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POLYMORPHISMS IN BETA AND KAPPA-CASEIN ARE NOT ASSOCIATED WITH MILK PRODUCTION IN TWO HIGHLY TECHNIFIED POPULATIONS OF HOLSTEIN CATTLE IN MEXICO

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ABSTRACT

The purpose of this study was to identify genotype variants in the beta-casein (CSN2) and kappa-case (CSN3) genes using the PCR-RFLP method in a population of 202 Holstein cattle from two highly technified farms in the state of Jalisco, Mexico. We further assessed the association of these variants with milk production during a second lactation period. In the first population (n = 102), the genotypic frequencies for CSN2 were A1 = 0.387 and A2 = 0.613, while for CSN3, they were A = 0.829 and B = 0.172. In the second population (n = 100), the genotypic frequencies for CSN2 were A1 = 0.430 and A2 = 0.570, while for CSN3, they were A = 0.795 and B = 0.205. No differences in frequency distribution were noted in either population, and both genes were in Hardy-Weinberg equilibrium. There was greater genetic diversity for the CSN2 gene (0.477 and 0.493) than for the CSN3 gene (0.286 and 0.328) in both populations, with no significant differences between them. The fixation index was low for both genes, suggesting that there was no significant decrease in heterozygosity. The same genetic changes occur in both genes, and give their high similarity index (0.99 and 0.99). The statistical association analysis between genotypes of both genes and milk production revealed there were no significant differences between populations. Furthermore, no significant additive or dominant effects of both casein genes on milk production were identified within our population samples.

Key words: beta-casein (CSN2), kappa-casein (CSN3), Milk production.

INTRODUCTION

Milk production is a quantitative trait that results from the interaction between genotype and environmental factors. Improvements in milk production depend on the implementation of a good animal selection program and appropriate environmental conditions (Eyduran *et al.*, 2013). The main goal of cattle farming is to optimize milk production, and selection and reproduction of animals with the most desirable genotypes serve as the basis for any genetic improvement process. Till now, several methods had been used for genotyping polymorphisms in milk proteins (Seibert *et al.*, 1985; Guy and Fenaille, 2006; Caroli *et al.*, 2009; Le *et al.*, 2012). However, the most commonly used technique is the polymerase chain reaction coupled to restriction fragment length polymorphisms (PCR-RFLP) (Hristov *et al.*, 2012).

Bovine milk contains four different types of caseins: alpha s1, alpha s2, beta, and kappa. These variants are expressed by autosomal co-dominant genes with multiple allelic variants, and all located on chromosome 6 (Çardak, 2005). The study about the influence of milk protein polymorphisms on milk production will better understand the genetic components of milk. These polymorphisms also offer an alternative for improving milk production (Vallas *et al.*, 2012). The polymorphisms of the CSN2 gene (alleles A1 and A2) and CSN3 gene (alleles A and B) have effect on milk production in previous studies (Freyer *et al.*, 1999; Rachagani and Dayal-Gupta, 2008).

Recently, several countries have become interested in developing genetic improvement strategies, with the goal of improving production traits. These strategies involve understanding the role of particular milk protein genes in each population of milk-producing cattle and incorporating genotyping as part of animal selection programs (Riaz *et al.*, 2011). Kamiński *et al.* (2007) mentioned that several reports regarding milk protein genes and their relationship to milk production parameters have been conducted in different parts of the world. However, information regarding casein genotypes in Mexican cattle is scarce. Kappa-casein has been considered as a molecular marker but has not been studied in relation to milk production (Cervantes *et al.*, 2007; Cortés-López *et al.*, 2012). According to the 2011

United Nations Food and Agriculture Organization (FAO) report regarding milk production, Mexico is the fifth largest milk producer in countries of Latin America and the Caribbean. Nonetheless, it is also one of the countries with the largest milk production deficit worldwide and the largest dairy product importer in the region. This situation highlights the need to assess the impact of genetic polymorphism markers in beta- and kappa-casein and their influence on milk production within certain country populations. The purpose of this study was to identify the A1 and A2 allele variants of beta-casein and the A and B alleles of kappa-casein using the molecular technique of PCR-RFLP. Furthermore, their effect on milk production was also assessed in two herds of Holstein cows in the state of Jalisco, Mexico.

