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Genetic variants of caseins and β-lactoglobulin in Lucerna cattle and their association with milk quality

Variantes genéticas de caseínas y β-lactoglobulina en el ganado Lucerna y su asociación con la calidad de la leche

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ABSTRACT

This study aimed to evaluate the variation in the genes *CSN1S1*, *CSN2*, *CSN1S2*, *CSN3* and *LGB* in the Lucerna breed (LUC). For this purpose, 94 LUC animals and 10 Hartón del Valle (HDV) animals were genotyped using the GGP™ Bovine 150 K chip, which contains four single-nucleotide polymorphisms (SNPs) in the *CSN1S1* gene, 14 in the *CSN2* gene, two in the *CSN1S2* gene, 12 in the *CSN3* gene and 11 in the *LGB* gene. Haplotypes and protein variants of α_{S1}-*CN*, β-*CN*, α_{S2}-*CN*, κ-*CN* and β-*LG* were reconstructed. Genotypic frequencies of the major variants were compared between sexes and generations. Hardy-Weinberg equilibrium, wF_{ST} and F_{IS} values were assessed for each of the SNPs. LD values and haplotype frequencies for the caseins were estimated and plotted. The *CSN1S1*B*, *CSN2*A²*, *CSN1S2*A*, *CSN3*A* and *LGB*B* variants were the most frequent in both breeds. Genotype frequencies did not vary between sexes, and only the *CSN2*A²A²* genotype varied between generations. Hardy-Weinberg equilibrium was maintained for all markers, except for one SNP in the *LGB* gene. The wF_{ST} values obtained were very low according to sex (wF_{ST}=0.0043) and generation (wF_{ST}=0.026). The F_{IT} value was -0.0086. Nineteen *CSN1S1-CSN2-CSN1S2-CSN3* haplotypes were found in LUC and eight in HDV with different frequencies. The genetic linkage between the casein genes was low. In conclusion, the genes studied are polymorphic, with a high frequency of variants related to milk production, quality and technological performance. These results can be used in assisted selection programs aimed at improving milk quality and processing traits.

Palabras clave:
Ganado criollo
Ligamiento genético
Frecuencias haplotípicas
Proteínas de la leche

RESUMEN

El objetivo de esta investigación fue evaluar la variación en los genes *CSN1S1*, *CSN2*, *CSN1S2*, *CSN3* y *BLG* en la raza Lucerna (LUC). Para ello, 94 animales LUC y 10 animales Hartón del Valle (HDV) fueron genotipados usando el chip GGP™ Bovine 150 K, que tiene cuatro SNPs en el gen *CSN1S1*, 14 en el gen *CSN2*, dos en el gen *CSN1S2*, 12 en el gen *CSN3* y 11 en el gen *LGB*. A partir de los cuales fueron reconstruidos los haplotipos y variantes proteínicas de la α_{S1}-*CN*, β-*CN*, α_{S2}-*CN*, κ-*CN* y β-*LG*. Se compararon las frecuencias genotípicas de las variantes más importantes entre sexos y generaciones. Se evaluó el equilibrio de Hardy-Weinberg y los valores de wF_{ST} y F_{IS} para cada uno de los SNPs. Se estimaron y graficaron los valores de LD y las frecuencias haplotípicas para las caseínas. Las variantes *CSN1S1*B, CSN2*A², CSN1S2*A, CSN3*A* y *BLG*B* fueron las más frecuentes en ambas razas. Las frecuencias genotípicas no variaron entre sexos, y solo el genotipo *CSN2*A²A²* varió entre generaciones. Todos los marcadores mostraron equilibrio de Hardy-Weinberg, excepto un SNP en el gen BLG. Los valores de wF_{ST} obtenidos fueron muy bajos por el sexo (wF_{ST}=0,0043) y generación (wF_{ST}=0,026). Mientras que, el valor de F_{IT} fue de -0,0086. Se encontraron 19 haplotipos *CSN1S1-CSN2-CSN1S2-CSN3* en LUC y ocho en HDV con frecuencias diferentes. El ligamiento genético fue bajo entre los genes de las caseínas. En conclusión, los genes evaluados son polimórficos y con alta frecuencia de las variantes relacionadas con mayor producción mejor calidad y rendimiento tecnológico de la leche. Estos resultados pueden ser utilizados en programas de selección asistida destinados a mejorar la calidad de la leche y los rasgos de procesado.

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The Lucerna cattle (LUC) is one of the two Colombian synthetic breeds, created in 1937 by crossbreeding the Hartón del Valle, Holstein and dairy Shorthorn breeds in proportions of 30, 30 and 40%, respectively (Giraldo et al. 2023). The creation of the breed was driven by the need for cattle to adapt to tropical environmental conditions, where hardiness, grazing ability, fertility, and longevity are essential (Molina et al. 2016). The LUC breed has an adult weight of 750-800 kg for males and 450-500 kg for females (Molina et al. 2016). Adjusted milk production at 305 days is reported to be 2,425±0.514 kg. This corresponds to an average of 0.15 kg per kg of cow per day of protein, 0.22 kg per cow per day of fat, and 0.59 kg per cow per day of total solids. Age at first calving and first calving interval averaged 41.84±5.01 months and 12.84±1.65 months, respectively. It has a low incidence of mastitis and a low somatic cell count (Rivera et al. 2019). In addition, studies show that it is minimally affected by ectoparasites, with 7.1±1.06 ticks per animal, and it maintains a low proviral load of bovine leukosis virus (Hernández-Herrera and Carrillo-González 2024). Currently, the breed is reported as not at risk according to the DAD-IS, and although its merits as a breed are clear, its use is not yet widespread in Colombia.

Milk is considered a high-value food from a nutritional perspective. Its composition varies within species due to environmental factors such as feed type, climate, lactation stage and milking interval, while breed and genetic composition are also determinants (Chessa et al. 2020). Essentially, the levels of α_{S1}-*CN*, β-*CN*, α_{S2}-*CN* and κ-*CN* κcasκeins and the properties of the micelles they form influence the nutritional quality and technological performance of milk (Kay et al. 2021). These proteins are encoded by four genes (*CSN1S1*, *CSN2*, *CSN1S2* and *CSN3*) located in tandem on chromosome 6 between positions 85.4 and 85.7 Mb (ARS-UCD1.2 genome assembly). Variants in the *CSN1S1* gene are associated with an increase in casein and whey protein (Mohan et al. 2021); some alleles of the *CSN1S2* gene are associated with increased milk production (Ardicli et al. 2018); the **A2* variant of the *CSN2* gene is associated with high yield and quality, as well as with a healthier product, since its digestion does not release the bioactive peptide β-casomorphin-7, which is considered a risk factor for endocrine, nervous, and cardiovascular diseases (Kay et al. 2021); finally, the structural stability of micelles, and thus cheese quality, has been associated with variants in the *CSN3* gene (Poulsen et al. 2017; Zepeda-Batista et al. 2017). As for whey proteins, the *LAA* gene, which codes for α-Lactalbumin (α-LA) protein, is located on chromosome 5 at position 31.2 Mb. β-lactoglobulin (β *-LG*) is encoded by the *LGB* gene and is located on chromosome 11 at position 103.2 Mb (Sanchez et al. 2020).

In general, the genetic variation in these genes responds to changes in whey protein, shortening curdling time and thus affecting cheese yield (Čítek et al. 2023; Dantas et al. 2023; Kay et al. 2021). The genetic variation at these *loci* has been extensively studied in several dairy breeds worldwide (Sanchez et al. 2020; Chessa et al. 2020; Villalobos-Cortés et al. 2023), including the Colombian Holstein breed (Padilla-Doval et al. 2021) and the Colombian Creole breeds for the *CSN3*, *LAA* and *LGB* genes (Rosero et al. 2012; Hernández-Herrera et al. 2024). However, the genetic variations responsible for milk production and quality in the LUC breed have not yet been thoroughly studied. The new market for "A2 milk" has created a need for certification of production systems specializing in this type of milk, as well as the initiation of genetic selection programs based on molecular markers. This research was therefore carried out with the aim of determining the genetic variation in the genes *CSN1S1, CSN2, CSN1S2, CSN3* and *LGB* in the LUC breed and its linkage map, as an input to be used in the development of breeding programs.

