Open Veterinary Journal, (2025), Vol. 15(11): 5971-5981
Research Article
DOI: *10.5455/OVJ.2025.v15.i11.52*

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Submitted: 31/12/2024 Revised: 29/09/2025 Accepted: 25/10/2025 Published: 30/11/2025

Frequency of A1 and A2 alleles of the β-casein gene in cattle in the Cajamarca region of Peru

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ABSTRACT

Background: Cow's milk β-casein has multiple genetic variants, the two most common of which are A1 and A2, which are encoded by the *CSN2* gene. Evidence suggests that the A1 variant may negatively affect human health. Type A2 milk is a safer alternative for human consumption because it is easier to digest than A1.
Aim: To determine the frequency of the A1 and A2 alleles of the β-casein gene in cattle.
Methods: Blood samples were collected from 103 cattle (26 males and 77 females), 71 crossbred, 30 Simmental, 1 Jersey, and 1 Holstein. The selection prioritized females due to their economic value for milk production, reproductive potential, and capacity to replace cows of high genetic value, whereas males are intended for sale for meat production. Analysis of the A1 and A2 alleles of exon 7 of the *CSN2* gene was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using the restriction enzyme D*deI*.
Results: The heterozygous A1A2 genotype was the most frequent (50.5%, $n = 52$), followed by the homozygous A2A2 genotype (35.0%, $n = 36$) and A1A1 genotype (14.6%, $n = 15$). The results showed that 33.8% (26/77) of the females and 38.5% (10/26) of the males had the A2A2 genotype.
Conclusion: PCR-RFLP genotyping allowed the genotypic frequencies of β-casein to be determined, with 33.8% and 38.5% A2A2 in females and males, respectively.
Keywords: A2 milk, Cattle, *CSN2* gene; Highland dairy.

Introduction

Milk is an essential component of the human diet (Dubagari *et al.*, 2022) at all life stages, consumed for its nutritional components and health benefits. It consists of approximately 90% water, and the remaining percentage of solids includes proteins, milk fats, minerals, and vitamins (Fernández *et al.*, 2015). Milk and milk products are considered to be nutritious foods because they contain bioactive components. However, in some cases, milk consumption can cause health-related problems, such as allergic reactions and gastrointestinal problems (Cieślińska *et al.*, 2022; Giribaldi *et al.,* 2022). Caseins deserve special attention among all the proteins present in milk (Shashank *et al.*, 2018; Cie *et al.*, 2019). Caseins constitute approximately 80% of the total proteins in cow's milk, with β-casein making up about 37% of the total caseins (Shashank *et al.*, 2018; Kaskous, 2020). The most common β-casein variants in cow's milk are A1 and A2 alleles, which differ at position 67 of exon 7 of the β-casein gene (*CSN2*); this mutation results in a histidine (His67) in the A1 β-casein variant and a proline (Pro67) in the A2 variant (Cieślińska *et al.*, 2022). Therefore, the presence of His67 during A1 milk digestion facilitates the proteolytic cleavage of β-casein, causing the release of the opioid peptide β-casomorphin-7 (BCM-7) (Giribaldi *et al.*, 2022).

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This peptide can cause adverse gastrointestinal effects (Giglioti *et al.*, 2020).

The release of BCM-7 by the gastrointestinal digestion of A1 and B variants of bovine β-casein, harms health (Shashank *et al.*, 2018). It is associated with lactose intolerance (He *et al.,* 2017), histamine secretion leading to allergy (Brooke-Taylor *et al.*, 2017), the development of cardiac diseases (Kamiński *et al.,* 2007), complications of the symptoms in type I diabetes (Elliott *et al.*, 1999), and various gastrointestinal problems, such as increased gas production, softer stools, bloating, and abdominal pain (Jianqin *et al.,* 2016). In line with these findings, regional studies, such as one conducted in Mexico, show similar findings. In an adult population with intolerance and regular consumption of dairy products, the consumption of A2/A2 milk significantly improved abdominal pain symptoms compared with conventional milk (Medel, 2024). These findings have raised concerns about the quality of milk and the diseases related to its consumption, as it can cause discomfort and allergic reactions in some individuals due to its protein components (Oliveira *et al.*, 2021). In contrast, the A2 variant is globally famous for its unique health benefits because it releases lower amounts of BCM-7 (De Noni *et al.,* 2009).

In this context, A2 milk has recently received attention from the dairy industry (Fernández-Rico *et al.*, 2022). The popularity of A2 milk is increasing worldwide, and new molecular techniques have been developed to develop reliable methods (Ardicli *et al.*, 2023). From this point of view, determining the cattle genotype in the Cajamarca region is necessary, with the possibility of selecting animals that produce A2 milk, representing a healthier option for human health. Given the beneficial characteristics of A2 milk for both industry and human health, it is essential to characterize the β-casein gene in cattle, especially in the Cajamarca region, which is the leading national producer of fresh milk, accounting for 17% of the national production (2,241,136 tons) (Red de Comunicación Regional, 2023). According to market research by Kantar Worldpanel, 41% of Peruvian households have at least one member with lactose intolerance (Redacción, 2019), representing an opportunity for the introduction of A2 milk as a differentiated product. Although there is currently no established market for this product, it has gained popularity in other countries in the region, such as Brazil (Mendes *et al.*, 2019) and Chile (Carvajal *et al.*, 2022), and there are government initiatives for the selective breeding of herds with the A2A2 genotype. Therefore, this study aimed to determine the frequency of the A1 and A2 alleles of the β-casein gene in cattle in the Cajamarca region of Peru.