MATERIALS AND METHODS

Animals and production data collection: For this study, a total of 202 Holstein cows that had undergone two or more lactation periods were included. These cows belonged to two technified milk-producing holdings: 102 cows from population 1 (P1) and 100 cows from population 2 (P2). Production records for the second lactation period, reported as kilograms of milk per cow and adjusted to 305 days, were obtained using the dairy farm software DairyCOMP 305® (Steve Eicker & Connor Jameson, Tulare CA, United States).

Genomic DNA extraction and genotyping: Genomic DNA was extracted from blood samples containing EDTA as an anticoagulant, following the protocol by Ayala-Valdovinos *et al.* (2000). Genotyping was conducted using PCR-RFLP primers and following the methods described by Miluchova *et al.* (2009) and Barroso *et al.* (1997) for beta and kappa-casein, respectively.

Statistical analysis: POPGENE© software version 1.32 (POPGENE, 1997) was used for the analysis of the following parameters: genetic and genotypic frequencies, Nei's observed heterozygosity, total heterozygosity, fixation index (Fis), and genetic distances between populations.

SPSS software version 17 (SPSS, 2008) was used for the association analysis between genotypes of the beta and kappa-casein genes and milk production. The analysis was conducted using a design containing three fixed effects and one random effect and took the following mixed statistical analysis into consideration:

$Y_{ijkl} = \mu + pop_i + (Beta\ Genotype)_j + (Kappa\ Genotype)_k + animal_l + e_{ijkl}$

Where:

"Y" is milk production during the second lactation period per cow, adjusted to two milkings and 305 days. "μ" is the general milk production mean. "pop" represents both populations (P1 and P2), $_i = 2$. "Beta Genotype" refers to the possible beta-casein genotypes for each animal (A1A1, A1A2, and A2A2), $_J = 3$. "Kappa Genotype" refers to the possible kappa-casein genotypes for each animal (AA, AB, and BB), $_k = 3$. "Animal" refers to the total random genetic component of each animal. $_l = 202$. "e" is the random error of each measurement.

Additivity and dominance: The additivity and dominance effects were calculated using the methodologies described by Falconer and Mackay (1996) and Knott *et al.* (1998). The additive effect was calculated as half of the difference between the homozygote genotypes (AA - aa)/2, while the dominant effect was calculated as the deviation of the heterozygote genotype from the homozygote genotype (Aa- 0.5 [AA + aa]). Afterwards, a regression analysis was conducted for each gene in each population using a fixed effects model that included the additive and dominant effects. The average values for milk production (in kilograms) during the second lactation period were adjusted to 305 days. To calculate the additive effect, one value was assigned to each genotype: 1, 0, and -1 for CSN2 A2A2, A2A1, and A1A1 or for CSN3 BB, AB, and AA. To calculate the dominant effect, the following values were assigned: 0, 1, and 0 for CSN2 A2A2, A2A1, and A1A1 or CSN3 BB, AB, and AA.

RESULTS AND DISCUSSION

Casein polymorphisms and population frequencies: The use of the PCR-RFLP technique for the identification of beta- and kappa-casein polymorphisms allowed for fast and accurate genotype assessments in the analysed animals. The genotype distributions for the CSN2 and CSN3 genes are shown in Table 1. The results indicated that the polymorphisms in both genes were similar within the populations (X^2, $\alpha = 0.38$). For beta-casein, the most frequent genotype in both populations was the heterozygous genotype, with no differences between them (X^2 $P > 0.05$). For kappa-casein, the AA homozygous genotype was the most frequent in both populations, also with no differences between them (X^2 $P>0.05$). Hallen *et al.* (2011) reported similar genotypic frequencies, where A1A2 (beta-casein) and AA (kappa-casein) were the most frequent genotypes in the red Swiss and Holstein Swiss cattle breeds. Rachagani and Dayal-Gupta (2008) obtained a similar frequency pattern for kappa-casein (AA>AB>BB) in Indian milk-producing cattle breeds.