MATERIALS AND METHODS

The Reserva Natural El Hatico provided 94 adult animals (20 males and 74 females) used in this study. Inclusion criteria were the lowest degree of relationships and clinical health. El Hatico is located in the municipality of El Cerrito, (Valle del Cauca, Colombia). At an altitude of 1,000 meters above sea level (masl), with a mean annual temperature of 24 °C, 75% humidity and 750 mm of annual rainfall, corresponding to a tropical dry forest (bs-T), according to the Holdridge classification system (Molina et al. 2016). In addition, 10 animals of the Hartón del Valle (HDV) breed from the Agricultural Laboratory of

the Mario González Aranda farm belonging to Universidad Nacional de Colombia, Palmira Headquarters, were used as a reference population.

DNA extraction

BD Vacutainer® tubes containing 7.2 mg EDTA were used to collect 3 mL of whole blood from the Coccygeal vein. Samples were transported to the Animal Genetics Laboratory of the Universidad Nacional de Colombia - Palmira for processing. Total DNA was extracted using the HigherPurity™ DNA Extraction and Purification Kit (Canvax®). The qualitative assessment of the DNA sample was conducted via electrophoresis on 0.8% agarose gels loaded with 6X BX/Loading Buffer (Canvax®), followed by staining employing GreenSafe DNA Gel Stain (Canvax®). To perform the quantitative analysis of the DNA, the Colibri spectrophotometer (Titertek Berthold Technologies GmbH & Co. KG) was utilized. DNA samples were dehydrated by sublimation in 1.5 mL conical tubes in a FreeZone freeze dryer. (Console FreeZone™ kit, Labconco™, Kansas City, MO, USA). Subsequently, the samples were dispatched to Neogen Genomics (https://genomics.neogen.com/en) for genotyping.

Genotyping

Animals were genotyped using the GeneSeek® Genomic Profiler™ Bovine 150K chip (140,668 SNPs). This particular genotyping array contains more than 200 single-nucleotide polymorphisms in major genes that have been associated with genetic diseases. In relation to the milk protein genes, it has been determined that there are four SNPs in the *CSN1S1* gene, 14 in the *CSN2* gene, two in the *CSN1S2* gene, 12 in the *CSN3* gene and 11 in the *LGB* gene (Hernández-Herrera et al. 2024). The genetic variants in question manifest in a triplicate pattern across the chip, thereby facilitating the process of genotyping.

From the SNPs in each gene, it is possible to reconstruct the variants **B*, **C* and **D* of the α_{S1}-*CN* protein; of the β-*CN*, the variants **A*^{1}, **A*^{2}, **B*, **H*^{2} and **F*; the two SNPs in the *CSN1S2* gene allow to identify the variants **A* and **D* of the α_{S2}-*CN*; in the κ-*CN* gene the variants **A*, **A*^{1}, **B*, **I* and **H* can be distinguished, while in the *LGB* gene the 11 genetic polymorphisms allow the reconstruction of the variants **A*, **B*, **C*, **D*, **E*, **F* and **H* of β-*LG* (Caroli et al. 2009; Sanchez et al. 2020; Hernández-Herrera et al. 2024).

Analysis of data

The allele frequencies of the 43 SNPs were estimated. From these, protein variants were reconstructed (Chessa et al. 2020) for all genes in the LUC and HDV breeds. Using a GLM model with Poisson family, the interaction between the main genotypes *(*BB*, **BC* and **CC* for the *CSN1S1* gene; **A*^{1}*A*^{1}, **A*^{1}*A*^{2} and **A*^{2}*A*^{2} for the *CSN2* gene; **AA*, **AD* and **DD* for the *CSN1S2* gene; **AA*, **AB* and **BB* for the *CSN3* gene and **AA*, **AB* and **BB* for the *LGB* gene) of each gene, sex and generation (generation 1: cattle born before 2010; generation 2: animals born between 2010 and 2015; generation 3: individuals born between 2016 and 2021) for the Lucerne breed only was explored, using R base (http://www.r-project.org/).

Deviations from Hardy-Weinberg equilibrium (HWE) and *wFST* values were quantified for each of the SNPs evaluated, using sex and generation as population structuring factors for casein. SNPs were pruned using sliding windows and a criterion r^2=0.2, and the LD value between them was estimated and plotted using the LD and LD plot functions, respectively, from the Gaston package see 1.5.9 (Dandine-Roulland and Perdry 2018) of R. Finally, the genotypes observed for each individual were combined, and the haplotypic frequencies of casein genotypes (*CSN1S1-CSN2-CSN1S2-CSN3*) were estimated by direct counting using R base (Chessa et al. 2020). These variants appear in triplicate within the chip, which ensures the genotyping process.

RESULTS AND DISCUSSION

Of the four polymorphisms present in the *CSN1S1* gene, the SNPs rs433385179 and rs132656458 were monomorphic. This meant that only the *CSN1S1*B* and **C* alleles were found in both breeds, with the former being the most common (Table 1). All SNPs were in HWE. Genotypic frequencies did not differ significantly between sexes (Figure 1A) or generations (Figure 2A).

Table 1. Allele frequencies of SNPs located in the *CSN1S1* gene and allele and genotypic frequencies of protein variants in the α_{S1}-*CN* in the breeds studied.

SNP / Frequency	*SCN1S1* variant											
	*B				*C				*D			
	LUC		HDV		LUC		HDV		LUC		HDV	
CSN1S1_1	-	-	-	-	-	-	-	-	G	A	G	A
Ala68Thr - 6:85418630 rs433385179	-	-	-	-	-	-	-	-	1.000	0.000	1.000	0.000
CSN1S1_2	T	A	T	A	-	-	-	-	-	-	-	-
Val91Asp - 6: 85420971 rs132656458	1.000	0.000	1.000	0.000	-	-	-	-	-	-	-	-
CSN1S1_3	G	A	G	A	-	-	-	-	-	-	-	-
31G>A - 6: 85428068 rs133474041	0.946	0.054	0.750	0.250	-	-	-	-	-	-	-	-
alphaS1Casein26181	-	-	-	-	A	G	A	G	-	-	-	-
Glu207Gly - 6:85427427 rs43703010	-	-	-	-	0.883	0.117	0.650	0.350	-	-	-	-
Allele frequency of the variant	0.882		0.650		0.118		0.350		0.000		0.000	
Genotypic frequency	LUC: BB=0.774 BC= 0.215 CC= 0.011											
	HDV: BB=0.400 BC= 0.500 CC= 0.101											

SNP: Single-nucleotide polymorphism, LUC: Lucerna breed, HDV: Hartón del Valle breed, A (Adenine), C (Cytokine), G (Guanine), and T (Thymine) represent allele frequencies found in each SPNs.

