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Influence of A1/A2 allelic variants of the CSN2 gene on milk composition and production in Holstein cows from Nariño, Colombia

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ABSTRACT

This study aimed to determine the allelic frequency and the effects of the A1 and A2 variants of the *CSN2* gene on milk production and quality in Holstein cows from five municipalities in Nariño, Colombia. Productive and compositional milk data were collected from 200 cows across 10 herds located in Pupiales, Pasto, Gualmatán, Guachucal, and Túquerres. The A1 and A2 variants of the *CSN2* gene were identified through allele-specific PCR using DNA extracted from blood samples. Associations between genotypes and milk production and composition traits were assessed using analysis of variance (ANOVA). The results showed allele frequencies of 0.46 for A1 and 0.54 for A2, and genotype frequencies of 0.23 (A1A1), 0.46 (A1A2), and 0.31 (A2A2). Observed heterozygosity (Ho) was 0.46, expected heterozygosity (He) was 0.47, and the polymorphic information content (PIC) was 0.37. Cows with the A1A1 genotype produced significantly more milk and total solids than A2A2 cows ($p < 0.05$). The A1 allele was associated with significantly higher yields of milk, fat, and protein ($p < 0.05$). A discriminant analysis revealed differentiation by municipality, suggesting that environmental factors influence variability in milk production and composition. It is concluded that the A1 allele is present at a moderately high frequency and is associated with enhanced productive traits in Holstein cows in the Department of Nariño. Therefore, the use of A2A2 genotype bulls with high genetic merit for productive traits is recommended, with the additional aim of promoting potential health benefits for milk consumers.

Keywords: β-casomorphin, β-casein, milk composition, molecular marker.

Influencia de las variantes alélicas A1/A2 del gen CSN2 sobre la composición y producción lechera en vacas Holstein en Nariño, Colombia

RESUMEN

El objetivo de este estudio fue determinar la frecuencia alélica y el efecto de las variantes A1/A2 del gen CSN2 sobre producción y calidad de la leche en vacas Holstein provenientes de

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cinco municipios de Nariño, Colombia. Se analizaron datos productivos y composicionales de la leche de 200 vacas de 10 hatos ubicados en Pupiales, Pasto, Gualmatán, Guachucal y Túquerres. Las variantes A1 y A2 del gen *CSN2* se identificaron mediante PCR alelo-específico a partir del ADN extraído de sangre. La asociación entre los genotipos y las características productivas y composicionales de la leche se evaluó mediante un análisis de varianza (ANOVA). Los resultados mostraron frecuencias alélicas de 0,46 para A1 y 0,54 para A2, y genotípicas de 0,23 (A1A1), 0.46 (A1A2) y 0,31 (A2A2), con valores de heterocigosidad observada (Ho) de 0,46, heterocigosidad esperada (He) de 0,47 y un contenido de información polimorfico (CIP) de 0,37. Las vacas con genotipo A1A1 produjeron significativamente más leche y sólidos totales que las vacas A2A2 ($p < 0,05$). El alelo A1 se asoció con mayores rendimientos ($p < 0,05$) de leche, grasa y proteína. Según un análisis discriminante, se observó diferenciación por municipio, sugiriendo que factores ambientales influyen en la variabilidad de la producción y composición de la leche. Se concluye que el alelo A1 está presente en una frecuencia moderadamente alta y además está asociado con aumento en las características productivas evaluadas en vacas Holstein del Departamento de Nariño, por lo que se sugiere usar toros con genotipo A2A2 de alto valor genético para características productivas, buscando, además, generar impactos positivos en la salud del consumidor de leche.

Palabras clave: β-casomorfina, β-caseína, composición láctea, marcador molecular.

INTRODUCTION

Colombia is among the leading milk producers in Latin America. Specifically, the department of Nariño contributes 6.03% of national milk production, with the Holstein breed being predominant in the region due to its high milk yield and favorable nutritional quality (Ministerio de Comercio, Industria y Turismo [MinCIT], 2021). Milk from cows contains approximately 45% β-casein among its protein content and provides essential nutrients such as carbohydrates, lipids, and micronutrients, including vitamins, calcium, and phosphorus (Padilla & Zambrano, 2021). The β-casein protein, encoded by the *CSN2* gene, exists in several allelic forms, of which A1 and A2 are the most common (Kamiński *et al.*, 2007; Padilla & Zambrano, 2021). These variants have different effects on milk composition, digestibility, and potential health impacts in humans (Laugesen and Elliott, 2003; Riaño and Narváez, 2015; Semwal *et al.*, 2022).

