

Polymorphism Of Three Milk Protein Genes in Mexican Jersey Cattle

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ABSTRACT: The main task was to estimate the allelic and genotypic frequencies, genetic diversity and polymorphic information content for the β -casein, κ -casein and β -lactoglobulin genes. Blood and frozen semen samples were collected of 453 Jersey individuals registered by the Mexican Jersey Cattle Association. 28 breed specific SNP primers for whole genes were used. The B allele of κ -casein had higher frequency (0.80) than the A (0.15) and E (0.05). For β -lactoglobulin, the highest frequency was for B (0.76), followed by A and C alleles (0.22 and 0.02, respectively). The β -casein allele with higher frequency was A² (0.62), followed by A¹ (0.18), A³ (0.09) and B (0.11). The average genetic diversity (H_e) was 0.424, with a locus effective allele number of 0.5. These results indicated a high allelic diversity of κ -casein, β -casein and β -lactoglobulin in Mexican Jersey cattle.

Keywords: κ -casein; β -casein; β -lactoglobulin; genetic polymorphism

Introduction

Frequencies for α S1-casein, α S2-casein, β -casein, κ -casein, α -lactalbumin and β -lactoglobulin have been reported for several dairy cattle populations. It is important to detect the genetic polymorphism for these genes since they determine cheese yield, rennet time, and curd firmness (Gibson and Rozzi (1990); Wedholm et al. (2006); Jōudo et al. (2008)). The objectives of this study were to determine the genotypic frequencies for β -casein, κ -casein, and β -lactoglobulin; to estimate the allelic frequencies, the genetic diversity and polymorphic information in Mexican Jersey cattle.

Materials and Methods

Samples for DNA extraction. Samples were collected from 453 Jersey individuals registered by the Mexican Jersey Association, and originated from Canadian, U.S., New Zealand, Australian and Mexican progenitors. Sampled cows had at least a calf, meanwhile sires needed to have at least two calves in different herds. DNA samples from cows were obtained from blood; frozen semen was used to obtain the DNA in sires.

Selection of SNP primers. Primers used to genotype cows/sires were designed using OligoAnalyzer 3.1® (Integrated DNA Technologies, 2012, Iowa, USA), reported in GenBank (NCBI, 2012), corresponding to the genes for each milk protein. Primers were synthesized by KBiosci-

ence (Massachusetts, USA). The changing nucleotides were marked with a different fluorophore at the SNP position to distinguish each one during the allele identification by real time PCR. The reverse sequences and their complements, the coefficients of hairpins formation, autodimerization and creation of heterodimers, the percentage of each nucleotide, and the fusion point for each sequence, necessary to design the thermo-cycling program, were determined with the same software.

Molecular validation of SNP primers. Molecular validation started by amplifying the previously designed primers to corroborate the *in silico* performance. With the SNP primers that amplified well, the resolution curve was verified to guarantee the correct measure of the sample. Total SNP primers for whole genes were 28. The blue sigmoid curves shown in Figure 1, are the selected SNP primers to genotype the DNA samples. The polymorphisms were identified with the UV-Vis of rtPCR thermal-cycler. The different fluorescence of SNP primers was used to detect the changing nucleotides.

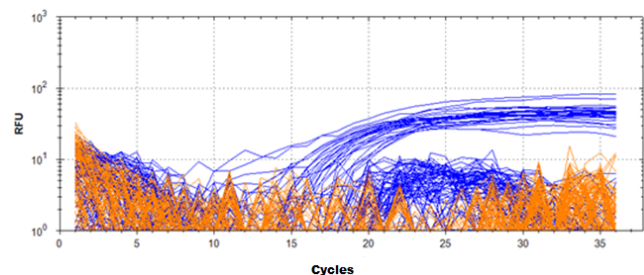


Figure 1. Molecular validation for SNP primers to genotype Jersey cattle.

RFU: relative fluorescence unit.

