Genotyping of kappa casein in crossbred cattle as a factor in managing the technological qualities of milk

E A Smirnova, T N Gryazneva, A K Smirnova and N V Pimenov

Federal State Budgetary Educational Institution of Higher Education «Moscow State Academy of Veterinary Medicine and Biotechnology - MVA named after K.I. Skryabin», 23, Akadamik Scriabin St., Moscow, 109472, Russia

Abstract. The article presents the results of a study of DNA isolated from the sperm of bulls-producers of the Belgian white-blue breed, images of the wool of cattle of the Holstein breed and crossbred calves obtained by crossing Holstins with the Belgian white-blue breed, for the presence of the B-allele gene kappa casein (CSN3) associated with a high content of protein in milk and optimal coagulation properties of milk used to produce cheese and cottage cheese. It was found that out of 33 animals studied, 26 individuals (78.9%) showed the AA genotype, which determines the inheritance of the ability of animals to produce milk suitable for drinking only, 7 individuals (21.2%) have the AB genotype. Animals with the AB genotype may produce milk suitable for the production of fermented milk products, but their quality will not be high enough. Therefore, calves and gobies with the AB genotype must be crossed with individuals having the AB genotype, then it is possible to obtain offspring with the BB genotype of milk kappa casein, which can be used to make high-quality cottage cheese, yogurt, cheese and other dairy products with a high protein content. Milk of cows with the genotype BB has the best technological qualities and does not require the addition of rennet enzyme in the production of lactic-acid products.

1. Introduction
Over the past 30 years, the Russian Federation has developed a well-developed livestock industry - dairy cattle breeding. The largest dairy breed is considered to be black-moth (Holstein), since cows of this breed give high milk yields, and breeding and farming receive subsidies from the state [1-5].

Currently, advances in molecular genetics have made it possible to identify genes associated with qualitative and quantitative features of cattle, including dairy productivity. The detection of preferred allelic variants of such genes allows, in addition to conventional animal selection and selection techniques, selection using DNA-level markers. The most informative in this regard are DNA-marker systems, namely test systems based on the analysis of polymorphism of structural genes involved in the formation and functioning of economically useful features [8, 9].

The attention of researchers has recently attracted the gene locus of one of the main milk proteins - kappa casein (CSN3). According to foreign researchers, the B allele of the CSN3 gene is associated with a higher protein content in milk, high yield of cottage cheese and cheese, as well as better coagulation properties of milk. The kappa-casein gene is one of the few known genes unambiguously associated with signs of dairy and technological properties of milk. The genotype of the bull according to kappa casein can serve as an additional criterion for the selection of animals. [6, 10-12].
recruiting breeding enterprises with producing bulls, it is necessary to take into account their genotype for kappa casein. The use of producer bulls without taking into account their genotypes leads to a decrease in the frequency of occurrence in the herd of desirable genotypes and a decrease in the quality of harvested milk. The b-allele of the kappa-casein gene is associated with higher protein content in milk, higher output of quality cottage cheese and cheese, as well as better coagulation properties of milk.

Kappa-casein has differences in structure and properties from other casein fractions and is the most important for obtaining cheese and curd mass. The structure and physicochemical properties of casein affect the technological properties of milk and the production of white milk products.

The kappa casein gene has been most studied by foreign scientists. The locus of kappa casein belongs to the synthetic group and is located on the sixth chromosome. The genotype of kappa casein in an animal is inherited according to the laws of Mendel by the type of codomination and serves as a lifelong marker, practically independent of changes in the environment and state of the body. To date, 13 kappa-casein alleles have been described: A, B, C, D, E, F, G, Az, H, X, I, A (I), J [2, 3, 5].

Many foreign researchers have found that the milk of cows with the HB genotype of kappa casein in a rennet-brooding sample in all periods of lactation belongs to class I, as high-quality (good). Milk of cows with the AAKappa-casein genotype is given to class II-III as low-quality (poor for the production of cottage cheese, yoghurt and cheese or satisfactory if used as drinking).

From the milk of cows with the genotype BB kappa-casein, elastic, large grain and cottage cheese are formed in high quality. Hard cheeses made from such milk are excellent in taste and nutrition, they are recommended for people with various pathologies of the digestive system and to strengthen immunity.