The β-casein variants differ in their amino acid sequences, particularly at position 67. In the A1 variant, the presence of histidine at this position allows for the release of β-casomorphin-7 (BCM-7) during digestion, whereas in the A2 variant, the presence of proline prevents this release (Küllenberg de Gaudry *et al.*, 2019; Padilla & Zambrano, 2021). BCM-7 is a bioactive peptide with opioid activity, released during the gastrointestinal hydrolysis of A1 β-casein in humans. Several studies have linked the consumption of A1 β-casein with health issues such as type 1 diabetes (Laugesen & Elliott, 2003), coronary heart disease (Küllenberg de Gaudry *et al.*, 2019),

ischemic heart disease (Laugesen & Elliott, 2003), atherosclerosis (Riaño & Narváez, 2015), and neurological disorders, including autism (Sokolov *et al.*, 2014) and schizophrenia (Küllenberg de Gaudry *et al.*, 2019). These associations are largely attributed to the ability of BCM-7 to cross the intestinal microvilli barrier, enter systemic circulation, and modulate the immune system (Kamiński *et al.*, 2007; Küllenberg de Gaudry *et al.*, 2019).

As a result, there has been increased consumer demand for A2 β-casein milk, especially in countries such as New Zealand, Australia, and the United Kingdom, where milk from cows producing only A2 β-casein is marketed as "A1-free milk," offering a more digestible and potentially safer alternative (Brooke-Taylor *et al.*, 2017). Nevertheless, in many countries, the A1 and A2 variants are not yet considered in genetic selection programs for cows and bulls, despite evidence linking these variants to productive traits such as increased yields of milk, fat, and protein (Winkelman & Wickham, 1996; Miluchová *et al.*, 2023; Olenski *et al.*, 2010).

Despite the relevance of β-casein variants, studies on the distribution and effects of A1/A2 in Colombia remain limited, particularly in the department of Nariño. Research on the *CSN2* gene in Colombian dairy herds is essential, as β-casein variants influence not only milk production and composition but also consumer health—especially among infants fed with formula milk. This study aimed to determine the frequency and effect of the A1/A2 allelic variants of the *CSN2* gene on milk production and quality in Holstein cows from five municipalities in the department of Nariño, Colombia.

MATERIALS AND METHODS

Population and sampling

A total of 200 adult Holstein cows were evaluated across 10 herds located in five municipalities within the dairy-producing zone of the Department of Nariño, Colombia. This region corresponds to a lower montane very humid forest zone (bmh-MB), situated at an altitude of 2,500–2,700 meters above sea level and characterized by an average temperature of 14 °C. A simple random sampling method was employed. In the municipality of Pasto, five herds were sampled; Guachucal had two herds; and Túquerres, Pupiales, and Gualmatán each had one herd. The average number of cows per herd was 20, ranging from 11 to 39.

Blood Sampling

Blood samples were collected using a chute to restrain the cows. Peripheral blood was drawn from the coccygeal vein, with 8 mL collected into Vacuette® tubes containing EDTA. Samples were kept refrigerated at 4 °C and transported to the Laboratory of the Research Group in Biochemistry and Genetic Studies (BIOGEN) at the University of Nariño.

DNA Extraction

For DNA extraction, 3 mL of blood were transferred into 15 mL Falcon® tubes and mixed with 6 mL of lysis buffer I, pH 7.6 (10 mM Tris-HCl, 320 mM sucrose, 5 mM $MgCl_2{\cdot}6H_2O$, and 1% Triton X-100). After vortexing and centrifugation at 4,000 rpm for 12 minutes, the supernatant was discarded, and the process was repeated using 8 mL of the buffer. The resulting pellet was resuspended in 5 mL of lysis buffer II, pH 8.2 (10 mM Tris-HCl, 400 mM

NaCl, and 2 mM Na_2EDTA), followed by the addition of 10 µL of proteinase K (CANVAX, Córdoba, Spain) at 2 mg/mL and 200 µL of 10% SDS (w/v). The mixture was incubated at 65 °C for 12 hours. Subsequently, 1.5 mL of saturated saline solution (6 M) was added, and the sample was centrifuged at 6,200 rpm for 10 minutes. DNA was precipitated using absolute ethanol at –20 °C, centrifuged at 4,000 rpm for 10 minutes, and the pellet was resuspended in 1 mL of TE buffer 1X (pH 8.0), then stored at –4 °C. DNA concentration and purity were assessed using a NanoDrop™ 2000 spectrophotometer, and integrity was verified by electrophoresis in 1% agarose gel (w/v).

