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The genotypic profile of milk proteins in Holstein cows raised in South of Brazil

Perfil genotípico de proteínas do leite em vacas da raça Holandês criadas no Sul do Brasil

El perfil genotípico de las proteínas de la leche en vacas Holstein criadas en el Sur de Brasil

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Fabrício Pilonetto
ORCID: https://orcid.org/0000-0002-2161-0831
University of São Paulo, Brazil
E-mail: fpilonetto@alumni.usp.br
Giovanni Coelho Ladeira
ORCID: https://orcid.org/0000-0003-2925-3266
University of Florida, USA
E-mail: g.coelholadeira@ufl.edu
Mayara Salvian
ORCID: https://orcid.org/0000-0002-8336-4082
University of São Paulo, Brazil
E-mail: mayara_salvian@hotmail.com
Aline Zampar
ORCID: https://orcid.org/0000-0002-2269-7932
University of Santa Catarina State, Brazil
E-mail: aline.zampar@udesc.br
Diego de Córdova Cucco
ORCID: https://orcid.org/0000-0001-5962-246X
University of Santa Catarina State, Brazil
E-mail: diego.cucco@udesc.br

Abstract
Genotyping in dairy cattle allows knowing the alleles and genotypes associated with milk proteins that affect yield and milk quality. This study aimed to verify the genotype profile for κ-casein I and II (κ-CN), α-s1-casein (α-CN) and β-lactoglobulin (β-LG), for Holstein cows raised in southern Brazil, where there is no scientific evidence on the presence of these alleles in dairy herds. Hair follicles samples from each animal were used for DNA extraction and genotyping. We calculated the allelic and genotypic frequencies for four types of milk protein variants in the population, and a chi-square test was employed to see whether the population of animals used was in Hardy-Weinberg equilibrium. Allele A and AA genotype showed the highest frequency for both κ-CN (56.77 to 80.36%). For α-s1-CN and β-LG, the B allele was the most frequent, as BB (80.26%) and AB (47.15%), respectively. Due to the presence of alleles associated with protein levels in milk, we concluded that these herd cows present milk technological properties interesting to produce dairy products.
Keywords: Allele frequency; Casein; Lactoglobulin; Milk yield.

Resumo
A genotipagem em bovinos leiteiros permite conhecer os alelos e genótipos associados às proteínas que afetam a qualidade e a produção de leite. Este estudo teve como objetivo verificar o perfil genotípico para κ-caseína I e II (κ-CN), α-s1-caseína (α-CN) e β-lactoglobulina (β-LG), para vacas Holandesas criadas no sul do Brasil, onde não há evidências científicas sobre a presença desses alelos em rebanhos leiteiros. Amostras de folículos pilosos de cada animal foram usadas para extração de DNA e genotipagem. Calculamos as frequências alélicas e genotípicas para quatro tipos de variantes de proteína do leite na população, e um teste qui-quadrado foi empregado para verificar se a população de animais utilizada estava em equilíbrio de Hardy-Weinberg. O alelo A e o genótipo AA apresentaram a maior frequência para ambos os κ-CN (56,77 a 80,36%). Para α-s1-CN e β-LG, o alelo B foi o mais frequente, assim como BB (80,26%) e AB (47,15%), respectivamente. Devido à presença de alelos associados aos níveis de proteína no leite, concluímos que essas vacas de rebanho apresentam propriedades tecnológicas do leite interessantes para a produção de produtos lácteos.
Palavras-chave: Frequência alélica; Caseína; Lactoglobulina; Produção de leite.

Resumen
El genotipado en bovinos lecheros permite conocer los alelos y genotipos asociados a las proteínas de la leche que afectan el rendimiento y la calidad de la leche. Este estudio tuvo como objetivo verificar el perfil genotípico de κ-caseína I y II (κ-CN), α-s1-caseína (α-CN) y β-lactoglobulina (β-LG), para vacas Holstein criadas en el sur de Brasil, donde no hay evidencia científica sobre la presencia de estos alelos en hatos lecheros. Se utilizaron muestras de

folículos pilosos de cada animal para la extracción de ADN y el genotipado. Calculamos las frecuencias alélicas y genotípicas para cuatro tipos de variantes de proteína de leche en la población, y se empleó una prueba de chi-cuadrado para ver si la población de animales utilizada estaba en equilibrio de Hardy-Weinberg. El alelo A y el genotipo AA mostraron la mayor frecuencia para ambos κ-CN (56,77 a 80,36%). Para α-s1-CN y β-LG, el alelo B fue el más frecuente, al igual que BB (80,26%) y AB (47,15%), respectivamente. Debido a la presencia de alelos asociados a los niveles de proteína en la leche, concluimos que estas vacas del hato presentan propiedades tecnológicas de la leche interesantes para la elaboración de productos lácteos.
Palabras clave: Frecuencia alélica; Caseína; Lactoglobulina; Producción de leche.

