines higher milk productivity and the suitability of milk for making cheese.

The homozygotes with the most common genotype AA of kappa-casein were most frequently detected in the actual genetic structure of the experimental cow population – 63.1%. The heterozygotes with the genotype of kappa-casein AB were slightly inferior to the above mentioned in the number – 31.6%. However, the share of the cows that had a rare homozygous genotype BB was 5.3% of the total array size. The expected genetic structure of the experimental herd approached the actual values of the array distribution and was 62.4; 33.2 and 4.4%, that indicated an increase in the role of the further selection work in the direction of the wide use of breeding bulls in custom mating, the bulls that are primarily the carriers of genotypes AB and BB and the consolidation of the herd for the milk quality – it suitability for making cheese.

The examination of the character of the actual distribution of the genotype frequencies and theoretically expected ones by Hardy-Weinberg law revealed the balanced state between their values, i.e. the absence of the probably significant shift of the genetic equilibrium when comparing any of the studied polymorphic variants of kappa-casein. It is noteworthy that the value of the criterion $\chi 2$, that was obtained when determining the genetic diversity of the frequency distribution, is significantly lower than the probable value ($\chi 2 = 0.23$).

The subtraction of gene frequencies of kappa-casein locus has indicated that allele A in the experimental array appeared with the frequency of 0.79, however a much lower distribution frequency was represented by a technologically desirable allelic variant B – 0.21, which was almost four times as low as the cows that carried allele A (Fig. 1).

As a result of the polymerase chain reaction the amplicon of the size of 271 bp was detected. The subsequent electrophoretic distribution of the amplicon after the restriction by the restriction enzyme Hind 1 made it possible to identify significant differences that were based both

Table 1. Genetic structure of the experimental cows by kappa-casein genotypes

Genotype distribution	n, heads	Frequency of genotypes						χ2
		AA		AB		BB		
		heads	%	heads	%	heads	%	
Actual	95	60	63.1	30	31.6	5	5.3	0.23
Expected		59.3	62.4	31.5	33.2	4.2	4.4	

on the presence of specific bands and on their absence. The size of the DNA fragments was within the range of 49–131 bp and genotype AA corresponded to three bands of the length 131, 91 and 49 bp, genotype AB – four bands of the length 222, 131, 91 and 49 bp, and the genotype BB was represented by only two bands of length 222 and 49 bp. That is, in addition to the same band for all three genotypes with the length of 49 bp, the bands with the length of 222, 91 and 131 bp were formed in the genotypes AA and AB while the band with the length of 222 bp was formed in the genotype BB. The graphical picture of electrophoregrams with the results of PCR of biological samples that were taken from the cows with different genotypes of kappa-casein is clearly shown in Fig 2.

The level of dairy productivity as well as other main economically useful traits is naturally determined, in addition to other things, by the breed and the genotype of animals. The relationship between genotypes by kappa-casein locus and quantitative and qualitative indicators of dairy productivity is shown in Table 2.

The analysis of the indices given in the table has given the grounds to state that the cows with AA genotype of kappa-casein were characterized by the highest milk yields, the cows with genotype AB were close to the above mentioned cows and the animals with genotype BB were characterized by the minimal milk yields that was caused by slight variability of milk yields and the number of samples of the latter genotype. The cows that were the carriers of genotype AA produced 204.5 kg or 3.3% more than the cows with genotype AB. While the productive potential of the cows with genotype BB was significantly lower than that of the cows with genotype AA by 422.8 kg or 6.6%, the statistically significant difference between them was $p < 0.05$ in favor of the latter. As for the cows with genotype BB, their milk production decreased not only to the level of milk production of the cows with genotype AA but also in comparison with the cows that were the carriers of genotype AB by 218.3 kg or 3.5% without a statistically signifi-