Materials and Methods

Location of the study site

Blood samples were collected from 103 calves in 16 localities located in 6 provinces (Chota, San Miguel, Hualgayoc, Cajabamba, Celendín, Cajamarca) of the Cajamarca region between July and September 2024 from cows of the beneficiary producers of the Proyecto de Mejoramiento Genético (PROMEG NACIONAL) product of artificial in-semination (Fig. 1).

The Cajamarca region is located 2660 m above sea level. Its average maximum temperature is 20.8°C, its annual temperature is 14.75°C, and its minimum temperature is 8.5°C. The average annual relative humidity is 68.3%, and the annual rainfall is 801.8 mm (Servicio Nacional de Meteorología e Hidrología del Perú, 2024).

Control samples

The hair follicle samples from *Bos taurus* calves, genotypically identified as A1A1, A1A2, and A2A2, were obtained directly from the previously selected and identified animals. The samples were sent to NEOGEN. First, the samples were genotyped with the single-nucleotide polymorphism (SNP) chip in the GeneSeek Genomic Profiler Bovine, which contains approximately 100,000 SNP markers and is explicitly designed for *B. taurus*. Finally, we performed the Igenity Basic test and the A2 evaluation (NEOGEN Company, Australia).)

Biological material

Blood samples were collected from cattle using a non-probabilistic convenience sampling method. Animals were selected based on sampling site accessibility and the willingness of farmers to voluntarily participate in the study. A total of 103 blood samples were taken from calves born to dams inseminated with semen from bulls of the Genetic Improvement Project (PROMEG NACIONAL), genotypically identified as Cuba A1A2, Pumpo A2A2, Delmon A1A2, Jakumaru A2A2, Jorge A1A2, Neymar A1A2). Of the 103 samples, 26 were males and 77 were females, representing 71 crossbred animals, including 30 Simmental, 1 Jersey, and 1 Holstein. Samples were collected in BD Vacutainer® tubes with EDTA as the anticoagulant with a needle 21. They were kept at a temperature of 4ºC during transport to the Laboratory of Biotechnology in Animal Health—Agricultural Experimental Station Baños del Inca, located in Baños del Inca, where the molecular analyses were performed.

DNA extraction

gDNA was extracted from bovine blood samples using the Wizard® Genomic DNA Purification kit (Promega, USA). For the procedure, 300 µl of whole blood and 5 µl of Proteinase K (20 mg/ml) were used, following the manufacturer's protocol. Subsequently, UV/VIS spectrophotometry (PCRmax Lambda, UK) was used to evaluate DNA purity and concentration, calculating the absorbance ratio of 260/280. Finally, the samples were adjusted to a 50 ng/µl concentration and stored at 4°C until further use (Samsung, RT38YNPP1/XPE, Mexico).

