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# STUDYING THE INFLUENCE OF THE PROTEIN COMPOSITION OF RAW MILK FROM COWS WITH DIFFERENT KAPPA-CASEIN GENOTYPES ON THE HARD CHEESE YIELD AND NUTRIENT CONTENT

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***Abstract***

*Milk that was obtained from cows with different genotypes for the kappa-casein gene in accordance with the requirements of normative documents corresponds to typical cow's milk in terms of physical and chemical parameters. According to the results of the conducted research, it was established that the sensory characteristics of the cheese are influenced by the genotype of the kappa-casein gene of the animals from whose milk it is made. In terms of the content of the main chemical components, cheeses made from BB milk had a higher content of solids and protein. Cheese made from the milk of cows with the AB genotype was characterized by a higher total content of amino acids (it was 16.6 mg/g). A higher yield of cheese was obtained from the milk of animals with the BB genotype (13.1%) compared to milk from animals with the AA and AB genotypes. The obtained results are of practical importance, since it is possible to take into account how changes in the kappa-casein genotype in raw milk can affect the yield of cheese, and therefore the profitability of its production.*

***Key words*****:** *milk, cheese, protein, kappa-casein, genotype, BB, amino acids, physicochemical characteristics*

## INTRODUCTION

Over the past decade, the processes of milk coagulation in cheese making have been widely studied. Scientists have concluded that milk protein fractions are the main factors in milk coagulation [13]. This explains their interest in studying the impact of animal genotype on the quantitative and qualitative characteristics of cows' milk productivity. Particular attention is paid to the study of polymorphism of milk protein genes, which can be markers of milk quality and technological indicators [5]. The researchers came to the conclusion that selection by the genotype of milk proteins can lead to the appearance of cows from which milk is obtained that is more suitable for the production of cheese [6]. A special role in this process is played by casein, which is a numerous protein of four fractions (α S1, α S2, β and κ). Between the animals of different cattle breeds there is a significant difference in the allelic variants of the genes encoding these proteins [2, 3]. The frequency of allele A in Ukrainian Black-and-White cattle is about 0.80, and allele B is 0.20 [8].

The kappa-casein fraction accounts for approximately 80% of the total milk protein and is involved in certain physiological processes [14]. This protein is a phosphoprotein containing 169 amino acids. It is encoded by the CSN3 gene. The most common variants of this gene are CSN3*A and CSN3*B, while the CSN3*E variant is rare. Scientists believe that the best variant of kappa-casein for the production of hard cheeses is the CSN3*B variant [1, 12].

Scientists have found that milk from animals of BB genotype was more suitable for cheese making both in terms of milk composition and its technological properties. It significantly

($p<0.05$) exceeded milk from animals with other kappa-casein genotypes in terms of protein, casein and solids content [4, 17]. Similar results were obtained by other scientists [5, 7].

It has been proven that the E allele is undesirable, which is associated with the deterioration of milk syrupability [11]. According to the results of genotyping of animals for the kappa-casein gene, breeders manage to improve breeding programs for breeding dairy cattle [9, 18]. The result of this work may be a decrease in the proportion of AA genotypes [16]. In turn, increasing the share of the desired BB genotype in local breeds will contribute to their preservation [15]. At the same time, other scientists state that the kappa-casein genotype did not affect the quantitative and qualitative characteristics of cow's milk [3].

In this context the purpose of the paper was to study of the influence of the protein composition of raw milk on the yield of hard cheese and the content of nutrients in it.

## MATERIALS AND METHODS

A commercial herd of Ukrainian Black-and-White dairy breed in Sumy region was chosen for the study. In this study, 10 kg of milk was collected during morning milking from each of nine cows with different kappa-casein genotypes (AA, AB and BB). The tested samples of Gouda hard cheese were produced from whole milk using traditional technology in accordance with the requirements of DSTU 6003:2008 "Hard cheeses. General technical conditions". Simultaneously, nine samples of cheese were prepared from cow's milk of different genotypes. 10 kg of raw milk was used to make cheese. Pasteurisation, fermentation, curdling and subsequent curd formation were carried out in a laboratory cheese dairy.