1. Introduction

Milk quality as well as solids, such as fat and protein, are important to the dairy industry due to their influence on the production of dairy products, such as cheeses (Cecchinato et al. 2015; Bittante et al. 2013). In addition, the animal genetic background is an important source of variation in milk quality, thus, the study of the genetic variations in the milk protein composition has become essential for the selection of the animals (Riaz et al. 2012). Within the milk protein, the kappa-casein I and II (κ-CNI and κ-CNII), α-s1-casein (α-s1-CN) and β-lactoglobulin (β-LG) are the most important protein variants and are present in greater proportion in milk (Visker et al. 2010).

In the south of Brazil, dairy cattle activity is one of the main income-generating activities, especially in the western Santa Catarina state, which is composed of small rural properties. However, there are few scientific reports on milk proteins allelic and genotypic frequencies in dairy cattle in Brazil, being unknown in the studied region. This study aimed to identify the genotype profile of milk proteins in Holstein cows from southern Brazil.

2. Methodology

A total of 391 Holstein cows, born between 2008 and 2015, and belonging to 30 farms in the Western region of Santa Catarina state, southern Brazil, were used in this study. This region has an average annual temperature between 12 to 26 ºC, and monthly rainfall of 125 to 175 mm (INMET, 2022). The cows were kept in freestall sheds and fed roughage and energy supplementation twice daily. Some exceptions were found on smaller farms, where cows were raised in a grazing system, with only energy supplementation. Independent of the farm, milked were twice a day.

The data was provided by DNA Genética do Brasil, headquartered in the Santa Catarina state (Brazil Southern). Hair follicles samples were collected from each cow and shipped to Zoetis Brazil for DNA extraction and quality control tests. Animals were nominated to CDCB (Council on Dairy Cattle Breeding), to obtain the genetic evaluation after a genomic test for 12,000 SNPs markers (SNP - Single Nucleotide Polymorphism), using chip CLARIFIDE® (12K Clarifide Plus, Zoetis). All the genotype results for milk yield, fat, protein and reproduction traits were validated by the genetic evaluation of the US Department of Agriculture/Council on Dairy Cattle Breeding (USDA-CDCB). In addition, genomic testing was performed to identify haplotypes for some genetic diseases in Holstein cattle (Pilonetto et al., 2019). From the genotyping panel, the genotypes of each animal were studied for casein and lactoglobulin genetics variants.

We calculated the allelic and genotypic frequencies for four genetic variants of milk protein, being the genotypes AA, AB, BB and BC, referring to kappa-casein I, II (κ-CN I and II), β-lactoglobulin (β-LG) and alpha-s1-casein (α-s1-CN). The frequencies for A, B, and C alleles were calculated by direct counting. A chi-square test (χ^2) was employed to see whether the population of animals used were in Hardy-Weinberg equilibrium to 0.05 significance value ($P < 0.05$) (Riaz et al. 2012), by R program 4.1.2 (R Code Team, 2021). Thus, the following equation was used:

$$\chi^2 = \sum \frac{d^2}{e}$$

where χ^2 is the chi-square value; d^2 the observed frequency; and e is the expected frequency of the genotypes AA, AB, BB, and BC.

3. Results

For κ-casein I and II, the highest frequency was for allele A (0.767 and 0.899, respectively), and for κ-casein I, genotype AA was the most frequent in the population with 56.77%, followed by AB and BB genotypes (39.84 and 3.39%, respectively). Likewise, for κ-casein II, the highest frequency was for AA (80.36%), followed by AB (19.05%) and BB (0.59%) (Table 1). About α-s1-casein, the genotyping detected the presence of three alleles (A, B and C). The B allele showed the highest frequency (0.853), followed by A and C (0.115 and 0.031, respectively) and BB genotype for α-s1-CN was the most frequent, with 80.26%, then AA (9.49%), BC (6.15%) and AB (4.10%).

For the β-lactoglobulin, the AB genotype was the most frequent (47.15%), followed by BB and AA (3.05 and 22.80%, respectively), and B allele greater predominance (0.536). Furthermore, the chi-square test showed that the cow population in this study is in Hardy-Weinberg equilibrium (HWE) for κ-casein II and β-lactoglobulin unlike of α-s1-CN ($P < 0.05$) and κ-CN ($P < 0.05$).

Table 1 - Genetic frequency for kappa-casein I and II (κ-casein I and II), alpha-s1-casein (α-s1-casein), and beta-lactoglobulin (β-lactoglobulin) in Holstein cows raised in southern Brazil.

Protein	Genotype frequency			Allelic frequency	χ^2
	Genotype	Animals	%		
κ-casein I	AA	218	56.77		
	AB	153	39.84	A 0.767	
	BB	13	3.39	B 0.233	5.035*
	Total	**384**			
κ -casein II	AA	270	80.36		
	AB	64	19.05	A 0.899	
	BB	2	0.59	B 0.101	0.747 NS
	Total	**336**			
α-s1-casein	AA	37	9.49		
	AB	16	4.10	A 0.115	
	BB	313	80.26	B 0.853	
	BC	24	6.15	C 0.031	246.520*
	Total	**390**			
β-lactoglobulin	AA	88	22.80		
	AB	182	47.15	A 0.464	
	BB	116	3.05	B 0.536	0.884 NS
	Total	**386**			

NS, not significant; χ^2, Chi-squared; *significant value (*P* < 0.05). Fonte: Autores.