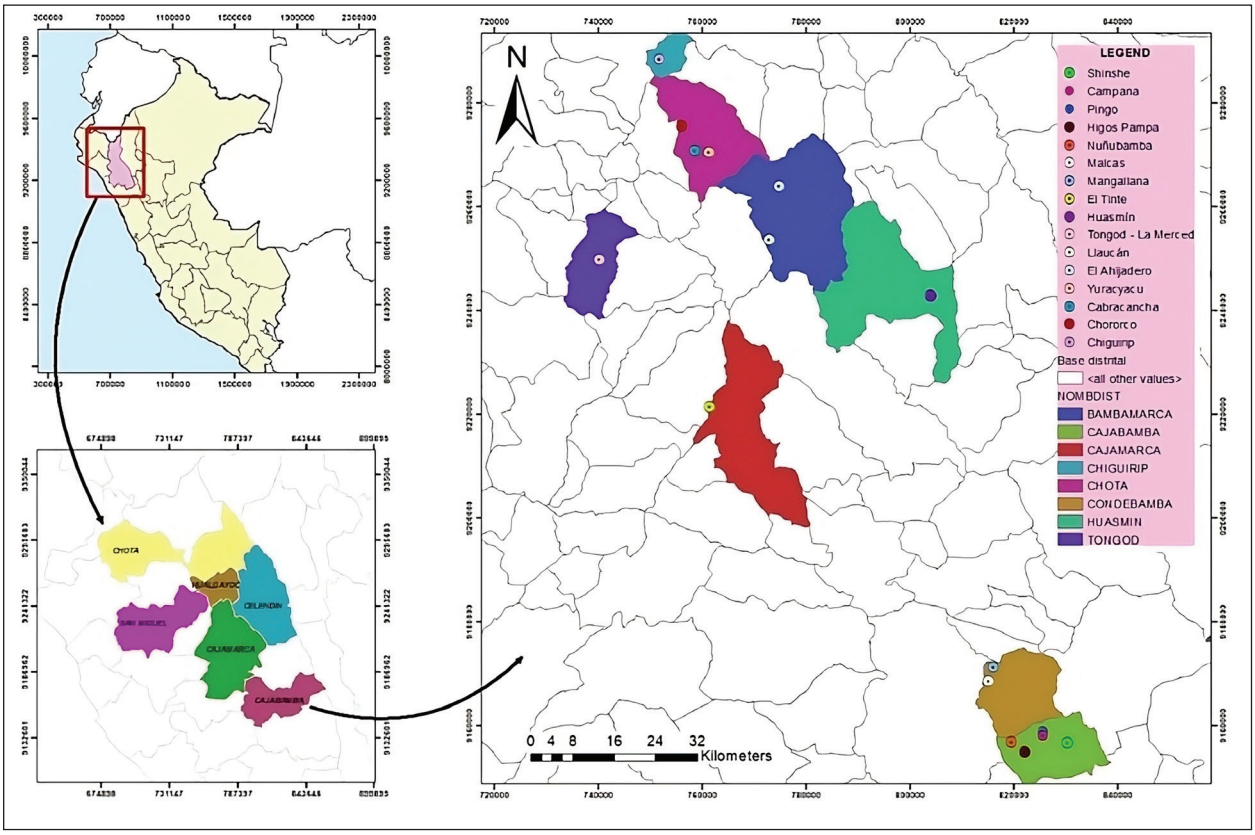

Fig. 1. Location map of the Cajamarca region.

PCR-restriction fragment length polymorphisms

Amplification of the 121-bp genomic region of exon 7 of the *CSN2* gene was performed at a final volume of 13 µl containing 4 µl of ultrapure H_2O, 6 µl of GoTaq® G2 Green Master Mix (Promega, USA), 1 µl of primer F (10 µM), 1 µl of primer R (10 µM), and 1 µl of DNA (50 µg/µl). The GoTaq® DNA Polymerase (Promega) was selected due to its robustness, reliability, and cost-effectiveness. Furthermore, this enzyme has been effectively used in previous genotyping studies (Antonopoulos *et al.,* 2021). The primers used were forward 5′-CCTTCTTCTTTCCAGGATGAACTCCAGG-3′ and reverse 5′- GAGTAAGAGGAGGGATGTTT TGTGGGAGGCTCT-3′ (Saran *et al.*, 2019). Polymerase chain reaction (PCR) amplification was performed under the following conditions: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 45 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 10 minutes (Antonopoulos *et al.*, 2021). PCR products were analyzed by horizontal electrophoresis (Labnet Enduro™ 250V, UK) at a constant voltage of 100 V for 40 minutes, using a 1.5% agarose gel stained with 5 µl SYBR Safe (Invitrogen, USA). The PCR products were visualized using a GelDoc Go imaging system (Bio-Rad, USA) under UV illumination, and a 100 bp DNA ladder (GeneRuler, SMO241-Thermo Scientific, Lithuania) was used.

Enzymatic digestion of 121 bp PCR products was performed using HpyF3I (D*deI*) restriction enzyme (10 U/µl) for 2 hours at 37°C in the following reaction mixture: 9 µl of ultrapure H_2O, 1 µl of 10 × Buffer Tango, 5 µl of PCR product, and 0.25 µl of D*deI* enzyme (Thermo Scientific, USA). Restriction fragments were analyzed by 3% agarose gel electrophoresis at 180 V for 23 minutes using the GeneRuler 100 bp DNA molecular weight marker (SMO241-Thermo Scientific, Lithuania).

Statistical analysis

Direct counting was used to determine genotypic and allele frequencies. Genotypic frequencies were calculated as the ratio of each genotype to the total number of individuals. Allele frequencies were calculated as a function of the total number of identified alleles. Statistical analysis included descriptive statistics using contingency tables, performed in IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA), and GraphPad Prism version 9.3.1 (GraphPad Software, San Diego, CA, USA) was used for graphing. The association between β-casein genotypes and race and province of origin was evaluated using Fisher's

exact test (>20 % of the cells had expected frequencies <5). Pearson's chi-square test was used to assess the association between genotype and sex because less than 20% of the cells presented low expected frequencies. A statistically significant association was considered when $p \leq 0.05$.

Ethical approval

The research was carried out in accordance with the Guidelines of Animal Research: Reporting of *in vivo* Experiments 2.0 (Würbel *et al*., 2020). This included the use of adequate restraint techniques and the avoidance of sudden movements during blood extraction from the coccygeal vein. The procedure was performed by professional veterinary medical personnel trained in the Faculty of Veterinary Sciences of the National University of Cajamarca; additionally, the study was conducted with the informed consent of the owners of the animals. After obtaining the farmers' authorization.

Results

PCR-RFLP analysis of CSN2

Genomic DNA was extracted from 103 blood samples from 26 male and 77 female calves. The extracted gDNA was evaluated by UV/VIS spectrophotometry, showing adequate concentration and purity, which allowed its subsequent analysis by PCR-RFLP to determine *CSN2* gene genotypes (Fig. 2).

The amplified exon 7 was then subjected to restriction enzyme digestion, and the resulting DNA fragments were visualized using 3% agarose gel electrophoresis (Fig. 3). Thus, digestion with DdeI generated three distinct banding patterns. An undigested fragment of 121 bp was obtained in the homozygous A1A1 genotype. Partial digestion produced three fragments of 121, 86, and 35 bp for the heterozygous A1A2 genotype. For the homozygous A2A2 genotype, complete digestion resulted in two fragments of 86 and 35 bp.