When preparing cottage cheese (9% fat content), it was found that the yield of the finished product from the milk of Holstein cows with the HB genotype of kappa casein was more than 10% higher than from the milk of cows with the AA genotype. At the same time, the consumption of whole milk per unit of production was the lowest [7].

Belgian experts have established the relevance of the use of Belgian blue and white milk (BBG), since after calving, cows produce milk, which is not inferior in quality to the product produced by representatives of dairy breeds. During lactation (about 250 days), about 4000 liters of milk with a fat content of 3.5 - 4% [5, 10] are fed from cows.

In the Russian Federation, it is also necessary to take into account the genotype of kappa-casein among bulls-producers when recruiting breeding enterprises. In bulls, the genotype of kappa casein can serve as an additional criterion in the selection of animals for breeding. Therefore, the study of the genotype of kappa casein in purebred animals, as well as crossed meat and dairy breeds for the formation of cattle herds with the genotype BB is an urgent task.

The aim of the study is to determine the genotype of kappa casein in cows of the Holstein and Belgian blue and white breeds, as well as crossbred calves and to assess the white-dairy properties of the studied animals.

2. Materials and methods
Research was carried out on purebred cattle of the Holstein and Belgian blue and white breeds, as well as on crossbred animals (hybrids Golshit and BBG).

The material for the study was the wool of cows, calves and fattening gобы, as well as the sperm of bulls.

To evaluate the locus of the kappa-casein gene, the wool of u27 crossbred animals (14 of them calves) obtained by crossing Holstein and BBG rocks was selected; in 2 cows of BBG breed and in 1 cow of the Holstein breed. Semen was selected from 3 bulls producing BBG breeds for the study.

DNA was isolated from each coat and sperm sample and PCR was performed using a Rotor Gene Q 5 Plex HRM detection amplifier. Restriction analysis was performed with the obtained amplicons using various restriction endonucleases (EaeI, Hinfl, HaeIII, Tael). The results were detected by
horizontal electrophoresis in a 2%-agarose gel. The incidence of genotypes was determined by the formula:

\[ p = \frac{n}{N} \]

where \( p \) is the frequency of occurrence of a particular genotype; 
\( n \) is the number of individuals having a certain genotype; 
\( N \)-number of individuals tested.

Studies were carried out in triplicate.

3. Results and discussion

The genotype of kappa casein BB was not found in any of the 33 animals examined (watch the table 1).

| № test | Number   | Breed | Sex         | Date of birth | The genotype of kappa casein |
|--------|----------|-------|-------------|---------------|-----------------------------|
| 1      | 3154     | CBa   | heifer      | 06.11.2019    | AA                          |
| 2      | 2014     | CBa   | heifer      | 22.01.2019    | AB                          |
| 3      | 3004     | CBa   | heifer      | 25.12.2019    | AA                          |
| 4      | 2032     | CBa   | heifer      | 12.02.2020    | AA                          |
| 5      | 3167     | CBa   | bull-calf   | 29.11.2019    | AA                          |
| 6      | 2034     | CBa   | heifer      | 13.02.2020    | AA                          |
| 7      | 3015     | CBa   | bull-calf   | 16.02.2020    | AB                          |
| 8      | 3007     | CBa   | bull-calf   | 26.12.2020    | AB                          |
| 9      | 3013     | CBa   | bull-calf   | 13.02.2020    | AA                          |
| 10     | 805819   | CBa   | heifer      | 01.07.2019    | AB                          |
| 11     | 844519   | CBa   | heifer      | 01.07.2019    | AA                          |
| 12     | 1/13     | CBa   | heifer      | 06.09.2019    | AB                          |
| 13     | 4/13     | CBa   | heifer      | 16.09.2019    | AA                          |
| 14     | 3112     | CBa   | heifer      | 16.09.2019    | AA                          |
| 15     | 3140     | CBa   | heifer      | 19.10.2019    | AA                          |
| 16     | 3150     | CBa   | heifer      | 30.10.2019    | AA                          |
| 17     | 3145     | CBa   | bull-calf   | 18.10.2019    | AA                          |
| 18     | 2083     | CBa   | bull-calf   | 14.08.2019    | AA                          |
| 19     | 3128     | CBa   | heifer      | 06.01.2019    | AB                          |
| 20     | 2115     | CBa   | bull-calf   | 16.09.2019    | AA                          |
| 21     | 3085     | CBa   | bull-calf   | 12.08.2019    | AA                          |
| 22     | 3145     | CBa   | bull-calf   | 18.10.2019    | AA                          |
| 23     | 3111     | CBa   | bull-calf   | 15.09.2018    | AA                          |
| 24     | 3073     | CBa   | bull-calf   | 06.08.2019    | AA                          |
| 25     | 3119     | CBa   | bull-calf   | 19.09.2019    | AA                          |
| 26     | 2        | CBa   | bull-calf   | 15.07.2001    | AA                          |
| 27     | 3078     | CBa   | heifer      | 15.07.2019    | AB                          |
| 28     | 3073     | HBb   | heifer      | 06.08.2019    | AA                          |
| 29     | 777      | BBBc  | cow         | 22.06.2016    | AA                          |
| 30     | 17000    | BBBc  | cow         | 17.09.2015    | AA                          |
| 31     | Maestro  | BBBc  | bull        | 23.04.2018    | AA                          |
The genotype of kappa-casein AA was found in 26 animals (78.9%), of which, in 20 crossbred (76.9%) and in 6 purebred (23%). Such animals will inherit a genotype that determines the production of milk with a low protein content, which can be used mainly as drinking.