Identification of CSN2 allelic variants via PCR-AS

The A1 and A2 alleles of the *CSN2* gene were identified using allele-specific PCR (PCR-AS), following the method of Ristanić *et al.* (2022) with slight modifications. Two separate PCR-AS reactions were performed using the following primers: IGBhF 5'-CTTCCCTGGGCCCATCCA-3' for the A1 allele, 5'-CTTCCCTGGGCCCATCCC-3' for the A2 allele, and the reverse primer IGBR 5'-AGACTGGAGCAGAGGCAAG-3'. Each PCR-AS reaction was carried out in a final volume of 20 µL, containing 75 ng of genomic DNA, 2 µL of 10X PCR buffer, 1 U of Green-Taq DNA polymerase (BrigthMAX™), 0.8 µL of 3 mM $MgCl_2$, 0.64 µL of 0.8 mM dNTPs, 0.75 µL of each primer at 5 pM, and ultrapure water. The thermal cycling conditions were as follows: initial denaturation at 94 °C for 5 min; 5 cycles of 94 °C for 30 s, 64 °C for 30 s, and 72 °C for 30 s; followed by 30 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 30 s; with a final extension at 72 °C for 5 min. PCR-AS products were resolved on 1.5% agarose gels (w/v, BrigthMAX™) at 110 V for 60 minutes and visualized using an ENDURO™ GDS UV transilluminator, with a molecular weight marker ranging from 25 to 700 bp (BrigthMAX™).

Determination of allelic and genotypic frequencies

Allelic frequencies were calculated by summing the number of homozygotes and half the number of heterozygotes. Genotypic frequencies were determined by dividing the number of individuals with a given genotype by the total number of individuals evaluated. Genetic diversity was estimated using observed heterozygosity (Ho), expected heterozygosity (He), and their respective standard errors (SE). The polymorphic information content (PIC) was also calculated using GenAlEx version 6.5 (Peakall and Smouse, 2012).

Determination of productive traits

Milk samples were collected from the selected cows following teat disinfection with 70% alcohol and discarding of the first milk streams. A total of 50 mL of milk was collected from all four quarters into labeled plastic containers with barcodes. Samples were stored in cooler boxes with ice packs at 4 °C and transported to the AGROSAVIA milk quality laboratory. The percentages of fat (FAT), protein (PRO), and total solids (TS) were analyzed using infrared spectroscopy (AOAC Official Method 972.16-2005, 21st Ed. 2019). Daily milk yield (MY) per cow and 305-day adjusted milk yield (MY_305) were recorded, along with herd, municipality, age, and parity data provided by the Nariño Society of Farmers and Ranchers (SAGAN). Based on MY_305, PRO, FAT, and TS values, the 305-day adjusted production

of fat (FAT_305), protein (PRO_305), and total solids (TS_305) was calculated.

Association analysis

Normality and homogeneity of variance were tested using the Anderson–Darling and Levene tests, respectively. ANOVA was performed to evaluate associations between productive traits (MY, FAT, PRO, TS, MY_305, FAT_305, PRO_305, and TS_305) and fixed effects: parity (NPAR), herd (HAT), municipality (MUN), age, and genotype (GENT). Age was grouped into four categories: E3 (2–3 years), E4 (4 years), E5 (5 years), and E6 (6–7 years). Herds were grouped into four categories: NPAR1, NPAR2, NPAR3, and NPAR4 or more. The fixed effects also included the A1 and A2 alleles and the genotypes A1A1, A1A2, and A2A2. The linear model used was:

Where *Y* represents the dependent variables (FAT, PRO, TS, MY, MY_305, FAT_305, PRO_305, and TS_305), and the independent variables are: NPAR = parity, with four levels (i = 1…4); AGE, with four levels (j = 1…4); HAT = herd, with 10 levels (k = 1…10); MUN = municipality, with five levels (l = 1…5); GENT = genotype, with three levels (m = 1…3); and ALLELE, with two levels (n = 1, 2). For fixed effects that showed significant differences in ANOVA, Tukey's multiple comparison test was applied ($\alpha \leq 0.05$). Statistical analyses were performed using R software v4.3.0 (R Core Team, 2024).

Genetic structure analysis

Hardy–Weinberg equilibrium was assessed based on allelic and genotypic frequencies using GenAlEx 6.5 (Peakall and Smouse, 2012). A chi-square (χ^2) goodness-of-fit test was also performed to confirm Hardy–Weinberg equilibrium. The entire Nariño region was considered the total population, while municipalities were treated as subpopulations. The inbreeding coefficient (Fis) and the effective number of alleles (Ne) were calculated using standard equations. Analysis of molecular variance (AMOVA) was conducted to evaluate genetic variation within and between groups, with significance set at $\alpha \leq 0.05$. The polymorphic information content (PIC) was also estimated.