4. Discussion

Based on previous studies of Poulsen et al. 2013 and Perna et al. 2016, the presence of genotypes associated with the κ-CN B allele suggests that the herd considered in this study has genetic potential to produce milk with desirable properties for the industrial dairy products manufacture. The authors reported genotypes linked to the B allele of κ-CN were associated with better coagulation and yield in milk derivatives production, mainly for cheeses. According to the results of our study, there is a possibility that this population has a higher protein content in milk in relation to fat content, due to the higher frequency of the AB genotype in relation to the BB.

Furthermore, Poulsen et al. (2013) investigated the effect of the B and C allele for α-s1-CN in Holstein cattle (B and C allele) and found a significant effect on rennet clotting time and curd firmness rate in animals that had these alleles. The research by Perna et al. (2016), and Gustavsson et al. (2014), found a greater association between milk yield and BB-genotype of α-s1-CN. Thus, our results for α-s1-CN indicate the genetic potential of the dairy herd to produce milk with important technological properties in the manufacture of dairy products, due to the higher frequency of the BB genotype and the B allele.

The β-lactoglobulin (β-LG) is a whey protein and an important industrial component for milk processing. In our study, the highest frequencies were of the AB genotype (47.15%) and the B allele (0.536). Similar results were reported by Bonfatti et al. (2010), in Simmental cattle, where the genotypic frequency for AB was 46.6%, but the A allele had the highest frequency (0.543). Lower levels of beta-lactoglobulin in milk are commonly reported in the literature when the B allele is more frequent in the population. In the study by Bedere and Bovenhuis (2017), this was proven and, in addition, the frequency of the A allele was associated with a higher concentration of β-LG in milk. In addition, Hallén et al. (2008) reported that homozygous BB animals should produce milk with lower β-LG content compared to AB heterozygotes.

Apparently, β-LG alleles A and B are found more frequently in dairy herds and are strongly associated with protein composition and industrial milk production. Therefore, we observed that the selection of animals for β-LG can be done according to the productive interest, since animals heterozygous for β-LG, especially those with genotype AB, may have higher protein content in milk. Thus, the results of our study suggest cows with a genetic profile for milk production with desirable quality properties for the manufacture of dairy products like cheese, due to higher proportion of animals in the population with AB and BB polymorphisms.

According to the Hardy-Weinberg Equilibrium presented in Table 1, κ-casein II and β-lactoglobulin are in genetic equilibrium, i.e., there was no selection, migration or mutation process for these populations, which is expected, since in Brazil it is not common to select for protein variants. In addition, the A allele of κ-CN II and the α-s1-CN B are close to the fixation and, due to the low frequency found, it may be difficult to change their frequencies in the population (Oner and Elmaci, 2006). However, the frequencies of β-LG and κ-CN I can be changed more easily, due to their greater distribution in the population. This information may contribute to the direction of mating in the region, due to the importance that the B allele of β-LG and κ-CN has on the protein concentration and physicochemical properties of milk (Perna et al. 2016; Gouda et al. 2013).

In Brazil, it is common to import genetic material from bulls with estimated genetic values outside the country. Thus, in many systems, the same bull is widely used for more than one generation of consecutive mattings, without parental control and pedigree registration. This is a practice that leads to an increase in the inbreeding coefficient, and declining the response to selection and genetic variability (Doublet et al. 2019; Pimentel et al. 2011; Brothersone and Goddard, 2005). We found alleles close to the fixation, consistent with the history of the studied region. This causes alarm about the genetic diversity decline of the dairy cattle population for milk quality of the studied region, requiring methods accessible to farms to select cattle to maximize genetic gain while maintaining genetic variation.

5. Conclusion

Although the frequencies of the B allele for κ-CN I and II were low, a genotypic profile of interesting proteins for the production of dairy products was reported, due to the higher frequencies of alleles and genotypes associated with casein and beta-lactoglobulin concentrations. Furthermore, this population of animals may have undergone an evolutionary process towards κ-casein I and α-s1-casein, due to not being in Hardy-Weinberg equilibrium. In the study region, animal reproduction occurs by artificial insemination, therefore, the importation of genetic material from bulls may have contributed to the genotypic imbalance for these traits. On the other hand, the fact that the A allele for κ-casein II and β-lactoglobulin is close to fixation implies the need to direct the mattings to avoid a decrease in the genotypic variability of dairy herds in southern Brazil. For a detailed study of the genetic structure and action of genes for milk proteins, phenotypic information and an enrichment of the genomic database should be essential.

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