The process of making samples of Gouda hard cheese in the laboratory includes the following stages: purified from mechanical impurities milk is pasteurised at a temperature of (72-75) °C for 20 s. Dried starter culture is added to the milk cooled to a temperature of (36±1) °C in the amount recommended by the manufacturer. The starter consists of mixed cultures of microorganisms – *Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis var. Diacetylactis* (Dalton, Italy). Next, a solution of calcium chloride (at the rate of 20-40 g per 100 kg of mixture) and rennet Albamax 600 (100 % chymosin) (Caglificio Clerici, Italy) is added. The mixture is fermented at a temperature of (36±1) °C until a dense curd is formed. Next, the curd is cut and processed (kneading, second heating at (39±1) °C, and drying). The formed curds are pressed and then salted in brine (salt concentration of 18-20%, temperature 10-14°C). The cheese is dried at a temperature of (10-12)°C for 4 hours. The dried cheese heads are coated with Polisved protective coating and sent for ripening at (12±2) °C for 30 days. The ripened cheese is stored in a refrigerator at (6±2) °C.

The quality of milk and cheese samples was evaluated according to generally accepted methods. Raw milk was analysed for quality parameters in accordance with DSTU 3662:2018, and cheese samples were analysed in accordance with DSTU 6003:2008.

To determine the density of milk samples, the aerothermic method was used (SSU 6082:2009). Determination of acidity (pH) of selected samples of milk and obtained cheese was carried out using the potentiometric method (SSU 8550:2015).

The mass fraction of solids in milk and cheese samples was determined by the method of drying to a constant indicator in accordance with DSTU 8552:2015. The mass fraction of protein was determined by the Kjeldahl method in accordance with DSTU ISO 8968-1:2005, DSTU 5038:2008. The mass fraction of fat was determined by the acid method (the Gerber method) in accordance with DSTU ISO 2446:2019.

Organoleptic characteristics of cheese samples were determined according to DSTU 6003:2008, with the recommendations described in the international standard ISO 22935-2:2023.

The analysis of amino acids in cheese samples was carried out by ion-exchange liquid column chromatography using an automatic amino acid analyser "T 339" (Czech Republic,

Prague). The procedure is as follows: a weighed sample (with a protein content of about 2 mg) is placed in the bottom of a test tube, 0.5 ml of distilled water and 0.5 ml of concentrated hydrochloric acid are added. The tube is cooled in a mixture of dry ice and acetone or liquid nitrogen. After the content of the tube is frozen, the air is removed from the tube using a vacuum pump to prevent oxidation of the amino acids due to hydrolysis. The tube is then sealed and placed in a constant temperature thermostat (106±1) °C for 24 hours. After hydrolysis has been completed, the tube is opened and cooled to room temperature. The content is quantitatively transferred to a glass weighing bottle and placed in a vacuum desiccator over granular caustic soda. The air is then removed from the desiccator using a water pump. After the sample is dried, 3-4 ml of deionised water is added to the weighing bottle and the drying procedure is repeated. The sample prepared in this way is dissolved in 0.3 n lithium citrate buffer (pH 2.2) and applied to the ion-exchange column of the amino acid analyser.

The research was conducted in triplicate. The obtained experimental data are presented in units of the international SI system.

The yield of hard cheese from the studied milk samples was calculated by the formula:

$$B = \frac{m_{cheese}}{m_{milk}} \cdot 100\%, \quad \text{.....} \quad (1)$$

where B – cheese yield, %;

$m_{cheese}$ – mass of cheese (30 days after production), kg;

$m_{milk}$ – mass of milk, kg.

Mathematical and statistical processing of the obtained results was carried out on a computer using MS Excel 2016 software.

## RESULTS AND DISCUSSIONS

The conducted analyzes proved that milk obtained from animals with different genotypes for the kappa-casein gene meets the requirements of the State Standard for fresh milk. Intergenotypic differentiation in terms of dry matter content, protein and fat content was established in favor of milk from animals with the BB genotype. A higher ratio of protein and fat is characteristic of milk from animals with the AB genotype (Table 1).

The photo shows the appearance of the experimental samples of hard cheese made from milk obtained from cows that differed in the kappa-casein gene genotype (Fig. 1).