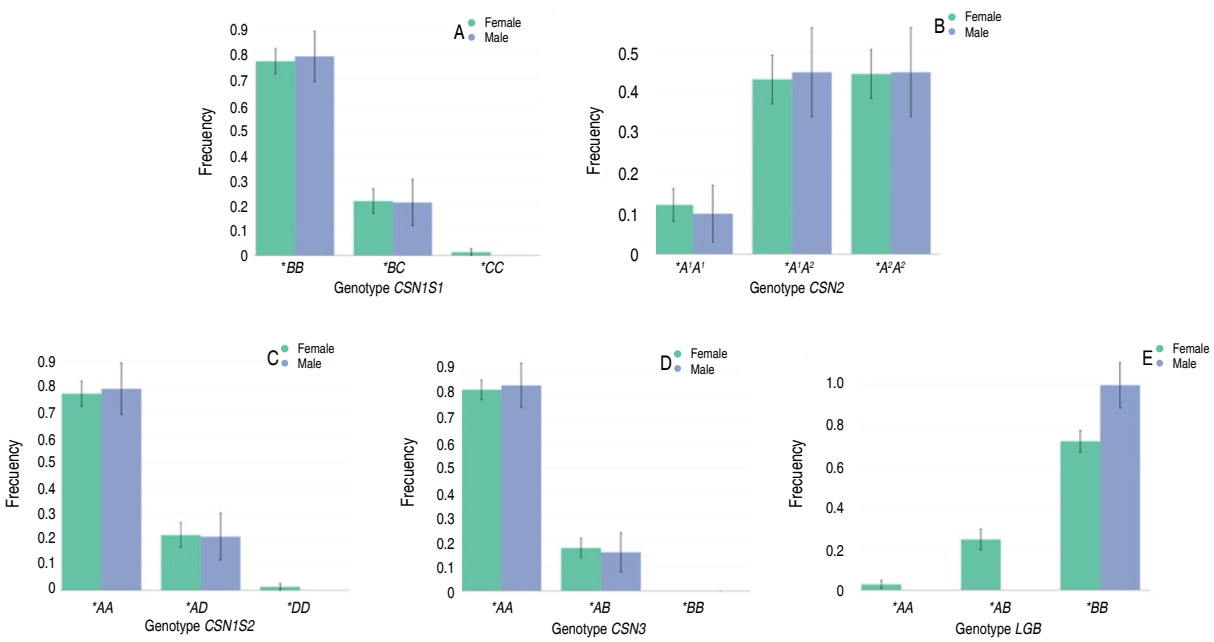

Figure 1. Genotypic frequencies of *CSN1S1* (A), *CSN2* (B), *CSN1S2* (C), *CSN3* (D), and *LGB* (E) genes by sex in the Lucerna breed.

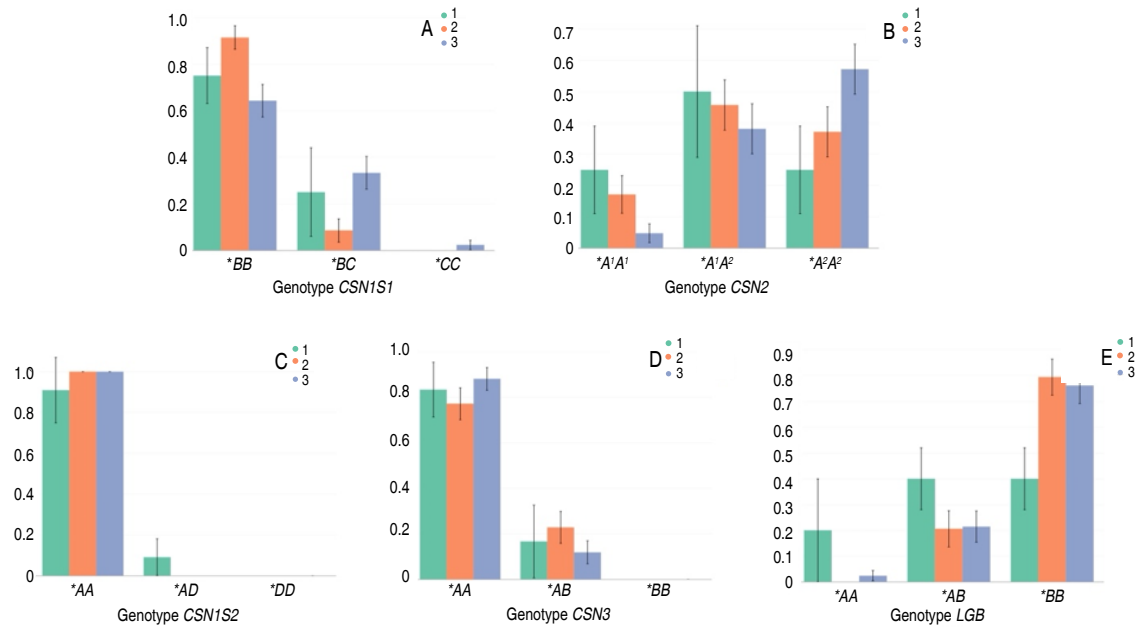

Figure 2. Genotypic frequencies of *CSN1S1* (A), *CSN2* (B), *CSN1S2* (C), *CSN3* (D), and *LGB* (E) genes by generation (generation 1: cattle born before 2010; generation 2: animals born between 2010 and 2015; generation 3: individuals born between 2016 and 2021) in the Lucerna breed.

Ten variants (**A*, **B*, **C*, **D*, **E*, **F*, **G*, **H*, **I* and **J*) are recognized in the α_{S1}-*CN* protein (Meier et al. 2019), from which only three (**B*, **C* and **D*) can be reconstructed with the SNPs present in the GeneSeek® Genomic Profiler™ Bovine 150 K chip, despite the highly polymorphic nature of the gene (Ahmed et al. 2017), but this is explained by the breeds used to develop the chip. The *CSN1S1*B* allele is reported to be the most frequent in several *Bos taurus* breeds, including Angler (1.0), Eringer (0.77), German Red Pied (0.93), German Yellow (1.0), Highland Cattle (1.0), Hinterwälder (0.93), Hungarian Grey Steppe (0.73), Jersey (0.68), Limpurger (1.0), Pinzgauer (0.74), Sarabi (0.54), Shorthorn (1.0) and Vorderwälder (0.87) (Gallinat et al. 2013). In the Holstein breed, the frequency of this allele is reported to be higher than 0.95 (Ardicli et al. 2018; Meier et al. 2019) and in the Simmental breed, 0.90 (Čítek et al. 2023). In the Colombian creole breed Blanco Orejinegro (BON), the frequency of the **B* allele reached 0.80 (Hernández-Herrera et al. 2024). On the other hand, in *Bos indicus* breeds, the *CSN1S1*C* allele is the most common (Butana=0.66, Gir=0.50, Golpayegani=0.48, and Sistani=0.88) (Gallinat et al. 2013).

In the LUC breed, the **BB* genotype was the most common (0.774). In contrast, the **BC* genotype was the most frequent in the HDV breed (0.500) (Table 1). It has been reported that animals with the homozygous **BB* genotype have higher milk production and better milk quality, as it contains higher percentages of fat and protein (Ardicli et al. 2018; Mohan et al. 2021; Čítek et al. 2023).

Of the 14 SNPs evaluated in the *CSN2* gene, 11 were fixed in the LUC breed and 13 in the HDV breed. This meant the presence of **A¹*, **A²*, **B,* and **F* variants of the β-*CN* protein in the LUC breed and only **A¹* and **A²* variants in the HDV breed (Table 2) 15 variants (**A¹*, **A²*, **A³*, **B*, **C*, **D*, **E*, **F*, **G*, **H¹*, **H²*, **I*, **J*, **K,* and **L*) have been reported for this protein (Meier et al. 2019). The frequency of the reference allele for this gene (*CSN2*A¹*) was low, highlighting the high frequency of the *CSN2*A²* variant in both breeds. This is in agreement with that reported for the breeds Eringer (0.54), German Yellow (0.52), Highland Cattle (0.81), Hinterwälder (0.79), Hungarian Grey Steppe (0. 67), Jersey (0.63), Limpurger (0.77), Pinzgauer (0.65), Sarabi (0.65), Hinterwälder (0.79), Hungarian Grey Steppe (0.63), Limpurger (0.77), Pinzgauer (0.65), Sarabi (0.75)

and Shorthorn (0.57) (Gallinat et al. 2013), Holstein (0.55) and Jersey (0.73) (Chessa et al. 2020), Karan Fries (0.60) (Mohan et al. 2021) and in the BON breed (0.51) (Hernández-Herrera et al. 2024). However, this variant is more common in *B. indicus* breeds (Ahmed et al. 2017; Gallinat et al. 2013). The *CSN2*I* allele has been reported at low frequency in the Black Pied (0.017) and Holstein (0.059) breeds from Germany (Meier et al. 2019), and, as reported in this study, the **B* and **F* alleles were found at low frequency in the BON breed (Hernández-Herrera et al. 2024). On the other hand, Padilla-Doval et al. (2021), who studied the Holstein breed in Colombia, reported that the **B* allele was the most common.