Discriminant analysis

Principal component analysis (PCA) was applied to the genetic data to address collinearity and high dimensionality in allelic frequencies. The most informative components were selected for discriminant analysis. The number of groups was determined using the Bayesian Information Criterion (BIC), which accounts for model complexity by applying a penalty. The contributions of individual alleles to the discriminant functions were assessed, along with the influence of each municipality. This approach enabled the evaluation of the independent effects of genetic and geographic factors on milk composition and yield traits.

Ethical considerations

This study was approved by the Research Ethics Committee of the University of Nariño under Approval Act No. 045, dated October 30, 2020.

RESULTS

The results of the allele-specific PCR (PCR-AS) for each genotype (figure 1) revealed allele frequencies of 0.46 for A1 and 0.54 for A2, with genotype frequencies of 0.23 for A1A1, 0.46 for A1A2, and 0.30 for A2A2. These findings offer insights into

the genetic distribution of the A1 and A2 variants in the Holstein cattle population sampled across the five municipalities of the Nariño department (table 1).

The Hardy–Weinberg equilibrium test, based on the chi-square statistic, revealed no significant deviation (χ^2 = 0.84; p = 0.36), indicating that the population is in equilibrium at the *CSN2 locus*. The effective number of alleles (Ne) was slightly lower, at 1.91 (SE = 0.068), compared to the total number of alleles (Na = 2) (table 1). Expected heterozygosity was 0.47 (SE = 0.021), while observed heterozygosity was slightly lower at 0.46 (SE = 0.066) (table 1), suggesting a minor deviation from expected genetic diversity. The inbreeding coefficient (Fis) was estimated at 0.03, indicating a low level of inbreeding within the population. Genetic differentiation among populations (Fst) was 0.05, and the polymorphic information content (PIC)

TABLE 1. Allelic and genotypic frequencies of A1/A2 variants and genetic structure in Holstein cows from the Nariño department

N	Alleles		Genotypes			Genetic structure						
	A1	A2	A1A1	A1A2	A2A2	Na	Ne	He	Ho	Fis	Fst	PIC
200	0.46	0.54	0.23	0.46	0.31	2.0	1.91	0.47	0.46	0.03	0.05	0.37

N: Sample size of Holstein cattle, Na: Number of different alleles, Ne: Effective number of alleles, Ho: Observed heterozygosity, He: Expected heterozygosity, Fis: Inbreeding coefficient within individuals, Fst: Fixation index, PIC: Polymorphic information content.

Source: own elaboration.

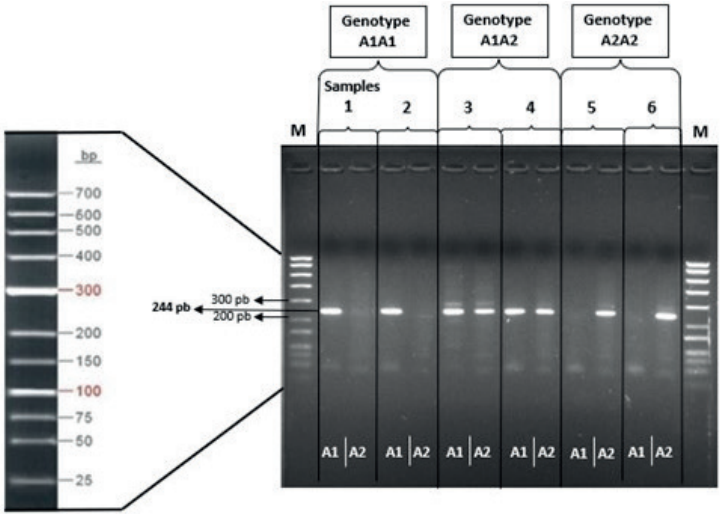

FIGURE 1. PCR amplification product obtained using the forward primer IGBhF (A1 allele), forward primer IGBpF (A2 allele), and reverse primer IGBR, from 200 Holstein cows.

Source: own elaboration.

was 0.37, both consistent with moderate genetic diversity at this locus in the studied population. Overall, these findings suggest that genetic diversity at the *CSN2* locus in Nariño is moderate.

The physicochemical analysis of milk revealed that the Holstein cows sampled across the five municipalities of the department of Nariño exhibited average values for productive and compositional traits that align with regional conditions, particularly given that these are not closed herds with animals of high genetic merit. On average, cows produced 13 ± 5.2 liters of milk per day, with a fat content of 3.4 ± 0.57%, protein content of 3.2 ± 0.27%, and total solids content of 11.3 ± 1.5%. Milk production adjusted to 305 days of lactation averaged 3,954 ± 1,594 liters, with protein yield at 134 ± 51.6 kg/lactation, fat yield at 133 ± 75 kg/lactation, and total solids yield at 454 ± 205 kg/lactation.

