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Polymorphism of three milk protein genes in Mexican Jersey cattle

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ABSTRACT

The objective 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 from 453 Jersey individuals registered by the Mexican Jersey Cattle Association. Twenty eight breed specific SNP primers for whole genes were used. The B allele of κ-casein had higher frequency (0.69) than the A (0.26) and E (0.05). For β-lactoglobulin, the highest frequency was for B (0.72), followed by A and C alleles (0.26 and 0.02, respectively). The β-casein allele with the highest frequency was A^2 (0.71), followed by A^1 (0.19), A^3 (0.05), B (0.04) and C (0.01). The average genetic diversity (He) was 0.53. The average locus effective allele number was 1.79. These results indicate a high allelic diversity for κ-caseín, β-casein and β-lactoglobulin that could be included in breeding programs in the population studied, aimed to improve the milk quality traits of economic importance.

1. Introduction

Mexican dairy cattle populations have been developed from imported genetic resources worldwide, incorporating a wide base of milk protein genes, including αS1-casein, αS2-casein, β-casein, κ-casein, α-lactoalbumin and β-lactoglobulin. Particularly, the Jersey cattle populations have been constituted by American, Canadian [1], European, Australian and New Zealander progenitors. Jersey populations of these countries have been reported with variable gene frequencies for milk protein genes [2,3,4].

The importance to detect the genetic polymorphism for milk protein genes in dairy cattle populations is their association with cheese yield, rennet time, and curd firmness [3,5,6]. Besides, most of the available technologies have breed specific developments; therefore, when this technology is applied on breeds different than the one used as a model, imprecisions at the presence of genetic marker level from 8.3 to 54.9% might appear [7,8]. Molecular technologies have been developed to detect alleles and frequencies within protein milk genes, including specific PCR sequences, restriction enzymes, and actually single nucleotide polymorphism [9,10,11]. The development of breed specific SNPs is necessary for genotyping and association mapping to milk traits. The objectives were to determine the allelic and genotypic frequencies, genetic diversity and polymorphic information for β-casein, κ-casein, and β-lactoglobulin in Mexican Jersey cattle populations.

2. Materials and methods

2.1. Samples for DNA extraction

Samples were collected from 453 Jersey individuals registered by the Mexican Jersey Cattle Association, originated from Canadian, U.S., New Zealand, Australian and Mexican progenitors, including 401 cows and 52 sires. Sampled cows had at least a calf, whereas sires needed to have at least two calves in different herds. DNA samples from cows were obtained from blood and frozen semen was used to obtain the DNA in sires.

2.2. Selection of SNP primers

Primers used to genotype individuals were designed using OligoAnalyzer 3.1® (Integrated DNA Technologies, 2012, Iowa, USA), corresponding to the exons that represent the open reading frame of β-casein (β-CSN-encoding gene CSN2), κ-casein (κ-CSN-encoding gene CSN3), and β-lactoglobulin (β-LGB-encoding gene LGB) milk proteins reported in GenBank (NC_007304.4, http://www.ncbi.nlm.nih.gov/genbank; [12]). 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 hairpin formation, autodimerization and creation of heterodimers, the

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percentage of each nucleotide, and the fusion point for each sequence, necessary to design the thermo-cycling protocol, were determined with the same software.

Total SNP primers for whole genes were 28, including 8 primer sequences for β-lactoglobulin, 10 for κ-casein and 10 for β-casein. Primers were synthesized by KBioscience (Massachusetts, USA).

Molecular validation was done 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.

2.3. Genotyping of DNA

The PCR amplification was done with 4.11 μL of reaction solution (KASPar V4.0 Master Mix, KBioscience, USA) and 20 ng of genomic DNA; for the negative template control, 4 μL of molecular grade water was used. The polymorphisms were identified with the UV–Vis of the rtPCR thermo cycler (CFX 96, Bio-Rad, California, USA). Different fluorescence of SNP primers were used to detect the changing nucleotides. The thermocycling protocol was: one cycle at 94°C for 15 min, 10 cycles at 94°C for 20 s and 65°C for a min, with a decrease of 0.8°C per cycle in the second step, and 35 cycles at 94°C for 20 s, followed by a cycle at 57°C for 20 s, including the fluorescence report for each cycle in the last step. With the 28 validated SNPs, it was possible to identify the most common alleles for the three milk proteins. This increased the probability of detection of those alleles previously reported as being of low frequency in *Bos taurus* populations [13].

2.4. 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 [14].

3. Results and discussion

3.1. Genotyping for the three milk proteins

Data of haplotype and genotype frequencies of three protein milk genes in 453 individuals representing the Mexican Jersey cattle gene pool, are reported.