The genotype of kappa-casein AB was found in 7 animals (21.2%), in 5 bodies and in 2 gobies. At the same time, all animals with the AB genotype were crossbred.

4. Conclusions

1 In cattle farms, it is necessary to purposefully form gene pools with certain gene combinations and select animals into herds, in accordance with the direction of production of dairy products (AA genotype - drinking milk, BB genotype - not only drinking milk, but also milk for the production of high-quality dairy products).

2 Most often (about 80%) in farms there are animals with the AA genotype, the milk of which is undesirable to use for the production of cottage cheese, cheese and other dairy products, since in order to improve the quality of the product, it is necessary to add rennet enzyme and other components that increase the cost of the final product. Milk from such cows is most suitable to use, such as drinking.

3 Cattle with the BB genotype of kappa casein are found in herds not selected for this reason, quite rarely, and animals with the AB genotype are detected in a little more than 20% of cases.

4 Animals with the AB genotype of kappa casein are promising for crossing with animals with the same genotype, since calves having the BB genotype (the law of splitting hereditary traits) can be obtained from them.

References

[1] Artemyev A M 2007 Dairy productivity and technological properties of milk of black-moth cows with various genotypes of kappa casein and calving seasons (Moscow) p 21

[2] Zobkova Z S, Zenina D V, Fursova P P and Gryazneva T N 2015 Milk processing 4(187) 12-5

[3] Borunova S M and Gryazneva T N 2016 Veterinary science, zootechny and biotechnology 9 30-6

[4] Gryazneva T N, Borunova S M, Bykanov A V, Degtyareva P A and Igumenshchev P A 2018 Effective livestock production 2(2) 41-6

[5] Zinovieva N A, Gladyr E A and Kostyunin O V 2004 Research methods in biotechnology of farm animals 7-22

[6] Zobkova Z S, Zenina D V, Fursova P and Gryazneva T N 2015 Dairy industry 3 80-3

[7] Marzanov N S, Devrishov D A and Marzanova S N 2019 Int. Academic and research Conf. Molecular and Genetic Technologies for the Analysis of an Expression of Genes of Efficiency and Resistance to Diseases of Animals (Moscow: Agricultural technologies) pp 124-30

[8] Faizov T K et al. 2014 Methodological recommendations for the typification of the kappa-casein gene, responsible for the white-dairy of cattle (Kazan: Kazan GAU) p 16

[9] Koochish I I et al. 2019 Int. Sci. and practical Conf. Molecular genetic technologies for the analysis of expression of genes of productivity and resistance to animal diseases (Moscow: Agricultural technologies) p 284

[10] Smirnova E A, Gryazneva T N and Smirnova A K 2020 Collection of articles of the international research competition Student of 2020 (Petrozavodsk: Publishing House of the
[11] Samusenko L and Himicheva S 2012 *Milk and dairy products. Production and realization* 2 pp 232-41

[12] Denicourt D, Sabour M P and McAllister A 1990 *Animal Genetics* 21 215-6