Additionally, ANOVA results showed a significant effect ($p < 0.05$) of both allele and genotype on milk composition and production traits. Cows with the A1A1 genotype produced 13.3% more milk per day, had a 13.5% higher 305-day milk yield, and 4.4% higher total solids content compared to cows with the A2A2 genotype ($p < 0.05$) (table 2). In the allelic comparison, cows carrying the A1 allele exhibited a 6.3% greater daily milk yield and a 6.6% greater 305-day milk yield than those carrying the A2 allele. Furthermore, A1 cows produced 8.5% more protein and 8.6% more total solids over 305 days than A2 cows, which showed lower yields across all production traits (table 2). These findings suggest that the A1 allele is associated with higher milk yield and improved compositional quality, particularly in fat and protein content.

The PCA plot of the overall genetic profile did not reveal clear separation

TABLE 2. Mean comparison test for milk composition and production traits in Holstein cattle.

		Composition			Production				
Genotypes	**N**	**FAT (%)**	**PRO (%)**	**TS (%)**	**MY (L/day)**	**MY_305 (L/lac)**	**FAT_305 (kg/lac)**	**PRO_305 (kg/lac)**	**TS_305 (kg/lac)**
A1A1	46	3.38	3.24	11.8^{a}	14.5^{a}	4423	148.2	153.1^{a}	523.6^{a}
A1A2	93	3.44	3.17	11.1^{b}	12.3^{b}	3762	127.2	127.3^{b}	424.6^{b}
A2A2	61	3.25	3.14	11.3^{ab}	12.8^{ab}	3895	129.4	130.4^{b}	445.2^{b}
Alleles	**N**	**FAT (%)**	**PRO (%)**	**TS (%)**	**MY (L/día)**	**MY_305 (L/lac)**	**FAT_305 (kg/lac)**	**PRO_305 (kg/lac)**	**TS_305 (kg/lac)**
A1	185	3.40	3.20	11.4	13.4^{a}	4090^{a}	138.7	140.1^{a}	473.8^{a}
A2	215	3.31	3.15	11.2	12.6^{b}	3837^{b}	128.5	129.1^{b}	436.3^{b}

N: Sample size. FAT (%): percentage of fat, PRO (%): percentage of protein, TS (%): percentage of total solids, MY: milk production per day, MY_305: Milk production per lactation of 305 days, FAT_305: Fat production per lactation of 305 days, PRO_305: Protein production of lactation of 305 days, TS_305: Production of total solids per lactation of 305 days. Different letters indicate significant differences between the means (p<0.05) according to the test of Tukey.

Source: own elaboration.

between groups based on genotype (figure 2A). Although considerable overlap was observed among genotypes, some distinction was noted for the A1A1 genotype, which exhibited a broader distribution along Dimension 1. Discriminant analysis based on genotype (figure 3) showed an improved ability to distinguish A1A1 (Area Under the Curve, AUC = 0.65) and A1A2 (AUC = 0.60) genotypes.

The A2A2 genotype was indistinguishable (AUC = 0.51) (figure 3). These results suggest that, while genetic constitution influences certain traits related to milk production and quality, its impact is particularly pronounced in cows with the A1A1 genotype.

On the other hand, substantial overlap was also observed among municipalities, although some differentiation was noted for Guachucal and Pupiales, which exhibited

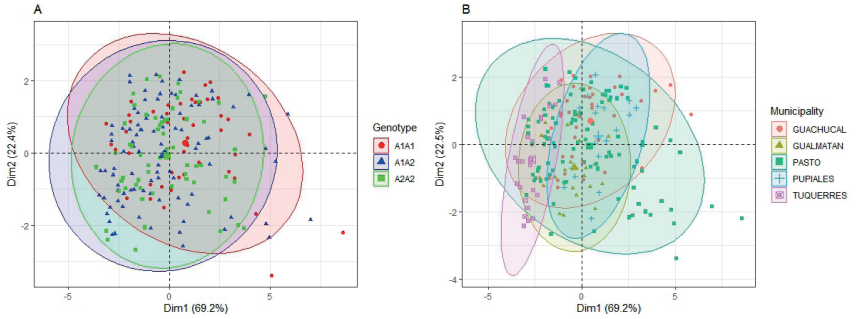

FIGURE 2. PCA score plots illustrate variation among genotypes and municipalities.

(A) Plot of the first two principal components (Dim1 and Dim2) showing the distribution of genotypes: A1A1 (red circles), A1A2 (blue triangles), and A2A2 (green squares). (B) Plot of municipalities: Guachucal (red circles), Gualmatán (yellow triangles), Pasto (green squares), Pupiales (purple diamonds), and Túquerres (pink squares). Ellipses represent 95% confidence intervals for each group. The first two dimensions explain 91.7% of the total variance, respectively.

Source: own elaboration.