Allelic and genotypic frequencies of CSN2

PCR-RFLP analysis revealed that the heterozygous A1A2 genotype was the most frequent (50.5 %, n = 52), followed by the homozygous A2A2 (35.0 %, n = 36) and A1A1 (14.6 %, n = 15) genotypes, which were the least frequent. In terms of allele frequency, the A2 allele was slightly more frequent than the A1 allele, with frequencies of approximately 60% and 40%, respectively (Fig. 4). Analysis of the breed distribution (Table 1) reveals that the A1A2 genotype predominates in Simmental calves (56.7%). A similar trend was observed in the Holstein/Simmental and Criolla/Simmental breeds, with 41.7% and 52% of heterozygotes, respectively. Regarding the A2A2 genotype, the Simmental breed and its crosses (Holstein/Simmental and Criolla/Simmental) had a higher number of A2A2 individuals (Fig. 5). Regarding the geographic distribution of genotypes, interesting variations were found among the 6 provinces included in the study (Table 2, Fig. 6). The A1A2 genotype was the most common in several provinces, except in Cajamarca and Celendín where the A2A2 genotype predominated with frequencies of 55.6% and 50.0%, respectively. A high prevalence of genotype A1A1 was observed in San Miguel (29.4%), the province with the highest proportion of this genotype. The genotypic frequency analysis according to cattle sex revealed that the A2 allele of interest was more frequent than the A1 allele in both females (59.75%) and males (61.6%). The A2A2 homozygous genotype was also observed, with a frequency of 33.8% in females and 38.5% in males (Table 3). Statistical analyses using Fisher's exact test for breed (p = 0.607) and province (p = 0.396) and Pearson's χ^2 test for sex (p = 0.875) showed no significant associations between genotypes and any of the variables analyzed.

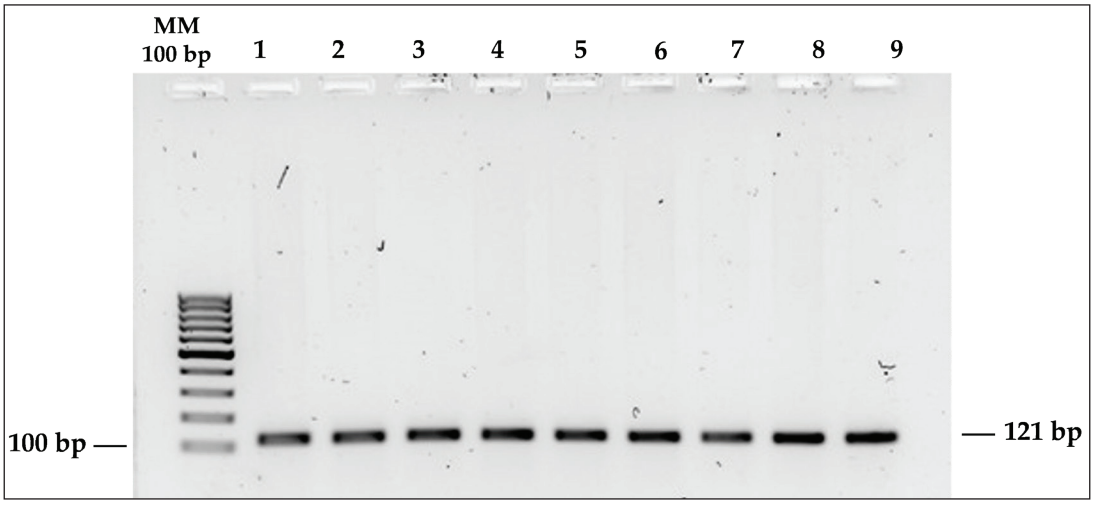

Fig. 2. 1.5% agarose gel of *CSN2* PCR products with an expected size of 121 bp. The 100 bp marker (MM) confirms the band size. All samples (1-9) show a sharp 121-bp band, indicating specific target gene amplification.