3.1.1. β-Casein

The alleles identified were A^1, A^2, A^3, B and C (Table 1); A^2 was the most frequent in the Mexican Jersey populations, 0.71. The frequency for this allele was similar to those reported, 0.58 to 0.65, for other Jersey populations [15,16,17]. This result suggests an absence of genetic selection for β-casein in Jersey cattle, including the Mexican populations. Some researchers have shown that the presence of the A^2 allele in dairy cattle produces high quality milk associated with diminished cholesterol and triglycerides in humans [18,19]. The fact that A^2 allele and A^2A^2 genotype were high in the Mexican Jersey populations is an important and distinctive aspect of this breed that could be used to improve the margin of profit for the milk producers. The frequency for the A^1 allele in this study, 0.19, was similar to that reported by Van Eenennaam and Medrano [2] in a US Brown Swiss population, 0.18, which also is close to the frequencies for this allele found in Guernsey and Jersey, breeds with high total solids in milk [16,20]. This is in contrast to the frequencies of the A^1 allele reported for Holstein populations, 0.49 to 0.95 [21,22].

The frequency of the A^3 allele was 0.05, similar to that reported by Ng-Kwai-Hang and Grosclaude [23], who found a frequency for this allele in US Holstein-Friesian and Brown Swiss of 0.04. Meanwhile, the B allele had low frequencies in the Jersey populations, 0.04, similar to the frequencies reported for Jersey in some American and German populations, as well as for American Brown Swiss [2,20,24]. The low allele frequencies of A^3 and B in this study, and the results reported by different authors in dairy breeds, suggest that these polymorphisms have little importance for breed differences in dairy cattle.

The most common genotype for β-casein was A^2A^2, 0.53. These results differ from those reported by Çardak [25] in a Turkish Holstein-Friesian population where the most frequent genotype was A^1A^2, 0.46. Furthermore, in this study the genotypes A^1A^3, A^1B, and A^2C were found, with frequencies lower than 0.05, which are similar to those reported by different authors, from 0.01 to 0.06, in US, New Zealand and Denmark Holstein, Brown Swiss and Ayrshire populations [16,20]. The genotypic frequencies found in the whole population were similar to those estimated only in females (Table 1). However, those corresponding to the male sub-population were different, with some absent genotypes (A^2A^3, A^1B, A^2C, A^3A^3, and BB). Hanusová et al. [26] found in Polish Holstein that one of the genotypes did not occur in the male subpopulation, even if it was present in the whole population. The absence of some alleles and genotypes in the male sub-population of this study might be a consequence of the reduced number of sampled individuals (n = 51 sires), even if the origin of sires is diverse.

3.1.2. κ-Casein

The alleles detected were A, B, and E (Table 2); B was the most common, 0.69. These findings were similar to the results reported for Colombian, German, and Chinese Jersey populations, 0.71 to 0.89 [11,27,28]. Some authors have shown that the presence of B allele in dairy cattle improves yield and quality of milk, raises milk casein fraction and diminishes whey protein fraction [3,6]. These results suggest an indirect genetic selection for the B allele in Jersey cattle, including the population studied, because the κ-casein alleles related to low total solid production, A (0.26) and E (0.05), are in lower frequencies than B allele. The frequency for the A allele in this population studied was similar to those reported by Trujillo et al. [27] and Ren et al. [11] in Jersey populations in Colombia and China, 0.26 in both populations, as well as in studies with Normande and Guernsey populations [27]. On the contrary, in Holstein there have been reported high frequencies, 0.68 to 0.89, for the A allele in several populations [22,29].

Jann et al. [28] and Boetcher et al. [30] reported in Holstein population frequencies for the E allele of 0.08 and 0.32, respectively. The frequencies for the A and E alleles in the Mexican Jersey populations, could be explained by the objective of selection over time for this breed, total solid production, common among most of the Mexican Jersey based milk producers. This is because these alleles influence positively milk production and negatively total solids in milk, and were indirectly selected against the genetic pool in Jersey.

Table 1
Allelic and genotypic frequencies of β-casein genes in the Mexican Jersey cattle populations.

	n	Allele frequencies					Genotype frequencies									
		A1	A2	A3	B	C	A1A1	A1A2	A1A3	A1B	A2A2	A2A3	A2B	A2C	A3A3	BB
Cows	401	0.22	0.69	0.04	0.04	0.01	0.06	0.30	0.01	0.01	0.50	0.03	0.04	0.01	0.02	0.01
Sires	52	0.12	0.86	0.01	0.01	N/P	0.02	0.20	N/P	N/P	0.73	0.03	0.02	N/P	N/P	N/P
Total	453	0.19	0.71	0.05	0.04	0.01	0.04	0.29	0.01	0.01	0.53	0.03	0.04	0.01	0.02	0.01