All SNPs analysed in the *CSN2* gene were in HWE. No differences in genotypic frequencies were found between the sexes (Figure 1B), but differences were found for the **A*^{2}*A*^{2} genotype in the third generation (P<0.05), which shows an increase in frequency with each generation (Figure 2B). The same trend of increase has been reported in the BON breed (Hernández-Herrera et al. 2024). The importance of the high frequency of this variant (β-*CN*A*^{2}) is understood when it is considered that the intestinal digestion of milk protein releases the bioactive peptide β-casomorphin-7 in animals with the β-*CN*A*^{1} genotype. This peptide has been implicated in the development of several diseases (Chessa et al. 2020; Villalobos-Cortés et al. 2023) and in the increase of both milk allergy and lactose intolerance (Muntean et al. 2022). In contrast, antithrombotic, antihypertensive, immunomodulatory, anticancer, and antimicrobial activities are recognized, as well as bioactive peptides resulting from protein digestion of the β-*CN*A*^{2}, **A*^{3}, **D*, **E*, **H2,* and **I* variants (Muntean et al. 2022). All of these variants have proline at position 67 of the protein instead of histidine (Caroli et al. 2009), producing what are known as A2-type milks.

Table 2. Allele frequencies of SNPs located in the *CSN2* gene and allele and genotypic frequencies of protein variants in the β-*CN* in the breeds studied.

SNP / frequency	*CSN2* variant											
	**A*^{1}				**A*^{2}		**B*		**F*		**H*^{2}	
	LUC		HDV		LUC	HDV	LUC	HDV	LUC	HDV	LUC	HDV
BCN_8491 Val247Ala - 6:85450908 rs715383373	T	C	T	C								
	1.000	0.000	1.000	0.000								
BCN_8463 6:85450976	C	T	C	T								
	1.000	0.000	1.000	0.000								
CSN2_2 His198Pro - 6:85451055 rs454083280	T	G	T	G								
	1.000	0.000	1.000	0.000								
CSN2_X14711_8219 His156Gln - 6:85451180 rs43703012	C	A	C	A								
	1.000	0.000	1.000	0.000								
CSN2_5 Met93Leu - 6:85451236	G	T	G	T								
	1.000	0.000	1.000	0.000								
CSN2_6 Leu88Ile - 6:85451248	C	A	C	A								
	1.000	0.000	1.000	0.000								
CSN2_X14711_8115 Gln72Glu - 6:85451284	C	G	C	G								
	1.000	0.000	1.000	0.000								
CSN2_8 Glu87Lys - 6:85452710 rs721259074	C	T	C	T								
	1.000	0.000	1.000	0.000								

Table 2

SNP / frequency	*CSN2* variant											
	**A¹*				**A²*		**B*		**F*		**H²*	
	LUC		HDV		LUC	HDV	LUC	HDV	LUC	HDV	LUC	HDV
CSN2_X14711_6687 Glu86Lys - 6:85452713 rs3423226649	G	A	G	A								
	1.000	0.000	1.000	0.000								
CSN2_X14711_6562 Arg75Cys - 6:85452837 rs799602970	C	T	C	T								
	1.000	0.000	1.000	0.000								
CSN2_7 His117Pro - 6:85451298 rs43703011					A	C	A	C				
					0.335	0.665	0.300	0.700				
CSN2_3 Arg172Ser - 6:85451132 rs43703013							C	G	C	G		
							0.979	0.021	1.000	0.000		
CSN2_1 Pro202Leu - 6:85451043 rs433954503									G	A	G	A
									0.995	0.005	1.000	0.000
CSN2_4 Met143Leu - 6:85451221 rs109299401											A	C
											1.000	0.000
Allele frequency of the variant	0.314		0.300		0.665	0.700	0.016	0.000	0.005	0.000	0.000	0.000
Genotipic frequency	LUC: A^1A^1= 0.085 A^1A^2= 0.436 A^1B=0.021 A^2A^2= 0.447 BF=0.011											
	HDV: A^1A^2= 0.600 A^2A^2= 0.400											

SNP: single-nucleotide polymorphism. LUC: Lucerna breed. HDV: Hartón del Valle breed. A (Adenine), C (Cytokine), G (Guanine), and T (Thymine) represent allele frequencies found in each SPNs.

There are at least 23 different brands of A2-type milk in markets around the world (Dantas et al. 2023). In Italy, for example, there has been selection for this allele since 1990, with a 10% increase by 2017 (Chessa et al. 2020). This research shows a significant increase in the **A²A²* genotype between generations 1 (β-*CN*A²A²*=0.25) and 3 (β-*CN*A²A²*=0.58). It is important to emphasize two points: first, the high frequency of this genotype in the LUC breed is likely due to its origin in the HDV breed; and second, there is no direct selection for this genotype in the herd under study. In addition, A2-type milk is not currently marketed in Colombia. Therefore, the increase in this genotype is likely due to the associated effects of this genotype, such as an increase in milk production, higher fat and protein content, and better yield in curd production (Mohan et al. 2021). Demand for this type of product may increase in the country as consumer awareness of functional foods increases (Dantas et al. 2023).

Five variants are known in the α_{S2}-*CN* protein, including **A*, **B*, **C*, **D,* and **E* (Meier et al. 2019). However, the genotyping chip only has two SNPs (rs441966828 and rs463985801) in the *CSN1S2* gene that allow analysis of the **A* and **D* variants. In the HDV breed, the SNPs were monomorphic and only the SNP *CSN1S2_2* varied in LUC (Table 3); therefore, the reference variant *CSN1S2*A* and the **AA* genotype were fixed in HDV and with a very high frequency in LUC.

Table 3. Allele frequencies of SNPs located in the *CSN1S2* gene and allele and genotypic frequencies of protein variants in the α_{S2}-*CN* in the breeds studied.

SNP / Frequency	*CSN1S2* variant							
	**A*				**D*			
	LUC		HDV		LUC		HDV	
CSN2S2_1 Ser68Phe - 6:85533780 rs441966828	C	T	C	T				
	1.000	0.000	1.000	0.000				
CSN1S2_2 Glu74Asp - 6:85536434 rs463985801					G	T	G	T
					0.995	0.005	1.000	0.000
Allele frequency of the variant	0.995		1.000		0.005		0.000	
Genotypic frequency	LUC: AA=0.989 AD= 0.011							
	HDV: AA=1.000							

SNP: single-nucleotide polymorphism. LUC: Lucerna breed. HDV: Hartón del Valle breed. A (Adenine), C (Cytokine), G (Guanine), and T (Thymine) represent allele frequencies found in each SPNs.

These results are similar to those reported in the BON breed (*CSN1S2*A*=0.997) (Hernández-Herrera et al. 2024) and in 20 other breeds studied (Gallinat et al. 2013; Ahmed et al. 2017; Meier et al. 2019). In contrast, the **D* allele has a high frequency (0.98) in the Holstein breed (Ardicli et al. 2018) and values between 2 and 10% in German breeds such as Angler, German Yellow, Hinterwälder, Limpurger, and Vorderwälder (Gallinat et al. 2013). In contrast, the *CSN1S2*E* variant was only found in the Sarabi breed (0.02) (Gallinat et al. 2013) and the *CSN1S2*C* variant in Angus with a frequency of 0.075. The positive effect of this gene on milk production and milk quality was attributed to the **AA* genotype (Gallinat et al. 2013). This research shows that there is no obvious selection in favor of the genotypes found in the *CSN1S2* gene, as the genotypic frequencies did not vary by sex (Figure 1C) or generation (Figure 2C) and were all in HWE.