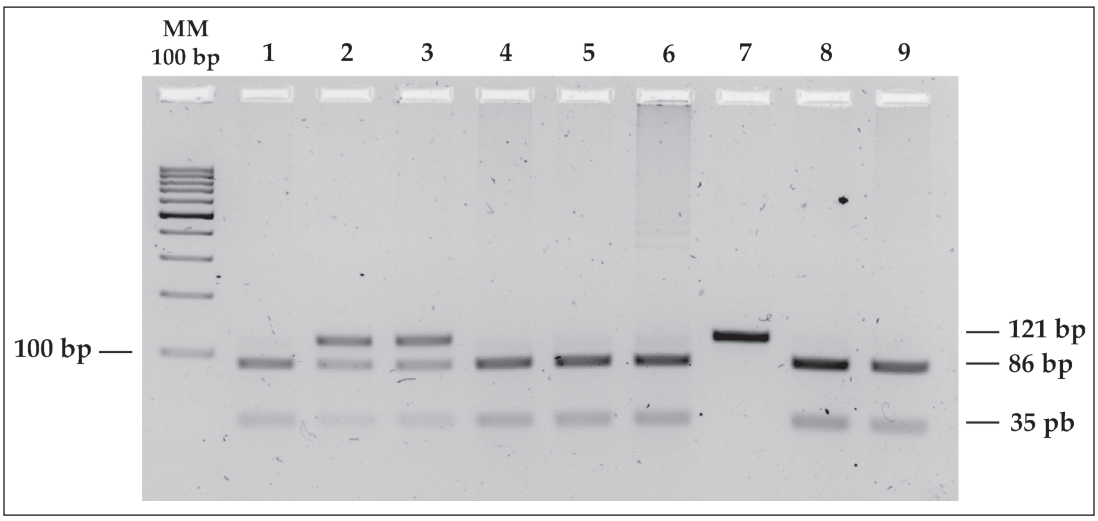

Fig. 3. Agarose gel electrophoresis of the PCR-RFLP analysis of the *CSN2* gene digested with the DdeI restriction enzyme. Bands 1,4-6,8-9 Genotype A2A2 (86, 35 bp), bands 2-3 Genotype A1A2 (121, 86, 35 bp), and band 7: Genotype A1A1 (121 bp).

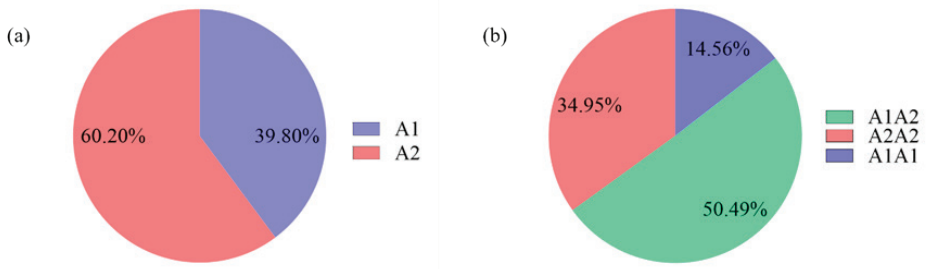

Fig. 4. (a) Allelic distribution of the *CSN2* gene in cattle from Cajamarca, Peru. (b) Genotypic distribution of the *CSN2* gene in the same population.

Discussion

The frequency of A1 and A2 alleles of the β-casein gene (*CSN2*) was determined in cattle born from dams artificially inseminated with semen from bulls with high genetic value in the Cajamarca region, Peru. This is the first report to provide an overview of the distribution of β-casein genetic polymorphisms associated with A2 milk production in animals born in the context of the National Genetic Improvement Project (PROMEG). The analysis of exon 7 of the *CSN2* gene, using the PCR-RFLP technique, made it possible to identify the A1A1, A1A2, and A2A2 genotypes, the latter of greater relevance because it is related to positive effects on human health and in the dairy industry. Allelic and genotypic frequencies were estimated in the sample analyzed (n = 103), and it was found that the A2 allele (60.25%) was more frequent than the A1 allele (39.80%). The heterozygous A1A2 genotype (50.5%) was the most frequent, followed by the A2A2 (35.0%) and A1A1 (14.56%) genotypes. These results are in agreement with those of studies conducted in crossbred populations, such as Holstein crossbred cattle in Bangladesh (Safa *et al.*, 2024) and Criollos in Mexico (Rivera *et al.*, 2016), where the A1A2 genotype was also the most frequent. However, they differ from reports in purebreds such as Holsteins in the United States or Japanese Jerseys (Nuomin *et al.*, 2022; Arens *et al.*, 2023), where A2A2 genotype frequencies exceed 45%–65%, reflecting decades of genetic selection directed to the A2 allele. In that sense, considering the frequency of the A2 allele (60.25%), it is recommended that to change the genetic composition of the herd toward A2A2 animals that only produce milk with the A2 variant of the β-casein, it is necessary to continue with the use of A2A2 bulls for insemination of cows carrying the A2 allele as indicated by Sebastiani *et al.* (2020). Likewise, the males identified as carriers of the genotype of interest in this study are of great utility because they can be used for future artificial insemination.

The high frequency of the heterozygous genotype (A1A2, 50.5%) could be related to the crossing of

Table 1. Allele and genotypic frequencies by breed.

