

GENOTYPING OF THE BETA-CASEIN A1 AND A2 VARIANTS IN CHILEAN DAIRY CATTLE

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SUMMARY

Bovine milk is a very important food for human health and nutrition. However, its consumption can also be associated with dysfunctions or pathologies in some consumers. The objective of this work was to evaluate the distribution of the β -casein A1 and A2 variants in the main dairy cattle breeds used in Chile. Blood samples were obtained from 134 cattle from southern Chile, belonging to different breeds: Holstein-Friesian (HF), Jersey (JE), Montbéliarde (MB), Overo Colorado (OC), and crossbreds (HYB). Genomic DNA was purified from the blood, and genotyping of the A1 and A2 variants was performed using allele-specific PCR. Genotypic and allelic frequencies were estimated by direct count, and the Hardy-Weinberg equilibrium was tested for using the chi-squared test, for a significance level of $P < 0.05$. Heterozygosity was evaluated by means of Wright's fixation index (FIS) using GenePop software. Genotyping of the variants showed variability in their distribution, with prevalence of A2 in almost all breeds (including HF), with the exception of OC. The χ^2 value indicated that the animal populations were in Hardy-Weinberg equilibrium with the exception of MB and JE ($p < 0.05$), with the latter showing a significant and positive FIS value suggesting inbreeding. In conclusion, our results, although preliminary, suggest that there is an opportunity to produce A2 milk in southern Chile and thus reduce the presentation of gastrointestinal dysfunctions in consumers who are affected by conventional milk.

KEY WORDS: milk quality, beta-casein, A2 variant



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INTRODUCTION

Many consumers worldwide are currently showing an interest in healthy and safe foods which additionally have properties preventing disease onset or progression. These foods are called functionals or nutraceuticals, as they contain compounds performing health functions in addition to nutrition. Bovine milk and dairy derivatives are rich in these compounds (Kay et al., 2021). On the other hand, the consumption of milk and dairy products has been associated with dysfunctions or pathologies in some consumers, such as some degree of gastrointestinal dysfunction and/or allergy associated with milk components, e.g. lactose or certain milk proteins (Pal et al., 2015; Sheng et al., 2019). Regarding the protein fraction, it has been suggested that one of the major proteins of milk, β -casein, could be responsible for some of these dysfunctions by generating a peptide in the gastrointestinal tract (Woodford, 2006; Pal et al., 2015).

Bovine β -casein has different forms or variants (15 have been described thus far), the most important being A1 and A2. These two variants differ in only one amino acid (proline to histidine at position 67) as result of a SNP in exon 7 of the *CSN2* gene, which codes for β -casein (Kamiński et al., 2007; Cieślińska et al., 2012). The A1 variant has been described as more hydrolysable and therefore more easily degraded by intestinal enzymes (e.g. elastase) than A2, generating some peptides. These include beta-casomorphin 7 (BCM-7,) which has opioid activity and therefore putative effects on the gastrointestinal, cardiovascular, and immune systems (Raies et al., 2014; Jianquin et al., 2016).

Several studies have suggested that the presence of this peptide derived from the A1 variant could be responsible for some of the alimentary dysfunctions in certain consumers of bovine milk (He et al., 2017; Ramakrishnan et al., 2020). On the other hand, milk containing only the A2 variant would not be associated with dysfunctions or discomfort and thus would be healthier for consumption, especially for people with intolerance or allergies. These associations have been postulated as the A1A2 hypothesis (Truswell, 2005); however, there is still debate about the true implications of these variants (Brooke-Taylor et al., 2017; Cieślińska et al., 2022; Kuellenberg et al., 2022).

The objective of this work was to evaluate the distribution of the β -casein A1 and A2 variants in the main dairy cattle breeds used for milk production in Chile.

Material and methods

Individual blood samples were obtained by caudal puncture using a vacuum blood collection tube with EDTA in accordance with protocols approved by the Institutional Animal Care and Use Committee of INIA (Institute for Agriculture Research in Chile). A total of 134 animals were used, representing the breeds Holstein-Friesian (HF), Jersey (JE), Montbéliarde (MB), and Overo Colorado (OC), as well as HF x OC crossbreds (HYB), distributed in several herds from the Los Ríos and Los Lagos districts, Chile. Genomic DNA was purified from 250 μ L of blood using the E.Z.N.A. Blood DNA Mini Kit (Omega Bio-Tek, USA) and quantified by spectrophotometry in an Infinity M200 Pro plate-reader (TECAN, Switzerland).

Genotyping of the A1/A2 variant was performed by allelic-specific PCR (AS-PCR), modifying the protocol described by Pabitra et al. (2022). Briefly, we amplify two or three products corresponding to

the A1, A2 or A1A2 genotype using four primers, two external and common and two internal and specific: Fc: 5-CCTTCTTTCCAGGATGAACTCCAG, FAS: 5-CTTCCCTGGACCCATCCA, Rc: 5-g AGCCTGACTCTCACTGATGTTG and RAS: 5-TAACAGCCTCCCACAAAACATC, based on NCBI sequence M55158.1. The A1 variant amplifies two fragments of 277 and 209 bp, whereas A2 amplifies two fragments of 277 and 108 bp. The heterozygote (A1A2) amplifies the combination of the three fragments: 277, 209 and 108 bp (Figure 1). Reactions were performed in a mixture with 100 ng DNA, 1x Green GoTaq® Flexi reaction buffer, 2U GoTaq G2 Flexi DNA Polymerase (Promega, USA), 2 mM Mg²⁺ and 0.5 mM dNTP mix in a final volume of 20 µL. The thermal cycling conditions were as follows: initial denaturation at 95°C for 5 min followed by 30 cycles at 95°C for 30 s, annealing at 55°C for 45 s, 72°C for 45 s, and a final extension at 72°C for 5 min. The amplicons were visualized on 2.5% agarose gels with GelRed (Biotium, UK) and UV-light (UVP PhotoDoc-It, USA). Genotypes of samples used as a positive control for each genotype were corroborated by commercial sequencing (AustralOmics, Valdivia, Chile).

