ASSESSMENT OF KAPPA-CASEIN GENE POLYMORPHISM IN ROMANIAN PINZGAU CATTLE: IMPLICATIONS FOR MILK PRODUCTION TRAITS

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Abstract

The study of milk polymorphism, focusing on k-casein is vital for improving milk quality and dairy production. K-casein is a key protein that influences milk's physical and chemical properties, including its ability to coagulate. The objective of this study was to investigate the genotype profile of the kappa-casein gene in Romanian Pinzgau cattle, specifically the Black and Red varieties. A total of 24 cows were genotyped for the kappa-casein gene using the PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) method. Three genotypes (AA, AB, and BB) were identified within the studied population. The frequency of the A allele was 0.681 in Black Pinzgau and 0.563 in Red Pinzgau, while the B allele exhibited frequencies of 0.319 in Black Pinzgau and 0.437 in Red Pinzgau. The higher frequency of the A allele in both Black and Red Pinzgau suggests a potential for greater milk volume but with potentially lower protein content and cheese-making efficiency. On the other hand, the presence of the B allele, especially in the Red variety, indicates a favorable genotype for dairy producers focusing on milk quality, particularly for cheese production.

Key words: *cattle, genotype frequency, k-casein, milk polymorphism*

INTRODUCTION

The genetic improvement of dairy cattle is a key aspect of enhancing milk production and quality. One of the primary targets in bovine genomics is the kappa-casein (CSN3) gene, which plays a crucial role in determining milk protein content, composition, and cheese-making properties. This gene encodes for kappa-casein, a protein that is critical for the stabilization of milk micelles, directly influencing the milk's ability to coagulate, a desirable trait in dairy products like cheese [1,2,3,4].

In recent years, there has been increased interest in studying gene polymorphisms, variations in DNA sequences that can influence traits such as milk yield, protein content, and fat percentage. The identification of specific polymorphisms, such as those found in the kappa-casein gene, can provide valuable insights into selective breeding strategies aimed at enhancing these economically important traits [4,5,6].

The Romanian Pinzgau cattle, a breed well adapted to the mountainous regions of Romania is known for its hardiness and moderate milk production. In addition, the Pinzgau cattle are renowned for their exceptional rusticity and adaptability, making them highly resilient to diseases and harsh environmental conditions, particularly in mountainous regions, where their robust nature ensures sustainable productivity even in challenging climates [3,7,8,9,10,11].

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However, there is limited information regarding the genetic factors affecting milk composition in this breed. Understanding the kappa-casein gene polymorphism in Romanian Pinzgau cattle could provide a pathway to improve milk production traits through targeted breeding programs.

This study focuses on the assessment of kappa-casein gene polymorphisms in Romanian Pinzgau cattle and their implications for key milk production traits, such as milk yield, protein content, and fat percentage. By identifying and characterizing specific genetic variations in this gene, the research aims to contribute to more effective breeding strategies for optimizing milk quality and enhancing the economic value of the Pinzgau cattle breed.

MATERIAL AND METHOD

The biological material used in the study consisted of a herd of Pinzgau cattle, specifically Black Pinzgau and Red Pinzgau, from the Vatra Dornei area. In total 24 full blood samples were collected, 14 of them cattle breed. A total of 24 blood samples were collected by puncturing the jugular vein, with 12 samples from each cattle variety. The samples were collected in EDTA (ethylenediaminetetraacetic acid) tubes to prevent coagulation until the genetic analysis was performed. The total genomic DNA was isolated using the Wizard Genomic DNA Purification kit provided by Promega. The total amount of DNA obtained after precipitation was resuspended in sterile distilled water and measured spectrophotometrically to determine the concentration in each sample. The integrity of the DNA was evaluated using agarose gel electrophoresis, and the purity of the samples was assessed based on the A260/A280 ratio.

For the genotyping of the animals in the study, focusing on the kappa-casein gene involved in milk protein synthesis, the following steps were carried out: in situ amplification of DNA fragments specific to the gene of interest using PCR, visualization of the amplicons (PCR products) in agarose gel, restriction of the amplification products with specific endonucleases using the RFLP technique, and visualization of the restriction fragments of different lengths through agarose gel electrophoresis.

RESULTS

Spectrophotometric analysis of DNA is crucial as it provides precise measurements of DNA concentration and purity, allowing researchers to ensure the quality of their samples before proceeding with downstream applications such as PCR, sequencing, and cloning. Following the spectrophotometric analysis of all DNA samples, the DNA concentration values obtained ranged from 74 to 260 ng/µl (Table 1).