The genotyping chip used detected 12 SNPs in the *CSN3* gene, five of which were fixed in the LUC strain and eight in the HDV strain. From these polymorphisms it is possible to reconstruct the **A*, **A*^{1}, **B*, **E*, **H* and **I* variants of κ-CN of the 14 reported (**A*, **A*^{1}, **B*, **B*^{2}, **C*, **D*, **E*, **F*^{1}, **F*^{2}, **G*^{1}, **G*^{2}, **H*, **I* and **J*) (Caroliet a. 2009; Gallinat et al. 2013; Meier et al. 2019). In both breeds studied, the **A* variant of κ-*CN* had the highest frequency, followed by the κ-*CN*B* variant in LUC and by κ-*CN*I* in HDV (Table 4). The other distinguishable alleles (κ-*CN*A*^{1}, **E,* and **H*) showed a low frequency in LUC and were absent in HDV.

The frequency found for the **A* allele in the LUC breed is higher than what was reported in many breeds around the world, including Angler (0.55), Butana (0.47), Eringer (0.50), German Red Pied (0.71), German Yellow (0.45), Gir (0.07), Hinterwälder (0.75), Holstein (0.52), Hungarian Grey Steppe (0.55), Jersey (0. 42), Karan Fries (0.62), Limpurger (0.80), Pinzgauer (0.59), Retinta (0.13), Sarabi (0.30), Shorthorn (0.60), Sistani (0.28) and Vorderwälder (0.78) (Gallinat et al. 2013; Ahmed et al. 2017; Meier et al. 2019; Chessa et al. 2020; Mohan et al. 2021); only slightly surpassed by Black Pied (0.83) (Meier et al. 2019). This gene has been studied in other breeds in Colombia. The frequency of the κ-CN*A variant has been reported as 0.68 in the BON breed (Hernández-Herrera et al. 2024), 0.68 in the HDV breed (Naranjo et al. 2007), and 0.80 in the Holstein breed (Padilla-Doval et al. 2021). In contrast, the κ-*CN*B* variant is reported at a high frequency in the Jersey breed (0.9) (Chessa et al. 2020), while the κ-*CN*H* variant is more common in *B. indicus* breeds such as Gir (0.5) and Sistani (0.5) (Gallinat et al. 2013).

The **BB* genotype was not found in the breeds studied, but the **AB* genotype was found (Table 4). Both genotypes are associated with higher milk production (Mahmoudi et al. 2020). In terms of quality, milk from animals with

Table 4. Allele frequencies of SNPs located in the *CSN3* gene and allele and genotypic frequencies of protein variants in the κ-*CN* in the breeds studied.

SNP / Frequency	*CSN3* variant																			
	*A				*A¹				*E				*B				*H				*I			
	LUC		HDV		LUC		HDV		LUC		HDV		LUC		HDV		LUC		HDV		LUC		HDV	
CSN3_AY380228_12690 Arg/His - 6:85656358	G	A	G	A																				
	1.000	0.000	1.000	0.000																				
CSN3_AY380228_12940 Thr= - 6:85656608	T	C	T	C																				
	1.000	0.000	1.000	0.000																				
CSN3_AY380228_12950 Arg164Ser - 6:85656618 rs110870535	C	A	C	A																				
	1.000	0.000	1.000	0.000																				
CSN3_AY380228_12951 Arg164His - 6:85656619 rs716557965	G	A	G	A																				
	1.000	0.000	1.000	0.000																				
CSN3_AY380228_13096 6:85656764	T	G	T	G																				
	1.000	0.000	1.000	0.000																				
CSN3_AY380228_13165 Ala235= - 6:85656833 rs110014544	A	G	A	G																				
	0.910	0.090	0.800	0.200																				
CSN3_AY380228_13111 Pro217= - 6:85656779 rs439304887					A	G	A	G																
					0.995	0.005	1.000	0.000																
CSN3_AY380228_13124 Ser222Gly - 6:5656792 rs43703017									A	G	A	G												
									0.995	0.005	1.000	0.000												
CSN3_AY380228_13068 Ile203Thr - 6:85656736 rs43703015													T	C	T	C								
													0.085	0.915	0.200	0.800								
CSN3_AY380228_13104_1 Ala215Asp - 6:85656772 rs43703016													A	C	A	C								
													0.888	0.112	0.800	0.200								
CSN3_AY380228_13065 Thr202Ile - 6:85656733 rs450402006																	C	T	C	T				
																	0.936	0.064	1.000	0.000				
CSN3_AY380228_12971 Ser171Ala - 6:85656639 rs43706475																					T	G	T	G
																					0.989	0.011	0.700	0.300
Allele frequency of the variant	0.819		0.550		0.005		0.000		0.005		0.000		0.112		0.200		0.048		0.000		0.011		0.250	
Genotypic frequency	LUC: AA=0.638 AB= 0.223 AE= 0.011 AH= 0.096 AI= 0.021 A1B= 0.011																							
	HDV: AA=0.200. AB= 0.400 AI= 0.300 II= 0.100																							

SNP: single-nucleotide polymorphism. LUC: Lucerna breed. HDV: Hartón del Valle breed. A (Adenine), C (Cytokine), G (Guanine), and T (Thymine) represent allele frequencies found in each SPNs.

the **AA* genotype has a lower protein content (*P*<0.05) (Mahmoudi et al. 2020). In contrast, milk from animals with genotype **BB* has higher protein, fat, calcium, and phosphorus content (Mahmoudi et al. 2020; Mohan et al. 2021). It also has a better fatty acid content and a higher yield in milk-to-cheese processing, understood by a higher structural stability of the micelles and a shorter coagulation time (Poulsen et al. 2017; Zepeda-Batista et al. 2017). IgE-binding epitopes common to the **A* and **E* variants are also known in the κ-*CN*, which could potentially be more allergenic than the **B* variant. However, bioactive peptides with antithrombotic, anti-inflammatory, and immune response modulating activity have also been described in this protein (Vargas-Bello-Pérez et al. 2019).

Due to the above-mentioned advantages of the *CSN3*B* variant, breeding programs have been initiated to increase its frequency. For example, in the Holstein breed in Italy, the frequency has increased from 0.18 to 0.35 in the last 17 years (Chessa et al. 2020). This does not seem to be the case in the LUC breed, where frequencies have not changed over time (Figure 2D), nor in the BON breed, where a tendency to decrease over time has been reported (Hernández-Herrera et al. 2024). None of the SNPs studied in the *CSN3* gene showed significant HWE deviations. The comparison of frequencies concerning sex in the LUC breed showed no differences (Figure 1D), contrary to those previously reported in the BON breed, where the **B* variant is more frequent in females (Hernández-Herrera et al. 2024).

Using the β-*LG*B* allele as a reference, 11 variants (**A*, **B*, **C*, **D*, **E*, **F*, **G*, **W*, **I*, **J,* and **W*) have been reported (Caroli et al. 2009). The 11 SNPs included in the genotyping chip, located in the LGB gene, allowed the reconstruction of the variants **A*, **B*, **D*, **G*, and **I* of β-*LG*, of which the last three showed low frequency in the LUC breed and absence in the HDV breed (Table 5). In both breeds, the **B* allele of beta-lactoglobulin was the most common, followed by the **A* allele. In the breeds Butana (0.46) (Ahmed et al. 2017), BON (0.67) (Hernández-Herrera et al. 2024), Holstein (0.70), and Simmental (0.59) (Čítek et al. 2023), the **B* allele was the most frequent. In contrast, in a previous study, the β-*LG*A* variant was reported to be the most frequent in BON (0.51) (Rosero et al. 2012), Gyr and Jersey breeds (Gai et al. 2021).