Cycles: DNA amplification cycles in the PCR.

Data analysis. Polymorphic amplicons were considered to estimate the allelic diversity and effective number of alleles. Allelic and genotypic frequencies were estimated using the software Popgen32 (Yeh et al. (1999)).

Results and Discussion

Genotyping for the three milk proteins. The results for the genotyping of β -casein, κ -casein and β -lactoglobulin are presented in Tables 1 to 6.

Table 1. Allelic frequencies for β -casein gene in Mexican Jersey cattle.

Alleles	Frequency		
	Sires	Dams	Total
A ¹	0.12	0.22	0.19
A ²	0.86	0.69	0.71
A ³	0.01	0.04	0.05
B	0.01	0.04	0.04
C	N/P	0.01	0.01

N/P: allele not present.

Table 2. Genotypic frequencies for β -casein gene in Mexican Jersey cattle.

Genotypes	Frequency		
	Sires	Dams	Total
A ¹ A ¹	0.02	0.06	0.04
A ¹ A ²	0.20	0.30	0.29
A ¹ A ³	N/P	0.01	0.01
A ¹ B	N/P	0.01	0.01
A ² A ²	0.73	0.50	0.53
A ² A ³	0.03	0.03	0.03
A ² B	0.02	0.04	0.04
A ² C	N/P	0.01	0.01
A ³ A ³	N/P	0.02	0.02
BB	N/P	0.01	0.01

N/P: genotype not present.

Table 3. Allelic frequencies for κ -casein gene in Mexican Jersey cattle.

Alleles	Frequency		
	Sires	Dams	Total
A	0.13	0.27	0.26
B	0.83	0.67	0.69
E	0.04	0.06	0.05

Table 4. Genotypic frequencies for κ -casein gene in Mexican Jersey cattle.

Genotypes	Frequency		
	Sires	Dams	Total
AA	0.04	0.04	0.04
AB	0.18	0.45	0.42
AE	N/P	0.02	0.02
BB	0.70	0.42	0.45
BE	0.08	0.04	0.05
EE	N/P	0.02	0.02

N/P: genotype not present.

Table 5. Allelic frequencies for β -lactoglobulin gene in Mexican Jersey cattle.

Alleles	Frequency		
	Sires	Dams	Total
A	0.13	0.28	0.26
B	0.82	0.71	0.72
C	0.05	0.01	0.02

Table 6. Genotypic frequencies for β -lactoglobulin gene in Mexican Jersey cattle.

Genotypes	Frequency		
	Sires	Dams	Total
AA	0.04	0.10	0.09
AB	0.18	0.37	0.34
BB	0.72	0.51	0.54
BC	0.04	0.02	0.02
CC	0.02	N/P	0.01

N/P: genotype not present

β -casein. The alleles identified were A¹, A², A³, and B; A² was the most common (0.71). The frequency for this allele is similar to those reported (0.58 to 0.65) for other Jersey populations (Bech et al. (1990); Winkelman and Wickham (1997); Miciński et al. (2007)). This result suggests an absence of genetic selection in Jersey cattle, including the Mexican population. Some researchers have shown that presence of the A² allele in dairy cattle produces high quality milk associated with diminished cholesterol and triglycerides in humans (Tailford et al. (2003); Kamiński et al. (2007)). The fact that A² allele and A²A² genotype were high in the Mexican Jersey herds is an important and distinctive aspect of this breed that could be used to improve the profit margin for the milk producers.

κ -casein. The alleles detected were A, B, and E; B was the most common (0.69). The frequency for this allele is similar to those reported (0.71 to 0.89) for Colombian, German, and Chinese Jersey populations (Trujillo et al. (2000); Jann et al. (2004); Ren et al. (2011)). Some researchers have shown that the presence of B allele in dairy cattle improves yield and quality of the milk, raises milk casein fraction and diminishes whey protein fraction (Wedholm et al. (2006); Jōudo et al. (2008)). These results suggest an indirect genetic selection for the B allele in Jersey cattle, including the Mexican population, because the κ -casein alleles are related to low total solids production, as A (0.26) and E (0.05) alleles had decreased over time. The fact that the B allele and the BB genotype were high in the Mexican Jersey herds is an important aspect of this breed that could be used to improve the profit margin for the producer of milk derivate products.