Table 1 Characteristics of the primer pairs used to amplify the gene sequences by PCR

No. of sample	DNA concentration (ng/µl)
1.	175.2
2.	110.3
3.	77.8
4.	251.6
5.	91.2
6.	79.8
7.	260
8.	144.9
9.	243.6

No. of sample	DNA concentration (ng/µl)
10.	122.6
11.	214.8
12.	221.7
13.	87.9
14.	132.7
15.	254.6
16.	193.6
17.	100.3
18.	89.8
19.	145.8
20.	201.5
21.	74.0
22.	207.5
23.	88.8
24.	104.3

The results obtained from the spectrophotometric quantification analysis demonstrated the effectiveness of the DNA extraction method as well as the purity of the samples.

The kappa-casein (κ-CN) gene plays a pivotal role in milk protein synthesis, significantly influencing the quality and coagulation properties of milk, which are essential for cheese production and overall dairy product quality [12,13].

Its symbol is „κ-casein (κ-CN)". To study this gene, its specific DNA sequence was amplified:

ATCATTTATGGCCATTCCACCA
AAGAAAAATCAGGATAAAACAGAA
ATCCCTACCATCAATACCATTGCTA
GTGGTGAGCCTACAAGTACACCTAC
CATCGAAGCAGTAGAGAGCACTGTA
GCTACTCTAGAAGCTTCTCCAGAAG
TTATTGAGAGCCCACCTGAGATCAA
CACAGTCCAAGTTACTTCAACTGCG
GTCTAAATACTCTAAGGAGACATCA
AAGAAGACAACGCAGGTAAATAAG
CAAAATGAATAACAGCCAAGATTCA
TGGACTTATTAATAAAATCGTAACA
TCTAAACTAGCGTAGATGGATAAAT
TAAA**TCTGTTACAGAGAAGGCGAA
ATGGGC**

For the PCR amplification of the gene of interest, primers from Table 2 were used.

The PCR reaction was performed in a final volume of 25 µl containing 25-100 ng of DNA, 20 pmoles of each primer, and mastermix (Dream Taq Green). The gene was amplified for a total of 35 cycles. The parameters of the amplification reaction are presented in Figure 1.

Table 2 Characteristics of the primer pairs (forward and reverse) used to amplify the gene sequences by PCR

Locus amplified	Sequenced (5′-3′)	Length of amplicon (bp)
κ-CN	5'-ATCATTTATGGCCATTCCACCAAAG-3' - Forward 3'-GCCCATTTCGCCTTCTCTGTAACAGA-5' - Reverse	350

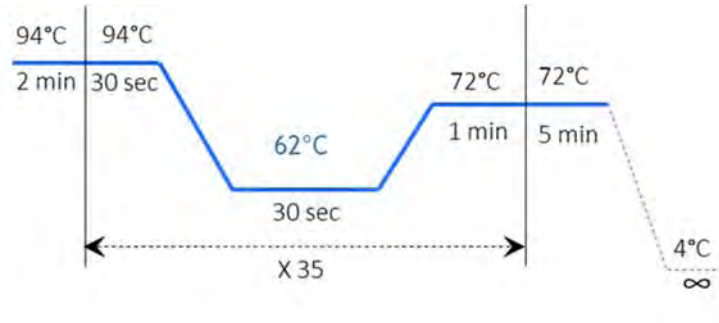

Fig. 1 Optimization PCR Program, κ-CN amplification

The PCR amplicons were checked in a 1% agarose gel, followed by migration at a voltage of 100 volts for 30 minutes. A 100 base pair molecular weight marker was used to estimate the size of the amplified fragments. The migration of the PCR products in the agarose gel revealed that there were no non-specific amplifications and no contamination, resulting in a high yield of copies of the gene sequence of interest (Figure 2).