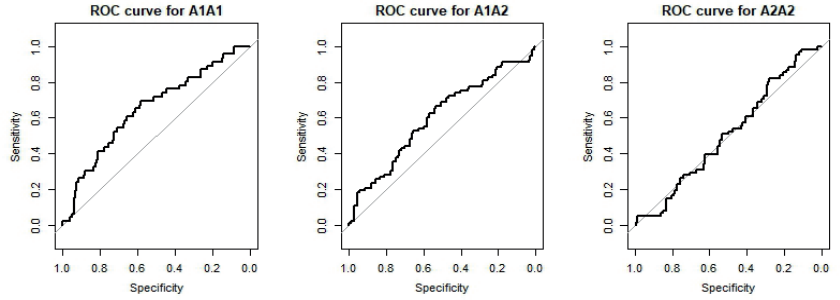

FIGURE 3. Receiver Operating Characteristic (ROC) curves for three different classes: A1A1, A1A2, and A2A2.

Each plot compares sensitivity (true positive rate) on the Y-axis against specificity (false positive rate) on the X-axis. The area under the curve (AUC) values were 0.65 for A1A1, 0.60 for A1A2, and 0.51 for A2A2.

Source: own elaboration.

broader distributions along Dimension 1 (figure 2B). Discriminant analysis based on municipality revealed a greater ability to distinguish Túquerres (AUC = 0.96) and Pupiales (AUC = 0.71), while it was less effective for Guachucal (AUC = 0.60), and indistinguishable for Gualmatán (AUC = 0.53) and Pasto (AUC = 0.44) (figure 4).

The most influential variables in the discriminant analysis were those with the highest contributions to Component 1, including protein yield (–0.65) and total solids (–0.49) at 305 days (table 3). For Component 2, the primary contributors were protein content (–0.97) and total solids (–0.26) (table 3). These results suggest that, in addition to genetic constitution, environmental or management-related factors may significantly contribute to the observed variability in milk composition and production across the different municipalities.

TABLE 3. Variables and their contributions to components 1 and 2 of the discriminant analysis.

Component 1		Component 2	
Variable	**Value**	**Variable**	**Value**
PRO_305	-0,65	PRO	-0,97
TS_305	-0,49	TS	-0,26
MY	-0,41		
MY_305	-0,41		
TS	-0,03		

Source: own elaboration.

DISCUSSION

The results indicate a balanced distribution of A1 and A2 alleles, with a slightly higher prevalence of the A2 allele. This balanced proportion suggests that there has been no strong historical directional selection favoring either allele of the *CSN2* gene in the study region (Padilla & Zambrano, 2021; Scott *et al.*, 2023).

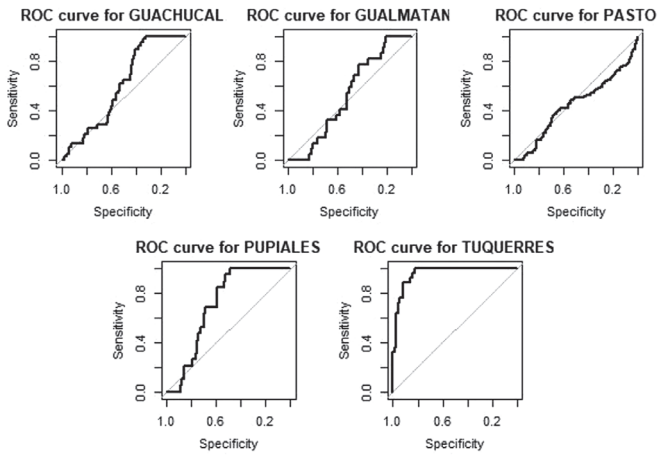

FIGURE 4. Receiver Operating Characteristic (ROC) curves for five municipalities: Guachucal, Gualmatán, Pasto, Pupiales, and Túquerres.

Each plot compares sensitivity (true positive rate) on the Y-axis with specificity (false positive rate) on the X-axis. The area under the curve (AUC) values were 0.60 for Guachucal, 0.53 for Gualmatán, 0.44 for Pasto, 0.71 for Pupiales, and 0.96 for Túquerres.

Source: own elaboration.

In several countries, a moderate predominance of the A2 allele has been reported in Holstein cattle. For example, studies in Latin American Holstein populations have shown allele frequencies similar to those observed in the present study. In the Peruvian Andes, Chaves (2023) reported an A2 allele frequency of approximately 0.57, compared to 0.43 for A1, closely aligning with the proportions found in Nariño. Similarly, research conducted in Mexico and other Latin American countries has reported a higher frequency of the A2 allele (around 0.60) compared to A1 (Manzano, 2017), further supporting our findings.