Table 1. Physicochemical characteristics of raw milk samples with different kappa-casein genotypes (3 samples of each genotype)

| Genotype | Acidity, units pH | Density, kg/м$^3$ | Mass fraction of solids, % | Mass fraction of protein, % | Mass fraction of fat, % |
|---|---|---|---|---|---|
| AA | 6.4±0.01 | 1,027±1.0 | 11.8±0.02 a** | 2.89±0.01a* | 3.65±0.01a** |
| AB | 6.4±0.01 | 1,026±1.0 | 11.2±0.02 | 2.83±0.01 | 3.07±0.01 |
| BB | 6.2±0.01 | 1,027±1.0 | 12.5±0.02 a**;b* | 2.93±0.01a* | 4.34±0.01a**;b* |

Note: a - in relation to the AB genotype; b - to the AA genotype; *P<0,05; *P<0,01

Source: Own research.


Fig. 1. Characteristics of the appearance of the obtained cheese samples (1 – made from the milk of animals with the AA genotype; 2 – made from the milk of animals with the AB genotype; 3 – made from the milk of animals with the BB genotype).

Milk from animals with the homozygous BB genotype was characterized by a higher yield of cheese (Fig. 2).

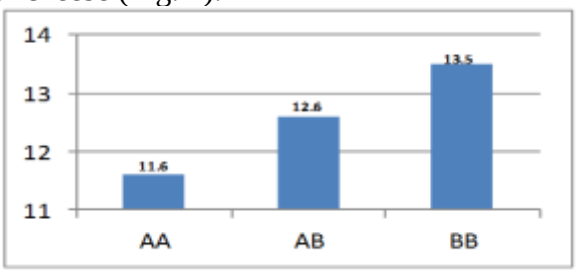


Fig. 2. Average yield of hard cheese from milk of cows with different kappa-casein genotypes, %

Source: Own research.

Based on the average results of the sensory analysis of the general characteristics of hard cheese conducted by the expert group, the specific features of such characteristics as appearance, taste, smell, consistency, colour, pattern, and head shape were identified.

The samples of hard cheese made from the milk of AA cows have an average score of 4.3 points for appearance, 4.3 points for taste and smell, 3.7 points for consistency, 4.3 points for colour, 3.3 points for the pattern on the cut, and 5 points for the shape of the cheese heads. The cheeses are characterised as satisfactory in appearance; with good taste but weak flavour; with satisfactory texture and uniform colour; with uneven slit-like arrangement of eyes on the cut.

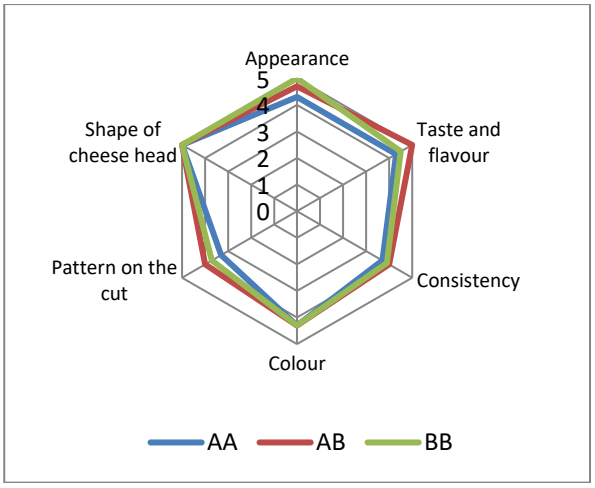


Fig. 3. Sensory profile of hard cheese samples
Source: Own research.

The samples of hard cheese from the milk of AB cows were rated on average at 4.7 points for appearance, 5.0 points for taste and smell, 4.0 points for consistency, 4.3 points for colour, 4.0 points for the pattern on the cut, and 5.0 points for the shape of the cheese heads. The cheese samples have a good appearance; excellent taste and flavour; good consistency; uniform colour and arrangement of the eyes on the cut (Fig. 3).

According to the obtained profiles of sensory analysis, the samples of hard cheeses made from the milk of BB genotype cows have an average score of 5.0 points for appearance, 4.5 points for taste and flavour, 3.9 points for consistency, 4.3 points for colour, 3.7 points for the pattern on the cut, and 5.0 points for the shape of the cheese heads.