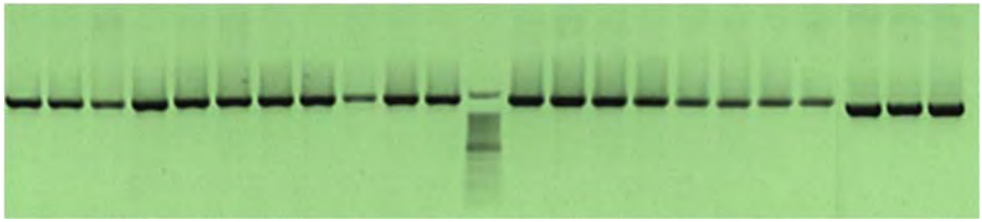

Fig. 2 Agarose gel electrophoresis of PCR amplicons

The determination of the existing genotypes at the k-CN gene level was achieved by specific cleavage of the amplicons with the restriction enzyme HindIII. This restriction enzyme cleaves the DNA molecule (amplicons) at specific regions, resulting in restriction fragments of different lengths. The restriction enzyme HindIII is a type II restriction endonuclease that recognizes the specific DNA sequence AAGCTT and cleaves it between the adenine and guanine nucleotides. This enzyme is widely used in molecular biology for cloning and gene manipulation, as it generates sticky ends that facilitate the ligation of DNA fragments.

After the migration of the PCR-RFLP products in agarose gel, the allele and genotype frequencies were calculated and interpreted. The data obtained for Black Pinzgau population are presented in Table 3 and for Red Pinzgau population are presented in Table 4.

Table 3 The polymorphism in the k-CN gene locus in the Black Pinzgau breed

Locus	Genotype	Genotype frequency	Allele frequency	
κ-CN	AA	0.464	p_A	0.681
	AB	0.434		
	BB	0.102	q_B	0.319

Table 4 The polymorphism in the k-CN gene locus in the Red Pinzgau breed

Locus	Genotype	Genotype frequency	Allele frequency	
κ-CN	AA	0.317	p_A	0.563
	AB	0.492		
	BB	0.191	q_B	0.437

In the Black Pinzgau population, a higher frequency of allele A (0.681) was recorded for the k-casein locus compared to allele B (0.319). The genetic structure of the population is dominated by the homozygous genotype AA, which has a frequency of 0.464 compared to the genotypes AB (0.434) and BB (0.102).

In the Red Pinzgau population, a higher frequency of allele A (0.563) was also recorded for the k-casein locus compared to allele B (0.437). In this case, the genetic structure of the population is dominated by the heterozygous genotype AB, which has a frequency of 0.492 compared to the genotypes AA (0.317) and BB (0.191).

DISCUSSIONS

The k-casein allele frequencies in the Black Pinzgau and Red Pinzgau populations indicate a strong presence of allele A, with frequencies of 0.681 and 0.563, respectively, which align with trends observed in other European dairy breeds known for similar high frequencies of the A allele (often ranging from 0.60 to 0.80). In the Black Pinzgau, the homozygous genotype AA predominates (0.464), reflecting selective breeding for milk production traits, while the Red Pinzgau shows a notable prevalence of the heterozygous genotype AB (0.492), suggesting a more diverse genetic structure that may enhance adaptability but indicates less targeted selection.

These findings underscore the potential benefits of increasing allele A frequencies to optimize milk production, as similar patterns have been reported in breeds like Brown Swiss and Jersey, where the A allele frequencies are also significantly high [14,15].

The genetic distribution highlights the importance of maintaining genetic diversity in breeding programs for long-term sustainability, adaptability, and resilience against diseases [15,16,17,18].

Future research should focus on monitoring these allele frequencies over time, particularly in response to breeding interventions, to ensure the balance between enhanced production traits and genetic diversity within these populations.

CONCLUSIONS

The analysis of k-casein allele frequencies in the Black Pinzgau and Red Pinzgau populations reveals a significant prevalence of allele A, which is associated with desirable milk production traits. The higher frequency of the homozygous genotype AA in the Black Pinzgau indicates a strong focus on selective breeding for enhanced milk yield and quality. In contrast, the predominance of the heterozygous genotype AB in the Red Pinzgau suggests a more diverse genetic pool, which may contribute to adaptability but could indicate a need for more focused selection strategies. Overall, these findings align with trends observed in other European dairy breeds, highlighting the potential advantages of increasing allele A frequencies in breeding programs.

To optimize milk production while maintaining genetic diversity, it is recommended that breeding programs for both Black Pinzgau and Red Pinzgau populations implement strategies that balance the selection for the A allele with efforts to preserve genetic variation. This could involve controlled mating programs that encourage the use of heterozygous

individuals to foster genetic diversity while gradually increasing the frequency of the A allele. Additionally, conducting longitudinal studies to monitor changes in allele frequencies and their impact on production traits will provide valuable insights that can inform future breeding strategies.

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