Breeds	Genotypic frequency			Allele frequency		Fisher's exact test (*p*-value)[1]
	A1A1	A1A2	A2A2	A1	A2	
Brown Swiss x Braunvieh (*n* = 11)	9.1% (*n* = 1)	63.6% (*n* = 7)	27.3% (*n* = 3)	40.9%	59.1%	0.607
Criolla x Braunvieh (*n* = 1)	100.0% (*n* = 1)	0.0% (*n* = 0)	0.0% (*n* = 0)	100%	0.0%	
Criolla x Gyr (*n* = 4)	25.0% (*n* = 1)	50.0% (*n* = 2)	25.0% (*n* = 1)	50%	50%	
Criolla x Simmental (*n* = 25)	12.0% (*n* = 3)	52.0% (*n* = 13)	36.0% (*n* = 9)	38%	62%	
Hereford x Simmental (*n* = 1)	0.0% (*n* = 0)	0.0% (*n* = 0)	100% (*n* = 1)	0.0%	100%	
Holstein (*n* = 1)	100.0% (*n* = 1)	0.0% (*n* = 0)	0.0% (*n* = 0)	100%	0.0%	
Holstein x Braunvieh (*n* = 2)	0.0% (*n* = 0)	100% (*n* = 2)	0.0% (*n* = 0)	50%	50%	
Holstein x Gyr (*n* = 3)	0.0% (*n* = 0)	33.3% (*n* = 1)	66.7% (*n* = 2)	16.65%	83.35%	
Holstein x Simmental (*n* = 24)	20.8% (*n* = 5)	41.7% (*n* = 10)	37.5% (*n* = 9)	41.65%	58.35%	
Jersey (*n* = 1)	0.0% (*n* = 0)	0.0% (*n* = 0)	100% (*n* = 1)	0.0%	100%	
Simmental (*n* = 30)	10.0% (*n* = 3)	56.7% (*n* = 17)	33.3% (*n* = 10)	38.35%	61.65%	
Total (*n* = 103)	14.6% (*n* = 15)	50.5% (*n* = 52)	35.0% (*n* = 36)	39.80%	60.20%	

[1]No statistically significant differences were found ($p > 0.05$). The number of animals for each frequency is indicated in brackets. For the *p* value column, the cells should be merged to avoid misunderstanding the total *p* value.

breeds carrying different alleles. In addition, since the population comes from rural areas where intensive genetic programs have not been developed, and it is a first generation of calves resulting from artificial inseminations, whose objective has not been to prioritize the selection of the A2 allele, the probability of higher frequencies of the A1A2 genotype is increased. Another reason for this phenomenon is that, in the absence of strong selective pressure, a natural genetic equilibrium, characterized by a higher number of heterozygous genotypes, may still be maintained (Cieślińska *et al.,* 2022). Sebastiani *et al.* (2020) indicated that in a population under Hardy–Weinberg equilibrium, one would expect to find 50% A1A2 heterozygotes, 25% A1A1 homozygotes, and 25% A2A2. It should be emphasized that the HW equilibrium was not calculated in this study because the basic assumptions for this test were not met.

The prevalence of A2 and A1 variants is associated with the animal breed, with the A2 variant being more common in Brown Swiss and Simmental cows (Kamiński *et al.,* 2007). Although analysis by breed is limited in our study due to the small size of some samples and sample sizes between genetic groups, an overall frequency of the A2A2 genotype was 35.0%. This frequency reflects current breeding practices in Peru, where A2 selection is not prioritized in regional programs such as PROMEG, only 2 of the 5 genotyped bulls had the A2A2 genotype, and this is only an additional trait considered in some service bulls. This contrasts with other studies where crossbred cattle populations have been reported as high as 52%–53% (Oliveira *et al.*, 2021) or 57.64% (Miluchová *et al.*, 2023).

In particular, in crosses with Simmental (Criolla × Simmental: 33.3 %; Hereford × Simmental: 36.0%; Holstein × Simmental: 37.5%), A2A2 frequencies were consistent with that reported by Şahin *et al.* (2022) for this breed (28%), suggesting that the Simmental breed shows a favorable genetic predisposition for breeding programs, although no statistically significant differences were found between breeds ($p = 0.607$). The high prevalence of the A2A2 genotype in the Simmental breed and its crosses is related to the parents.

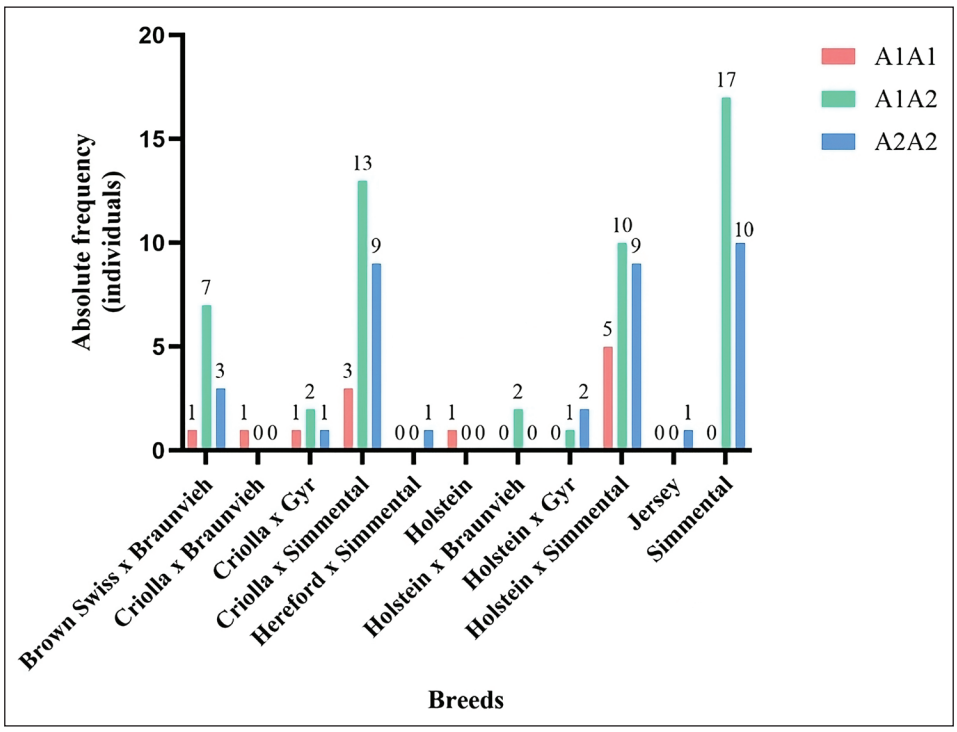

Fig. 5. Distribution of A1A1, A1A2, and A2A2 genotypes in bovine breeds.