Allelic and genotypic frequencies of A1 and A2 variants were estimated by direct count, and the Hardy-Weinberg equilibrium (HWE) was tested by the chi-squared test for a significance level of $P < 0.05$. Heterozygosity was evaluated employing the FIS estimator, using GenePop 4.7.5 software (Rousset, 2008).

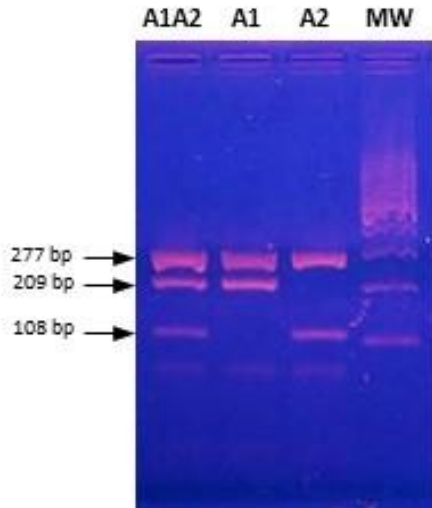


Figure 1. Amplification of the A1, A2 and A1A2 variants of the beta-casein gene by AS-PCR from cattle blood samples. MW, standard molecular weight (GeneRuler 100 bp DNA ladder, Fermentas); 1, 2 and 3: A1A1, A1A2 and A2A2 genotypes; 4, negative control.

RESULTS AND DISCUSSION

This is the first report concerning the β -casein A1/A2 variants in Chile. Genotyping of the A1/A2 CSN2 marker showed variability in their distribution (Table 1). The frequency of A2 ranged between 0.48 and 0.75, prevailing in HF (0.75) and JE (0.73), while it was less frequent but close to 50% in the other breeds (MB, OC and HYB). Only OC displayed a higher frequency for the A1 variant (0.52). The χ^2 value indicated that the animal populations were in Hardy-Weinberg equilibrium, except for JE and MB ($p < 0.05$). These two breeds were introduced to southern Chile more recently than HF. Regarding heterozygosity, all FIS values were positive and non-significant except for JE, which had a positive and significant value (0.5045; Table 1), showing a deficit of heterozygotes that suggests some degree of inbreeding. This could be due to the use of semen with limited genetic diversity available in Chile. In accordance with several international breeding trends (Sanchez et al., 2020; Kamiński et al., 2023), Chile has set the goal of increasing milk solids, and therefore this is one of the main criteria considered for breeding (Levicoy et al., 2023). However, the distribution of germplasm is still limited.

Table 1.

Allele frequencies (%), Hardy-Weinberg equilibrium and heterozygosity for the A1/A2 variants in dairy cattle

Breed	n	Allele frequencies		HWE	Heterozygosity	
		A1	A2	χ^2	FIS	P value
Holstein-Friesian	24	0.25	0.75	0.30	-0.0918	0.8429
Jersey	24	0.27	0.73	5.36*	0.5045	0.0282**
Montbéliarde	24	0.42	0.58	7.07*	-0.0653	0.7614
Overo Colorado	24	0.52	0.48	0.17	-0.5329	0.9995
Crossbred	38	0.45	0.55	1.11	-0.1597	0.9023

HWE: Hardy-Weinberg equilibrium; χ^2 , chi-square test. *HWE deviation; ** $p < 0.05$.

The high level of representation of the A2 variant found in the tested population was consistent with several reports worldwide. This distribution was present in all populations tested, including the Holstein-Friesian, Jersey, and Montbéliarde biotypes. It has been concluded that A2 was the original variant, while A1 arose by natural mutation (g.6570C>A). Taurine breeds from northern Europe display high frequencies, but reports indicate that the Holstein-Friesian breed and its crosses show this trend as well (Sebastiani et al., 2020; Ivanković et al., 2021). Regarding the Jersey biotype, the frequency determined (0.73) was similar to other reports (Jensen et al., 2012; Şahin & Boztepe, 2022). Looking at the entire population, our results show that the A2 variant was represented by 32% of the animals, whereas heterozygotes (A1/A2) reached 51%, highlighting the possibility of using these animals in

breeding programmes to increase the frequency of the allele in herds. Thus, our results, although preliminary, suggest that representative dairy herds from southern Chile display the A2 variant. This presents the opportunity to produce milk and dairy products with this healthy allele for consumers with special nutritional needs.

In addition, the protocol described was confirmed to enable fast, economic and reliable genotyping and certification of milk quality in terms of β -casein A1/A2 variants, which is necessary for food authenticity.

CONCLUSION

Our results show that the A2 beta-casein variant was present in the most important cattle breeds used for milk production in Chile, with a high distribution, especially in Holstein-Friesian and Jersey. Such animals could be used as breeding stock to increase the A2 distribution among herds and thus increase the potential production of even healthier milk.

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