Other reports suggest that animals with the β-*LG*AA* genotype produce more milk. On the contrary, the β-*LG*BB* genotype produces higher protein, fat, and shorter rennet formation time (Gai et al. 2021; Čítek et al. 2023). This genotype showed a frequency of around 30% in the breeds analyzed (Table 5). At least two interesting bioactive peptides (LQKW 58-61 and WYSLAMAASDI 19-29) have been reported in β-*LG*, which, through their antioxidant activity, reduce stress granule formation, scavenge 2,2'-azino-bis-acid radical cations, and increase myotube differentiation in skeletal muscle, resulting in an anti-aging effect (Kim et al. 2019; Čítek et al. 2023). However, these beneficial effects have not been associated with any particular genetic variant.

In this gene, the variant LGB_X14710_5263 (rs109625649) showed significant deviations from HWE. However, this did not imply differences in the genotype frequencies between sexes (Figure 1E) and between generations (Figure 2E), although the **BB* genotype was more common in males and the third generation. As mentioned above, there was no selection for this genotype in the herd studied, but it is possible that there was indirect selection due to the milk traits of these animals.

Regarding the population structure in the LUC breed, the *wFST* values obtained were low, considering all SNPs and as structuring factors sex (*wFST*=0.0043) and generation (*wFST*=0.026). In the BON breed, using the herd as a structuring factor, the reported F_{ST} is 0.035, 0.003 by sex, and 0.001 by year (Hernández-Herrera et al. 2024). The *wFST* value for the variant associated with the β-*CN*A²* protein was 0.0512 (*P*<0.05). While the estimated F_{IT} value was -0.0086 (*P*>0.05), which shows that there is no inbreeding, evaluated from the milk genes.

It has been proposed that, due to the physical proximity of the casein genes, they are more likely to be inherited as a linkage group rather than independently (Villalobos-Cortés et al. 2023). To test this, the 32 SNPs analyzed in caseins and the 11 SNPs in beta-lactoglobulin were pruned. Finally, 14 SNPs were informative in the casein genes and five in the *LGB* gene. Figures 3A and 3B show the heat maps of the LODs obtained. The highest LOD value was found between the SNPs CSN3_AY380228_13165

Table 5. Allele frequencies of SNPs located in the *LGB* gene and allele and genotypic frequencies of protein variants in the β-*LG* in the breeds studied.

SNP / Frequency	*LGB* variant													
	*B		*A		*D		*H		*G		*I			
	LUC		HDV		LUC		HDV		LUC		HDV		LUC		HDV	
LGB_X14710_3080	C	T	C	T												
Pro66Ser - 11:103257043																
rs3423333957	1.000	0.000	1.000	0.000												
LGB_X14710_5962	C	T	C	T												
Pro142Leu - 11:103259931																
rs3423321021	1.000	0.000	1.000	0.000												
LGB_X14710_5970	G	T	G	T												
Asp145Tyr - 11:103259939																
rs475846954	1.000	0.000	1.000	0.000												
DB-2053-seq-rs209836063	T	C	T	C												
T>C - 11:103280858																
rs209836063	1.000	0.000	1.000	0.000												
LGB_X14710_3982					C	T	C	T								
Asn79= - 11:103257948																
rs110180463					0.868	0.132	0.950	0.050								
LGB_X14710_5174					T	C	T	C								
Asn104= - 11:103259143																
rs110641366					0.866	0.134	0.650	0.350								
LGB_X14710_5263					C	T	C	T								
Ala134Val - 11:103259232																
rs109625649					0.493	0.507	0.313	0.687								
LGB_X14710_3065									G	C	G	C				
Glu61Gln – 11:103257028																
rs211077340									0.995	0.005	1.000	0.000				
LGB_X14710_4003_1													G	C	G	C
Lys70Asn - 11:103257969													0.994	0.006	1.000	0.000
LGB_X14710_4027																
Ile94Met - 11:103257993																
rs3423333959																
LGB_X14710_5233																
Glu124Gly - 11:103259202																
rs3423334663																
Allele frequency of the variant	0.585		0.650		0.388		0.350		0.005		0.000		0.005		0.000	
Genotipic frequency	LUC: AA= 0.043 AB= 0.691 BB=0.223 BD= 0.011 BI= 0.0021 GH= 0.011															
	HDV: AB= 0.700 BB=0.300															

SNP / Frequency	*G*				*I*			
	LUC				HDV			
LGB_X14710_3080								
Pro66Ser - 11:103257043								
rs3423333957								
LGB_X14710_5962								
Pro142Leu - 11:103259931								
rs3423321021								
LGB_X14710_5970								
Asp145Tyr - 11:103259939								
rs475846954								
DB-2053-seq-rs209836063								
T>C - 11:103280858								
rs209836063								
LGB_X14710_3982								
Asn79= - 11:103257948								
rs110180463								
LGB_X14710_5174								
Asn104= - 11:103259143								
rs110641366								
LGB_X14710_5263								
Ala134Val - 11:103259232								
rs109625649								
LGB_X14710_3065								
Glu61Gln – 11:103257028								
rs211077340								
LGB_X14710_4003_1								
Lys70Asn - 11:103257969								
LGB_X14710_4027	C	G	C	G				
Ile94Met - 11:103257993								
rs3423333959	0.994	0.006	1.000	0.000				
LGB_X14710_5233					A	G	A	G
Glu124Gly - 11:103259202								
rs3423334663					0.989	0.011	1.000	0.000
Allele frequency of the variant	0.005		0.000		0.005		0.000	
Genotipic frequency								

SNP: single-nucleotide polymorphism. LUC: Lucerna breed. HDV: Hartón del Valle breed. A (Adenine), C (Cytokine), G (Guanine), and T (Thymine) represent allele frequencies found in each SPNs.

(κ-*CN*A*) and CSN3_AY380228_13068 (κ-*CN*B*), followed by another pair of SNPs also determining the **A* and **B* variants of the κ-*CN* gene (*CSN3_AY380228_13165* and *CSN3_AY380228_13104_1*) and the SNPs determining the κ-*CN*B* variant (*CSN3_AY380228_13068* and *CSN3_AY380228_13104_1*). The *CSN2S2_1* SNPs, which determine the **A* variant of the α_{S2}-*CN* gene, showed a high LOD value with the *CSN3*_AY38022228_13165 variant, which determines the κ-*CN*A* variant. Among the SNPs of the β-*CN* gene, the LOD values were low (LOD<0.07) and presented an average value between κ*CN* and β-*CN*. The SNP *CSN2_7*, which determines the most studied variant of β-*CN (*A2*), did not show LOD values higher than 0.15 with other polymorphisms (Figure 3A). Then, the SNP alphaS1Casein26181 of the α_{S2}-*CN*C* gene showed mean LD values with the SNP determining the **B* variant of the same gene, with the SNP *CSN2S2_1* determining the **A* variant of the α_{S2}-*CN* gene, and the variant *CSN3*_AY380228_13065 coding for the **H* allele of the κ-*CN*. The linkage values between the informative SNPs of the *LGB* gene were low (LOD<0.03), except between *LGB*_X14710_3982 and *LGB*_X14710_5174 (LOD=0.50), which both determine the **A* allele of the β-*LG* (Figure 3B). These results show that the linkage between casein genes is low, and the highest linkage is found between SNPs of the same gene. A similar conclusion is presented by Hernández-Herrera et al. (2024) in BON cattle.