Table 2. Allelic and genotypic frequencies by province.

Province	Genotypic frequency			Allele frequency		Fisher's exact test (*p*-value)[1]
	A1A1	A1A2	A2A2	A1	A2	
Cajabamba (*n* = 14)	14.3% (*n* = 2)	57.1% (*n* = 8)	28.6% (*n* = 4)	42.85%	57.15%	0.396
Bambamarca (*n* = 19)	15.8% (*n* = 3)	52.6% (*n* = 10)	31.6% (*n* = 6)	42.1%	57.9%	
Celendín (*n* = 18)	5.6% (*n* = 1)	44.4% (*n* = 8)	50.0% (*n* = 9)	27.8%	72.2%	
San Miguel (*n* = 17)	29.4% (*n* = 5)	58.8% (*n* = 10)	11.8% (*n* = 2)	58.8%	41.2%	
Cajamarca (*n* = 9)	0.0% (*n* = 0)	44.4% (*n* = 4)	55.6% (*n* = 5)	22.2%	77.8%	
Chota (n = 26)	15.4% (n = 4)	46.2% (n = 12)	38.5% (n = 10)	38.5%	61.6%	

[1]No statistically significant differences were found ($p > 0.05$). The number of animals for each frequency is indicated in parentheses.

Although the dam's genotype is unknown, the A2A2 parent bulls used in artificial insemination were known to be Simmental. Therefore, together with the breed's natural predisposition for the A2 allele (Kamiński *et al.,* 2007), this may have influenced the high frequency of the A2A2 genotype in this breed. These results underline the need to cover a larger number of samples in future studies to properly evaluate genotype-breed associations, considering the possible selection biases of sires.

The relationship of genotypes with respect to the province of origin and sex was also evaluated; however, no significant statistical differences were found. In this case, the limited sample size limited the analysis depth, highlighting the need for future studies to include larger and more balanced sample sizes to improve statistical power. Nevertheless, an exploratory analysis of the data

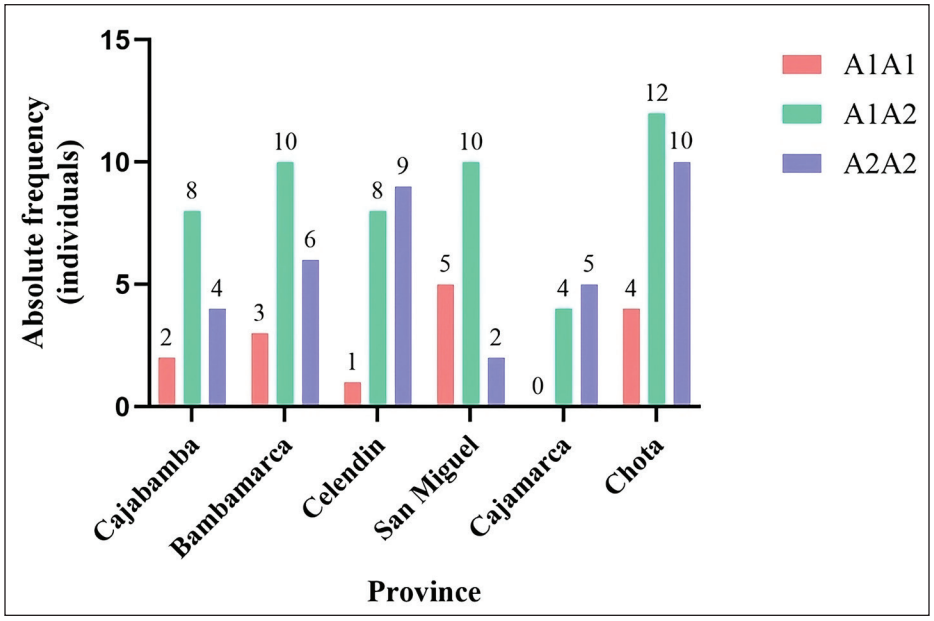

Fig. 6. Distribution of A1A1, A1A2, and A2A2 genotypes by province.

Table 3. Allelic and genotypic frequencies according to sex.