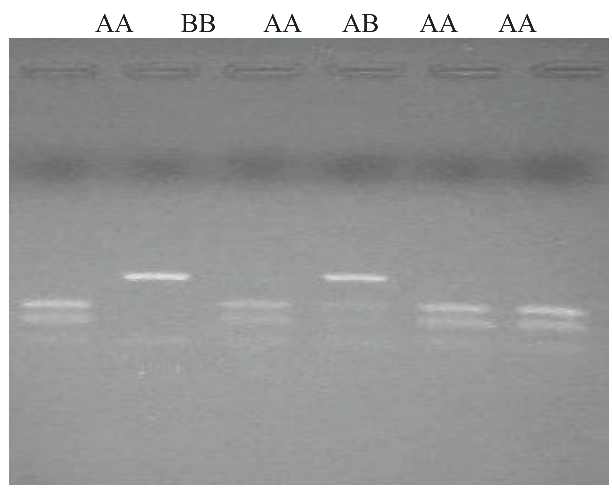

Fig. 2. Electrophoregram of the products of kappa-casein gene amplification

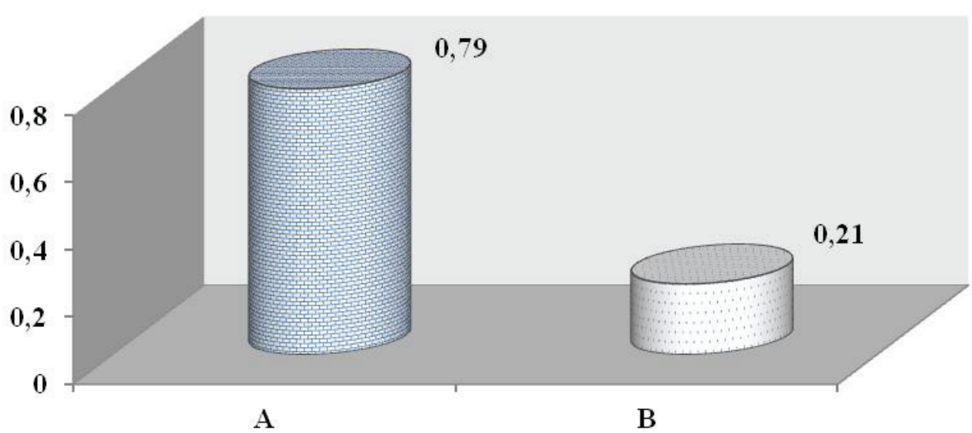

Fig. 1. Distribution of allele frequencies by kappa-casein locus

Table 2. Milk productivity and milk quality in cows with different genotypes of kappa-casein, M ± m

Indicator	Genotype		
	AA	AB	BB
Number of animals, heads	60	30	5
Milk yield for 305 days of lactation, kg	6357.6 ± 120.80*	6153.1 ± 135.60	5934.8 ± 141.30
Milk yield in terms of basic fat content (3.4%), kg	7105.6 ± 172.94	7166.5 ± 169.24	6999.7 ± 279.0
Mass fraction of fat in milk, %	3.80 ± 0.05	3.96 ± 0.05#	4.01 ± 0.08#
protein, %	3.14 ± 0.03	3.23 ± 0.10	3.56 ± 0.14##
Content of milk fat, kg	241.59 ± 5.88	243.66 ± 5.75	237.99 ± 9.49
protein, kg	199.63 ± 4.57	198.75 ± 7.65	211.28 ± 10.83
The ratio of mass fractions protein: fat	0.84 : 1	0.82 : 1	0.89 : 1

**p < 0.05 – the probability of difference was calculated for the cows with genotype BB;*
#p < 0.05 – ##p < 0.01 – the probability of difference was calculated for the cows with genotype AA.

cant difference between them. When transferring the milk yields to the basic fat content the equivalent regularity was observed and the cows with genotype AA dominated by this indicator over the cows with genotype AB by 60.9 kg or 0.9% and BB – by 166.8 kg or 2.4%.

However, a slightly different regularity was revealed for some other indicators of milk production. In particular, the milk of higher quality with the increased mass fraction of fat by 0.21% ($p < 0.05$) was produced by the cows with genotype BB as compared to the cows that were the carriers of the genotype AA, although the statistical significance of the intergroup difference as for the mass fraction of protein was significantly higher by 0.42% ($p < 0.01$) in favor of the former. The influence of kappa-casein on the mass fraction of protein in milk in the animals with genotype AB was expressed in lesser extent than the influence of fat (0.09%), as compared to the cows with genotype AA, the revealed difference between them was 0.16% that was also statistically significant in favor of the former ($p < 0.05$).

Trustworthy fluctuations of the level of milk productivity and qualitative composition of milk due to the increase in the mass fraction of protein in the dry matter of the cows with genotype BB led to the increase in the content of milk protein by 11.65 kg or 5.9% as compared to the cows of the same age that carried genotype AA and by 12.53 kg or 6.3% as compared to the cows with genotype AB. However, the difference revealed for these indicators did not become statistically significant in any of the groups

At the same time, not only the actual content of the main components of milk but also their ratio is of the key importance to make cheese of high quality. In particular, the amplitude of fluctuations of the ratio of the mass fraction of protein to fat in the milk of the cows in all the groups slightly widened towards the fat content: one part of fat accounted for an average of about 0.82–0.89 parts of protein that indicated the high suitability of milk for cheese making. A slight increase in the values of the ratio of mass fraction of fat to the mass fraction of protein in the milk of the cows with genotype AB and BB was to some extent associated with the decrease in the mass fraction of protein while increasing the mass fraction of fat.

Conclusions

It has been stated that the results of the conducted research have proved the polymorphism of the studied array of the cows of the Ukrainian Black-and-White dairy breed. The polymorphism was represented by three genotypes AA, AB and BB with a predominance of the allele A in the allelic spectrum. However, when assessing the correspondence of the actual frequency of the occurrence with the theoretically expected one in the locus that provided the synthesis of kappa-casein, the genetic balance was maintained.

It has been found that the animals that were homozygous by the AA genotype of kappa-casein exceeded the cows of the same age with genotypes BB and AB in the level of milk yields for 305 days of lactations. However, despite the intergroup differences in milk productivity, the highest level of mass fraction of protein and fat was characteristic to the cows with genotype AB and BB as compared to the cows with genotype AA that indicated the higher quality of their milk and the suitability of the milk for making cheese.

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