β -lactoglobulin. The alleles present in the sampled cows were A, B, and C; B was the most common (0.72). The B allele frequency estimated was higher than those reported for other dairy cattle populations. Some authors (Miciński et al. (2007); Meza-Nieto et al. (2010); Ren et al. (2011)) genotyped the β -lactoglobulin loci in Holstein, Braunvieh, Jersey, and Milking Tropical Creole populations, estimating allelic frequencies for the B allele from 0.31 to 0.65. The B allele of the β -lactoglobulin gene improves the production of milk total solids; and the differences between the dairy cattle populations frequencies around the world, suggest that its selection is related with the B allele in the κ -casein gene in dairy cattle.

Genetic diversity. The analyzed loci were 100% polymorphic, similar to the findings for Colombian Creole, Brahman, Holstein, Braunvieh, and Tropical Milking Creole populations (Cervantes et al. (2007); Rosero et al. (2009)). These results suggest that the loci frequency is constant regardless of breed or origin of the population. The most polymorphic gene was β -casein with five alleles. This result is similar to those reported for Colombian Brahman (Rosero et al. (2009)). The following more polymorphic genes were κ -casein and β -lactoglobulin with three alleles. When comparison for the average observed alleles with the average effective alleles through the three loci was made, it was observed 30% less effective alleles than those that were observed (Figure 2). This suggests that there is not Hardy–Weinberg equilibrium at any loci.

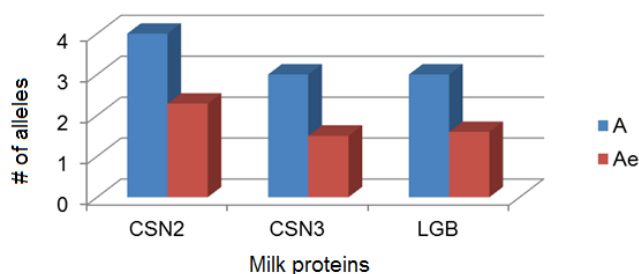


Figure 2. Observed alleles (A) and effective alleles (Ae) for three milk protein genes in the Mexican Jersey population.

Heterozygosity and Hardy-Weinberg Equilibrium. According to Table 7, the studied population showed up differences between observed (H_o) and the expected (H_e) heterozygosity. These results are similar to those reported for Turkish dairy cattle populations (Dinç (2009)). This indicates that H_o doubled H_e in breeds where reproductive management and genetic improvement programs have been applied. The difference between the H_o and H_e population and the X^2 value ($P < 0.05$), confirmed the absence of Hardy-Weinberg equilibrium at the three loci. A similar result was reported by other authors for Mexican and foreign populations of Holstein, Brahman, Braunvieh and Jersey (Hernández et al. (2006); Rosero et al. (2009); Ren et al. (2011)).

Table 7. Estimated values of observed heterozygosity (H_o) and expected heterozygosity (H_e) for the loci CSN2, CSN3, and LGB in the Mexican Jersey population.

Locus	H_o	H_e	X^2
CSN2	0.60	0.46	0.033*
CSN3	0.50	0.45	
LGB	0.50	0.41	
Population	0.53	0.44	

* X^2 test P value. (P value < 0.05 , not consistent with Hardy Weinberg Equilibrium).

Conclusion

The sampled Mexican Jersey population has an extensive genetic pool. The most common alleles were A2 for β -casein, and B for both κ -casein and β -lactoglobulin. The Mexican Jersey population has the potential to be used in extensive selection and genetic improvement programs, aimed to improve the milk quality traits of economic importance.

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