Sex of the calf	Genotypic frequency			Allele frequency		χ^2 (*p*-value)[1]
	A1A1	A1A2	A2A2	A1	A2	
Female (*n* = 77)	14.3% (*n* = 11)	51.9% (*n* = 40)	33.8% (*n* = 26)	40.25%	59.75%	**0.875**
Male (*n* = 26)	15.4% (*n* = 4)	46.2% (*n* = 12)	38.5% (*n* = 10)	38.5%	61.6%	

[1]No statistically significant differences were found ($p > 0.05$). The number of animals for each frequency is indicated in parentheses.

revealed that the provinces of Cajamarca (55.6%) and Celendín (50.0%) exhibited higher frequencies of the A2A2 genotype, which could represent an opportunity for a future genetic selection program aimed at A2 milk production. Therefore, it is necessary to continue promoting the genetic improvement of cattle in the region through artificial insemination programs using semen from bulls identified as A2A2 or the transfer of A2A2 embryos. Likewise, the identification of β-casein polymorphisms should continue, not only in Cajamarca but also in other regions with high dairy production in the country, considering expanding the sample size to more representative breeds. However, there are challenges in terms of costs, infrastructure, and access to livestock areas that must be addressed for the successful implementation of a genetic improvement program. In addition, as in the case of Colombia (Martínez y Mendoza, 2025), there needs to be prior awareness of the advantages of A2 milk, since obtaining samples was difficult due to mistrust among producers in some areas during fieldwork. This could have a positive economic impact, considering that A2 milk is gaining popularity in the global market (Alfonso *et al.,* 2019; Fernández-Rico *et al.,* 2022). In this sense, the choice of A2A2 milk-bearing animals is fundamental, as it brings added value to local dairy products, considering that A2 milk stands out for its benefits in human health. Several studies have shown that consumption of A2 milk by lactose-intolerant people causes fewer gastrointestinal symptoms than conventional milk (Jianqin *et al.,* 2016; He *et al.,* 2017). In terms of milk technological characteristics, the A2 variant is associated with higher milk protein yield (Olenski *et al.,* 2010) and 2.1% higher milk yield per day (Morris *et al.,* 2005) compared to those with the A1 variant. Therefore, the identification of the A2A2 genotype offers an important opportunity for the Cajamarca dairy industry to produce differentiated milk.

The PCR-RFLP technique is widely accepted for β-casein gene genotyping and has been reported in several previous studies (Saran *et al.*, 2019; Antonopoulos *et al.,* 2021; Miluchová *et al.,* 2023). In a comparative analysis of molecular and biochemical

methods for the detection of A1 and A2 alleles, Vigolo *et al.* (2022) concluded that PCR-RFLP and PCR-ARMS are rapid and highly reliable allele-specific techniques. In this context, sequencing in the present work was not considered necessary because it has been previously demonstrated that PCR-RFLP is a highly reliable technique that alone provides reliable results that allow discriminating the A1A1, A1A2, and A2A2 genotype, based on the size of bands visualized in the agarose gel (Vigolo *et al.,* 2022; Raschia *et al.,* 2023). Also, this analysis was no longer considered as it does not provide significant advantages in terms of accuracy, given the increased costs for sequencing. However, its use could be recommended in future research as a confirmatory method for detecting allelic changes. However, despite its reliability, the PCR-RFLP technique can be expensive and requires procedures that can be complex compared to other alternatives (Vigolo *et al.,* 2022). Therefore, in regions such as Cajamarca, where cattle raising is mostly practiced in rural areas under traditional breeding systems and where advances in genetic improvement and specialized infrastructure (biotechnological laboratories) are limited, the use of other genotyping methodologies for future genetic selection programs in the region should be suggested. These techniques should be accurate, economical, fast, easy to apply, and less invasive. Vigolo *et al.* (2022) recommend the use of automated dairy matrix DNA extraction combined with Amplification refractory mutation system PCR for large-scale *CSN2* gene genotyping as a rapid and inexpensive alternative. Likewise, Albiero *et al.* (2024) conducted a comparative study of various methods for the determination of A2A2 animals, where the use of the commercial lateral flow immunoassay (2-MiLK TEST® from Scienco Biotech) is highlighted, which is capable of detecting A2A2 genotypes with a sensitivity and specificity of 100%. Therefore, this technique is very useful for its application in rural contexts because of its speed and simplicity.

Conclusion

Genotypic identification of the β-casein gene is crucial as it allows targeting inseminations and matings to increase the frequency of the A2A2 genotype while reducing the presence of the less favorable genotypes (A1A1 and A1A2). This would facilitate the exclusive production of A2 milk, which is already marketed in many countries and represents an excellent opportunity to improve local milk production. It would also position Cajamarca as a pioneer in A2 milk production in Peru. In conclusion, the PCR-RFLP technique was used to determine the genotypes of the β-casein gene, finding that the Simmental breed and its crosses have a higher frequency of the A2A2 genotype and that the provinces of Cajamarca and Celendín could be potential A2 milk producers.

Acknowledgments

The authors would like to acknowledge the Proyecto de Mejoramiento Genético Nacional-PROMEG CUI 2432072 funded this research.

Conflict of interest

The authors declare no conflicts of interest.

Funding

This study received partial funding from "Proyecto de Mejoramiento Genético Nacional-PROMEG CUI 2432072".

Authors' contributions

Conceptualization by the MCG and MRJ ALBQ and ATS methodology Internal research on VTC and JBA Resources CQP and WAG Formal analysis of MCG and MCR Visualization WAG. Funding; Acquisition; Project supervision; Project administration CQP. All authors have read and accepted the revised version of the manuscript.

Data availability

Data supporting the results and conclusions of this research are available upon reasonable request from the corresponding author, MCG, MCR, ATS, and ALBQ.

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