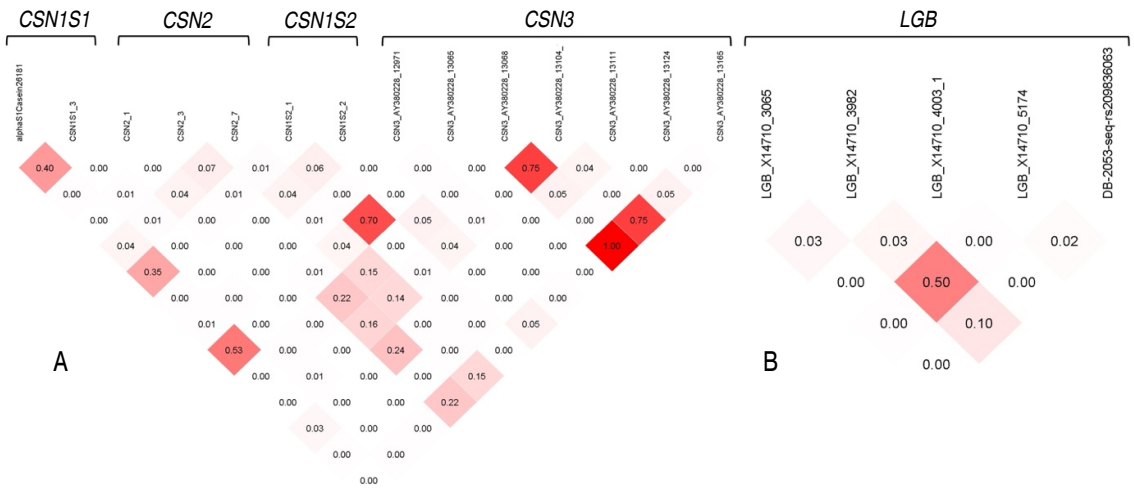

Figure 3. Linkage disequilibrium heat map of SNPs after pruning in the casein (A) and beta-lactoglobulin (B) genes in the Lucerne breed.

Twenty-two *CSN1S1-CSN2-CSN1S2-CSN3* haplotypes were found, 19 in the LUC breed and eight in the HDV breed, of which five were shared between breeds and three were unique to the HDV (Table 6). Haplotype *BB-A^2A^2-AA-AA* had the highest frequency (0.312), followed by haplotypes *BB-A^1A^2-AA-AA-AA* (0.194) and *BB-A^1A^2-AA-AB* (0.118) in the LUC breed. The other haplotypes had frequencies of less than 6.5%. In the HDV breed, the most common haplotypes were *BB-A^1A^2-AA-AA-AI* and *BC-A^1A^2-AA-AA-AB* (0.2), which were rare in LUC. Coincidentally, the three most common haplotypes in the LUC breed were also common in the BON breed. In contrast, of the two most common haplotypes in the HDV breed, one was absent in BON, and the other had a frequency below 5% (Hernández-Herrera et al. 2024). Using haplotypes reconstructed from *CSN1S1-CSN2-CSN1S2-CSN3* alleles—rather than from genotypes as in the present study—84 haplotypes were identified in the Butana breed, with the most frequent being *C-A2-A-A* (0.156) (Ahmed et al. 2017). On the other hand, using only *CSN2-CSN3* genes, the most common haplotype was *A^2-A* in Holstein (0.474) and *A^2-B* (0.646) in the Jersey breed (Chessa et al. 2020).

This research showed a low level of linkage between casein genes. Nevertheless, milk, being a composite of several proteins, could have synergistic relationships that affect its quality and technological performance. For

this reason, the effects of *CSN1S1-CSN2-CSN3* gene haplotypes on milk traits were studied. Thus, the most frequent haplotype *BB-A^2A^2-AA* (*CSN1S1-CSN2-CSN3*) in the LUC breed (31.2%) has contradictory reports. Perna et al. (2016) demonstrated that this haplotype is associated with low cheese yield when coagulation is induced by rennet. In contrast, when coagulation is induced by acidic methods, traits related to cheese formation are significantly improved (Ketto et al. 2017). Similarly, the second most common haplotype in LUC (*BB-A^1A^2-AA*, 19.4%) was associated with better curdling time, better curdling structure, and higher cheese yield (Ketto et al. 2017). On the other hand, the *BB-A^2A^2-BB* haplotype was associated with higher milk protein and fat content and better rennet formation (Perna et al. 2016). This haplotype had a low frequency in the LUC breed.

Table 6. Haplotype frequencies of the *CSN1S1-CSN2-CSN1S2-CSN3* genes in the Lucerna and Hartón del Valle breeds.

No.	Haplotype *CSN1S1-CSN2-CSN1S2-CSN3*	LUC	HDV
1	*BB-A^1A^1-AA-AA*	0.043	-
2	*BB- A^1A^1-AA-AB*	0.022	-
3	*BB- A^1A^1-AD-AB*	0.011	-
4	*BB-A^1A^2-AA-AA*	0.194	-
5	*BB- A^1A^2-AA-AB*	0.118	-
6	*BB- A^1A^2-AA-AE*	0.011	-
7	*BB- A^1A^2-AA-AI*	0.022	0.200
8	*BB-A^1B-AA-AB*	0.022	-
9	*BB-A^2A^2-AA-AA*	0.312	-
10	*BB- A^2A^2-AA-AB*	0.011	-
11	*BB- A^2A^2-AA-II*	-	0.100
12	*BB-BF-AA-AA*	0.011	-
13	*BC- A^1A^2-AA-A1B*	0.011	-
14	*BC- A^1A^2-AA-AA*	0.032	0.100
15	*BC- A^1A^2-AA-AB*	0.032	0.200
16	*BC- A^1A^2-AA-AH*	0.022	-
17	*BC- A^2A^2-AA-AA*	0.043	0.100
18	*BC- A^2A^2-AA-AB*	0.011	0.100
19	*BC- A^2A^2-AA-AH*	0.065	-
20	*BC- A^2A^2-AA-AI*	-	0.100
21	*CC- A^2A^2-AA-AB*	-	0.100
22	*CC- A^2A^2-AA-AH*	0.011	-

LUC: Lucerna breed. HDV: Hartón del Valle breed.

Finally, these results justify the efforts made by private and public bodies to conserve Colombian Creole breeds and to promote the development of current conservation programs into genetic breeding programs, not only to promote the use of adapted breeds, but also as breeds capable of producing high-quality milk.

CONCLUSION

Only 46 and 23% of the SNPs evaluated were found to be polymorphic in the LUC and HDV breeds, respectively. Nevertheless, it was possible to reconstruct the most significant variants (*CSN1S1*B*, β-*CN*A^2*, κ-*CN*B,* and β-*LG*B*) from the perspective

of their association with milk production, nutritional quality, and technological performance. These variants exhibited high frequency in both breeds, with minor variations attributable to the limited number of HDV animals utilized and the geographical origin of the LUC breed in HDV. The linkage found between single-nucleotide polymorphisms located in the same gene was high. In contrast, the linkage between the casein genes was found to be low, suggesting that they are independently inherited. The high frequency of these favorable alleles in the Lucerna population—despite the absence of targeted selection—suggests a strategic opportunity to incorporate this information into genetic selection programs aimed at improving both productive efficiency and milk compositional and technological quality. Furthermore, the low genetic linkage among the loci studied suggests that independent selection for each gene is feasible, enabling greater genetic gain without constraints imposed by linkage disequilibrium. This genetic characterization provides a foundation for implementing marker-assisted selection (MAS), advancing towards genomic selection, and promoting the broader use of the Lucerna breed not only for its tropical adaptability but also for its potential to produce high-quality milk.

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ETHICAL CONSIDERATIONS

All processes within the experimental analysis were carried out in accordance with the guidelines proposed in 'The international guiding principles for biomedical research involving animals' (CIMOS and ICLAS 2012).

CONFLICT OF INTERESTS

The authors declare no competing interests.