Globally, many dairy herds have increased the frequency of the A2 allele due to its potential advantages. A widespread preference for A2 β-casein has been documented in countries such as New Zealand (Winkelman & Wickham, 1996), various European nations (Barłowska *et al.*, 2022; Ladyka *et al.*, 2023), and several Asian countries, including China (Dai *et al.*, 2016) and India (Jawane *et al.*, 2018). In these regions, the frequency of the A2 allele ranges from 0.51 to 0.97, reflecting a general preference for A2 β-casein. For instance, studies in New Zealand reported an A2 frequency of 0.51 (Winkelman & Wickham, 1996), while in India, some populations showed frequencies as high as 0.97 (Jawane *et al.*, 2018), largely attributable to recent selection programs aimed at producing A1-free milk due to its perceived health benefits (Semwal *et al.*, 2022).

These values contrast with specific cases where the A1 allele is more prevalent. For example, in Pakistan, an A1 allele frequency of 0.67 has been reported—significantly higher than that of the A2 allele (Ayaz *et al.*, 2023). Such exceptions are often associated with the specific objectives of the dairy industry. In cheese production, for instance, the A1 β-casein variant may offer certain technological advantages, leading to a preference for A1-producing animals within those systems (Vigolo *et al.*, 2023). This underscores the complex relationship between genetic selection goals and the diverse demands of dairy production.

In Colombia, the findings of this study align with the international trend of coexistence between both β-casein variants. However, national studies on the distribution of A1/A2 alleles remain limited, particularly in Andean dairy regions such as Nariño. The concurrent presence of both alleles at similar frequencies suggests that local herds have been managed through mixed reproductive practices—such as the use of bulls or semen from diverse sources—without intentional selection for either β-casein variant.

From a public health perspective, the continued presence of a considerable frequency of the A1 allele implies that cows are still producing milk containing A1 β-casein—an important consideration given its potential health risks, particularly for newborns. Several studies have associated A1 β-casein consumption with an increased risk of chronic conditions, including type 1 diabetes (r = 0.92) (Laugesen & Elliott, 2003), coronary heart disease (Küllenberg de Gaudry *et al.*, 2019), ischemic heart disease (r = 0.86) (Laugesen & Elliott, 2003), atherosclerosis (Riaño & Narváez, 2015), sudden infant death syndrome (Wasilewska *et al.*, 2011), and neurological disorders such as autism (r = 0.85) (Sokolov *et al.*, 2014) and schizophrenia (Küllenberg de Gaudry *et al.*, 2019). Nonetheless, further research is needed to provide conclusive evidence supporting the hypothesis that consumption of A1-type milk increases the risk of these diseases

As interest in A2 milk continues to grow—driven by its potential health benefits, such as reduced gastrointestinal discomfort and a lower risk of chronic diseases—genetic improvement programs have increasingly prioritized the selection of the A2 allele (Dantas *et al.*, 2023; Żbik *et al.*, 2024). For dairy producers in Nariño, understanding the genetic composition of their herds and the advantages of A2-type milk may create new market opportunities, particularly in response to rising global demand and its positive implications for human health.

The genetic analysis of the Holstein cow population in Nariño enabled the determination of the effective number of alleles at the *CSN2* locus, revealing an allelic structure consistent with findings from other studies (Duifhuis *et al.*, 2014; Miluchová *et al.*, 2014; Ardicli *et al.*, 2024). However, the effective number of alleles in this population was slightly lower than that reported in countries such as Pakistan, suggesting a potential reduction in genetic diversity, possibly related to the indirect selection of phenotypic traits associated with production (Ayaz *et al.*, 2023). The observed and expected heterozygosity values indicate substantial genetic diversity within the population. The slight difference between these values, along with the polymorphism information content, suggests a minor deviation from Hardy–Weinberg equilibrium. This deviation may be attributed to evolutionary forces such as incipient inbreeding, natural selection acting against heterozygotes, or the influence of genetic drift in populations with a reduced effective size. Notably, the low inbreeding coefficient supports the hypothesis that inbreeding, while present, has not yet reached concerning levels. This finding is consistent with populations managed under semi-intensive systems or using assisted reproductive technologies, as reported in countries like Slovakia (Miluchová *et al.*, 2014) and Turkey (Ardicli *et al.*, 2024). Moreover, the effects of genetic drift may be linked to the limited effective population size, which can lead to random fluctuations in allele frequencies.

Analogous situations have been reported in Holstein herds managed under semi-intensive systems in Latin America and other regions, where the periodic introduction of external genetics and sire rotation help maintain genetic variability and prevent high levels of inbreeding (Manzano, 2017). For example, a study on Mexican cattle observed a similar genetic equilibrium at casein loci, attributing this stability to the use of imported semen from diverse sources (Duifhuis-Rivera *et al.*, 2014).