In terms of appearance, the cheeses are characterised by experts as cheeses with a good oval; with a good taste but a weakly expressed flavour; with a satisfactory consistency and uniform colour; with an uneven arrangement of eyes on the cut.

Table 2 shows the results of the study of physicochemical characteristics of hard cheese from milk obtained from cows of different kappa-casein genotypes.

The composition of cheese differed to some extent, depending on the kappa-casein genotype.

Intergenotypic differentiation according to the chemical composition of cheese was established. The cheese obtained from milk from cows with the AB genotype prevailed in terms of dry matter content, AB - in terms of protein content, and BB - in terms of fat content.

The amino acid profile of the hard cheese samples was analysed using a chromatograph. Figure 4 shows the average results.

It was found that samples of cheese made from milk from AA genotype cows were characterised by the lowest content of essential amino acids: leucine (1.21 mg/g), lysine (0.93 mg/g), phenylalanine (0.69 mg/g), threonine (0.50 mg/g), histidine (0.33 mg/g), valine (0.39 mg/g).

Table 2. Physicochemical characteristics of cheese samples from milk of cows with different kappa-casein genotypes (3 samples each)

| Kappa-casein genotype | Acidity, units pH | Mass fraction of solids, % | Mass fraction of protein, % | Mass fraction of fats, % |
|---|---|---|---|---|
| AA | 5.44±0.01 | 51.6±0.20 | 13.5±0.10$^{b**}$ | 36.1±0.10 |
| AB | 5.16±0.01 | 64.5±0.20$^{a**}$ | 17.2±0.10 | 35.6±0.10 |
| BB | 5.33±0.01 | 63.6±0.20$^{a**}$ | 13.0±0.10$^{b**}$ | 38.1±0.10$^{b**}$ |

Note: a - in relation to the AA genotype; b - to the AB genotype; *P<0.05; *P<0.01
Source: The authors' own research.

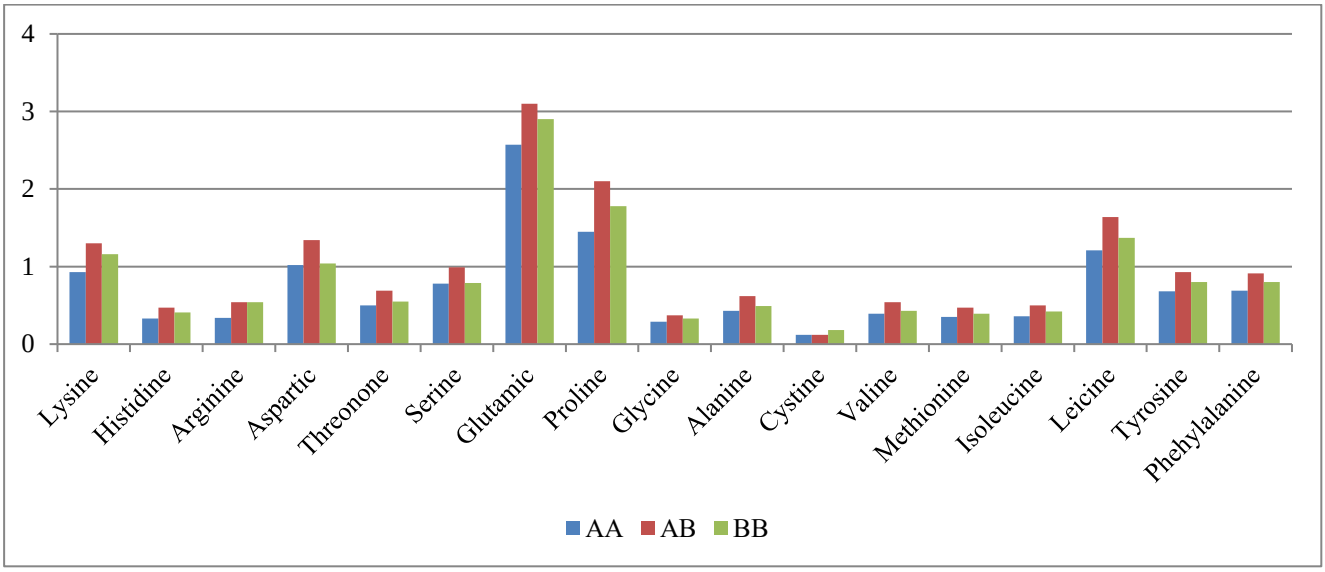


Fig. 4. Average amino acid profile of hard cheese samples made from milk of cows of different genotypes, mg/g
Source: The authors' own research.