REFERENCES

Ahmed A, Rahmatalla S, Bortfeldt R et al (2017) Milk protein polymorphisms and casein haplotypes in Butana cattle. Journal of Applied Genetics 58(2): 261-271. https://doi.org/10.1007/s13353-016-0381-2

Ardicli S, Soyudal B, Samli H et al (2018) Effect of *STAT1*, *OLR1*, *CSN1S1*, *CSN1S2*, and *DGAT1* genes on milk yield and composition traits of Holstein breed. Revista Brasileira de Zootecnia 47: e20170247. https://doi.org/10.1590/rbz4720170247

Caroli AM, Chessa S and Erhardt GJ (2009) Invited review: Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition. Journal of Dairy Science 92(11): 5335-5352. https://doi.org/10.3168/jds.2009-2461

Chessa S, Gattolin S, Cremonesi P et al (2020) The effect of selection on casein genetic polymorphisms and haplotypes in Italian Holstein cattle. Italian Journal of Animal Science 19(1): 833-839. https://doi.org/10.1080/1828051X.2020.1802356

Čítek J, Samková E, Brzáková M et al (2023) *CSN1S1* and *LALBA* polymorphisms and other factors influencing yield, composition, somatic cell score, and technological properties of cow's milk. Animals 13(13): 2079. https://doi.org/10.3390/ani13132079

Dandine-Roulland C and Perdry H (2018) Genome-Wide data manipulation, association analysis and heritability estimates in R with gaston 1.5. Human Heredity 83(1): 1-29.

Dantas A, Kumar H, Prudencio E et al (2023) An approach on detection, quantification, technological properties, and trends market of A2 cow milk. Food Research International 167: 112690. https://doi.org/10.1016/j.foodres.2023.112690

Gai N, Uniacke-Lowe T, O'Regan J et al (2021) Effect of protein genotypes on physicochemical properties and protein functionality of bovine milk: A review. Foods 10(10): 2409. https://doi.org/10.3390/foods10102409

Gallinat JL, Qanbari S, Drögemüller C et al (2013) DNA-Based identification of novel bovine casein gene variants. Journal of Dairy Science 96(1): 699-709. https://doi.org/10.3168/jds.2012-5908

Giraldo N, Viveros A, Rincón J and González-Herrera L (2023) Curva de primera lactancia en ganado bovino con diferente proporción Lucerna y su relación con factores ambientales asociados a la producción de leche. Livestock Research for Rural Development 35(Article # 1).

Hernández-Herrera D and Carrillo-González D (2024) Association of BoLA-DRB3 Alleles with the Progression of Bovine Leukosis in the Lucerna Breed. Tropical Animal Science Journal 47(4): 417-425. https://doi.org/10.5398/tasj.2024.47.4.417

Hernández-Herrera DY, Rincón-Floréz JC and Pulido-Hoyos MN (2024) Milk protein polymorphisms and casein haplotypes in Blanco Orejinegro cattle of Colombia. Revista de Ciências Agroveterinárias 23(1): 117-129. https://doi.org/10.5965/223811712312024117

Kay S, Delgado S, Mittal J et al (2021) Beneficial effects of milk having A2 β-Casein protein: Myth or reality?. The Journal of Nutrition 151(5): 1061-1072. https://doi.org/10.1093/jn/nxaa454

Ketto I, Knutsen T, Øyaas J et al (2017) Effects of milk protein polymorphism and composition, casein micelle size and salt distribution on the milk coagulation properties in Norwegian Red cattle. International Dairy Journal 70: 55-64. https://doi.org/10.1016/j.idairyj.2016.10.010

Kim Y, Kim J, Cheon S et al (2019) Alpha-casein and beta-lactoglobulin from cow milk exhibit antioxidant activity: A plausible link to antiaging effects. Journal of Food Science 84(11): 3083-3090. https://doi.org/10.1111/1750-3841.14812

Mahmoudi P, Rostamzadeh J, Rashidi A et al (2020) A meta-analysis on association between *CSN3* gene variants and milk yield and composition in cattle. Animal Genetics 51(3): 369-381. https://doi.org/10.1111/age.12922

Meier S, Korkuć P, Arends D and Brockmann G (2019) DNA

sequence variants and protein haplotypes of casein genes in German Black Pied cattle (DSN). Frontiers in Genetics 10. https://doi.org/10.3389/fgene.2019.01129

Mohan G, Kumar A, Khan S et al (2021) Casein (CSN) gene variants and parity affect the milk protein traits in crossbred (*Bos taurus* x *Bos indicus*) cows in sub-tropical climate. Tropical Animal Health and Production 53(2): 289. https://doi.org/10.1007/s11250-021-02736-w

Molina I, Angarita E, Mayorga O et al (2016) Effect of *Leucaena leucocephala* on methane production of Lucerna heifers fed a diet based on *Cynodon plectostachyus*. Livestock Science 185: 24-29. https://doi.org/10.1016/j.livsci.2016.01.009

Muntean I, Bocsan I, Wiest L et al (2022) Predictive factors for oral immune modulation in cow milk allergy. Nutrients 14(3): 494. https://doi.org/10.3390/nu14030494

Naranjo J, Posso A, Cárdenas H and Muñoz J (2007) Detección de variantes alélicas de la kappa-caseína en bovinos Hartón del Valle». Acta Agronómica 56 (1): 43-48. https://revistas.unal.edu.co/index.php/acta_agronomica/article/view/568

Padilla-Doval J, Zambrano-Arteaga JC, Echeverri-Zuluaga JJ and López-Herrera A (2021) Análisis genético de cinco polimorfismos de nucleótido simple de caseínas lácteas obtenidos con chips genómicos en ganado Holstein de Antioquia, Colombia. Revista de la Facultad de Medicina Veterinaria y de Zootecnia 68 (2): 137-149 https://doi.org/10.15446/rfmvz.v68n2.98026

Perna A, Intaglietta I, Gambacorta E and Simonetti A (2016) The influence of casein haplotype on quality, coagulation, and yield traits of milk from Italian Holstein cows. Journal of Dairy Science 99(5): 3288-3294. https://doi.org/10.3168/jds.2015-10463

Poulsen N, Gregersen V, Maciel G et al (2017) Novel genetic variation associated to *CSN3* strongly affects rennet-induced milk coagulation. International Dairy Journal 71: 122-30. https://doi.org/10.1016/j.idairyj.2017.03.012

Rivera J, Chará J, Murgueitio E and Molina J (2019) Feeding leucaena to dairy cows in intensive silvopastoral systems in Colombia and Mexico. Tropical Grasslands 7(4): 370-374. https://doi.org/10.17138/tgft(7)370-374

Rosero J, Álvarez L, Muñoz J et al (2012) Allelic frequency of the kappa-casein gene in colombian and creole cattle breeds. Revista Colombiana de Ciencias Pecuarias 25(2): 173-182. https://doi.org/10.17533/udea.rccp.324744

Sanchez M, Fritz S, Patry C et al (2020) Frequencies of milk protein variants and haplotypes estimated from genotypes of more than 1 million bulls and cows of 12 French cattle breeds. Journal of Dairy Science 103(10): 9124-9141. https://doi.org/10.3168/jds.2020-18492

Vargas-Bello-Pérez E, Márquez-Hernández R and Hernández-Castellano L (2019) Bioactive peptides from milk: animal determinants and their implications in human health. Journal of Dairy Research 86(2): 136-144. https://doi.org/10.1017/S0022029919000384

Villalobos-Cortés A, Rodriguez G, Castillo H and Franco S (2023) Characterization of casein variants in the Guaymi and Guabala breeds through a low-density chip arrangement. Journal of Applied Animal Research 51 (1): 69-73. https://doi.org/10.1080/09712119.2022.2154216

Zepeda-Batista J, Saavedra-Jiménez L, Ruíz-Flores A et al (2017) Potential influence of κ-casein and β-lactoglobulin genes in genetic association studies of milk quality traits. Asian-Australasian Journal of Animal Sciences 30(12): 1684-1688. https://pubmed.ncbi.nlm.nih.gov/28728383/