The impact of *CSN2* gene allelic variants on milk yield and quality remains a subject of ongoing debate within the scientific community. Some studies report that the A2 variant is associated with higher milk and protein yields, whereas the A1 variant is linked to increased fat percentages (Winkelman & Wickham, 1996; Miluchová *et al.*, 2023). Other research suggests that heterozygous A1A2 cows may outperform homozygous individuals in terms of milk and protein production, while some authors have found no significant association between these genetic polymorphisms and milk production traits (Manga & Dvorak, 2010). Although the present study identified associations between *CSN2* alleles and certain productive traits, no human intervention has been applied to select cows based on A1A1, A1A2, or A2A2 genotypes. Nevertheless, public health studies suggest a potential health risk associated with milk from both A1A1 and A2A2 cows (Küllenberg de Gaudry

et al., 2019; Borş *et al.*, 2024), underscoring the need for further research and cautious consideration in breeding strategies.

Efforts to promote the production of A2 milk—free of A1 β-casein—may lead to the implementation of rigorous breeding programs, similar to those established in New Zealand, which include strict genetic selection, separate grazing, and controlled feeding of A2A2 cattle. This study underscores the importance of using semen from bulls with high genetic merit for productive traits and confirmed A2A2 genotypes, with the goal of increasing the frequency of the A2 allele and delivering potential health benefits to consumers. Companies such as Genética Selecta, GENEX, SEMEX, and ABS Global offer semen from elite A2A2 bulls, supporting its adoption in Colombian dairy regions, including the Department of Nariño. As the Colombian dairy industry continues to advance alongside developments in animal genetics, the incorporation of A2A2 genotype animals into dairy herds is a strategic step toward promoting consumer health—particularly for newborns who consume infant formula.

With respect to the discriminant analysis, the results revealed a complex interaction between genetic and environmental factors influencing milk production and composition in Holstein cows from the municipalities of Nariño. Although some genetic distinctions were evident—particularly for the A1A1 genotype—the observed overlap among genotypes and municipalities suggests that genetic constitution alone does not fully account for the variability in productive traits. These findings align with previous studies indicating that milk yield and composition are polygenic traits influenced not only by genetic factors (Padilla & Zambrano, 2021) but also by environmental variables such as diet (AlSuwaiegh *et al.*, 2022), climate, and management practices (Gareli *et al.*, 2023).

The limited capacity of the analysis to clearly distinguish the A2A2 genotype and certain municipalities—despite local differences such as those observed in Túquerres—suggests that regional factors, including feeding and herd management practices, play a crucial role in trait expression and variation in milk yield (Padilla & Zambrano, 2021; AlSuwaiegh *et al.*, 2022). Moreover, the significant contribution of protein yield and total solids as discriminant variables highlights their importance in production environments, as these traits are highly responsive to nutritional inputs and management intensity (Gareli *et al.*, 2023). Their relevance also reinforces their utility in the genetic selection of cows and bulls. Overall, the results support the notion that optimizing milk production and quality requires a comprehensive approach that integrates both *CSN2* genotype and environmental influences.

It is important to note that in the present study, 200 individuals were randomly sampled from the municipalities with the highest milk production in the southern region of the Department of Nariño. However, environmental factors—such as management techniques, milking practices, and nutrition—were not evaluated. For future research, it is recommended to expand the sample size by including cattle from additional milk-producing municipalities. Additionally, complementing genotype determination with more advanced techniques, such as sequencing, and evaluating other alleles potentially associated with production traits would provide a more comprehensive understanding of the genetic influences on milk yield and quality.

CONCLUSIONS

The A1 genetic variant was associated with higher milk yield, protein content, and total solids compared to the A2 variant. Homozygous A1A1 animals exhibited the highest values across the productive parameters evaluated, while heterozygous A1A2 individuals showed only a weak association with these traits.

Genetic analysis of the *CSN2* locus in the Holstein cow sample revealed a moderate genetic structure, accompanied by indications of a slight decline in genetic diversity. While the observed heterozygosity reflects considerable genetic variation, a mild deviation from Hardy–Weinberg equilibrium was detected.

Based on the findings, it is recommended to increase the frequency of the A2 allele in the dairy region of the Department of Nariño through targeted improvement strategies, such as artificial insemination with elite A2A2 sires that possess high breeding values for productive traits. This approach aims to promote the production of milk with potential health benefits and a lower likelihood of causing dietary intolerance in consumers.

CONFLICTS OF INTEREST

No conflicts of interest were reported by the authors.

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DECLARATION ON THE USE OF ARTIFICIAL INTELLIGENCE

The authors declare that no artificial intelligence tools were used during the development of the research or in the preparation of the manuscript resulting from this study.

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