The samples also contain the following nonessential amino acids: glutamic acid (2.57 mg/g), aspartic acid (1.02 mg/g), proline (1.45 mg/g), serine (0.78 mg/g), tyrosine (0.68 mg/g).

The samples of cheese made from milk of AB genotype cows had the highest content of essential amino acids among the studied ones: leucine (1.63 mg/g), lysine (1.30 mg/g), phenylalanine (0.91 mg/g), threonine (0.69 mg/g), histidine (0.47 mg/g), valine (0.54 mg/g). The samples contained the following nonessential amino acids: glutamic acid (3.10 mg/g), aspartic acid (1.34 mg/g), proline (2.09 mg/g), serine (0.99 mg/g), tyrosine (0.93 mg/g). The cheese samples from milk of BB genotype cows differed in the average content of essential amino acids among the studied ones: leucine (1.37 mg/g), lysine (1.16 mg/g), phenylalanine (0.80 mg/g), threonine (0.55 mg/g), histidine (0.40 mg/g), valine (0.43 mg/g).

The samples contained the following nonessential amino acids: glutamic acid (2.90 mg/g), aspartic acid (1.04 mg/g), proline (1.78 mg/g), serine (0.80 mg/g), tyrosine (0.80 mg/g).

Cheese samples obtained from milk from cows with AB and BB genotypes are characterized by both higher product yield and better quality characteristics. In order to breed animals with such genotypes, we recommend using stud bulls with BB genotype for reproduction (Fig. 5).

Our previous studies [10] identified stud bulls of domestic breeds, including the Ukrainian Black-and-White dairy breed, with the desired genotype.

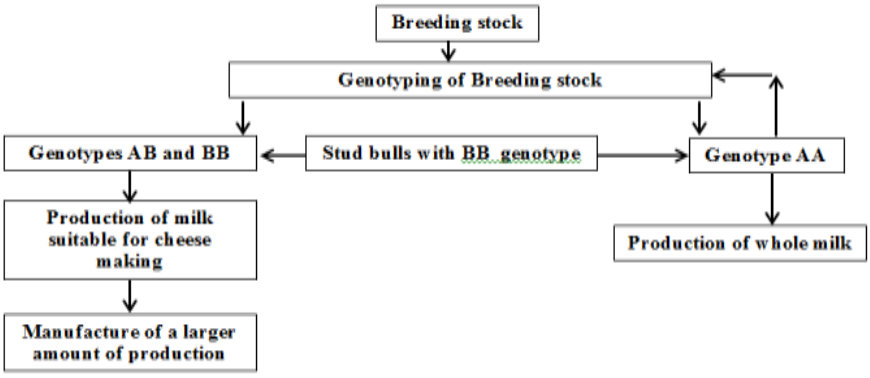


Fig. 5. Scheme of producing milk suitable for cheese making
Source: The authors' own research.

## CONCLUSIONS

The studies have established that the physicochemical characteristics of raw milk from cows with different kappa-casein genotypes (AA, AB, BB) are common for fresh cow's milk production and meet the requirements of regulatory documents.

Milk from cows with different genotypes for the kappa-casein gene has certain differences in chemical composition. Milk from animals with the BB genotype prevails in terms of dry matter, fat and protein content. The effect of the genotype of the cows from which the milk was obtained on the sensory characteristics of the cheese was also determined. However, in terms of the content of the main chemical constituents, cheeses made from BB milk had a higher content of solids and protein.

The intergenotypic differentiation by the amino acid profile of cheese was established. Samples from animals with the AB genotype had a higher total amino acid content. Genotype for the kappa-casein gene had an effect on cheese yield. Thus, the output from the milk of animals with the BB genotype was greater and amounted to 13.1%.

These results are interrelated with the chemical composition of milk and the optimal protein: fat ratio in the initial milk samples.

A scheme for producing raw milk suitable for cheese making has been developed and implemented at the experimental farm.

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