Despite the differences in the number of animals of each genotype, there were no significant differences ($P>0.05$) between the observed and expected Hardy-Weinberg frequencies for both beta and kappa-casein in either population. In milk-producing cattle, when milk protein genes are in genetic equilibrium, it is an indicator of the absence of selection processes (Gouda *et al.*,

2013). This situation is confirmed in the present study, where, in both cattle populations, molecular markers for milk production and casein haplotypes are not considered as animal selection criteria.

Regarding genotypic frequencies, the A2 allele of beta-casein was the most frequent allele in both populations. This finding is in agreement with previous studies by Keating *et al.* (2008), Värv *et al.* (2008), Hassan *et al.* (2010), Hallen *et al.* (2011), and Molee *et al.* (2011). However, it is opposite to the findings of Szymanowska *et al.* (2004), Miluchova *et al.* (2009), and Hanusová *et al.* (2010); the latter group of studies reported that the A1 allele was the most frequent allele. For kappa-casein, the A allele was the most frequent allele in both populations. This finding is in agreement with previous studies by Biase *et al.* (2005), Rachagani and Dayal-Gupta, (2008), Värv *et al.* (2008), Garcia-Botaro *et al.* (2011), Riaz *et al.* (2011), Hallen *et al.* (2011), Molee *et al.* (2011), and Gouda *et al.* (2013). Meanwhile, few studies report a greater frequency of the B allele, but these studies were conducted in breeds that were unrelated to the Holstein breed and were instead more directly related to the *Bos indicus* breed (Rohallah *et al.*, 2005; Pacheco-Contreras *et al.*, 2011). Up until this study, there had not been any selection process favouring either variant of beta or kappa-casein in either population. Therefore, the variations in allelic frequencies described in this study could arise from the association between genotypic variants and classic selection criteria, applied either within each development or by genetic drift (Heck *et al.* 2009).

Diversity and genetic distances: The detailed genetic analysis for each gene was conducted independently and shown in Table 2. Similar heterozygosity values were noted in both populations (P>0.05). Overall, there was greater heterozygosity for the CSN2 gene compared to the CSN3 gene, which was caused by the decreased genotypic frequency of the B allele of kappa-casein. The observed heterozygosity indicates that the proportion of heterozygous individuals is approximately one-half for the beta-casein gene and approximately one-fourth for the kappa-casein gene. Värv *et al.* (2008) obtained heterozygosity frequencies similar to the results measured in this study (i.e., 0.522 for the CSN2 gene and 0.237 for the CSN3 gene). When both casein genes are compared, Nei's expected heterozygosity and the total heterozygosity show that there is greater genetic diversity for CSN2 than for CSN3. This difference could be due to the genotypes of the bulls provided by the genetic companies supplying the semen that is used in these farming developments, which could have greater genotype diversity for the beta-casein gene than for the kappa-casein gene. However, the genetic diversity is similar for both genes in both populations, despite the fact that semen is provided by a greater number of genetic companies in population 2 compared to population 1. The fixation index indicates the degree to which heterozygosity decreases. In this study, the fixation index for the CSN2 gene was larger in population 1 than in population 2, and on the contrary result was found for the CSN3 gene. In contrast, Värv *et al.* (2008) reported very low and negative fixation indices in Holstein cattle for beta and kappa-casein: -0.003 and -0.004, respectively. This disparity is likely due to the continuous genetic flux found in the Holstein breed worldwide.

The genetic distances were calculated from the genotype data for each gene. Both populations were genetically very close for both casein genes. The similarity/genetic distances between populations were 0.9995/0.0005 for CSN2 and 0.9978/0.0022 for CSN3.

Effect of casein loci on milk production: The average milk production adjusted to 305 days is shown by genotype in Table 3. Statistical analysis revealed no significant effect (P>0.05) of the presence or absence of a particular genotype on milk production for neither beta nor kappa-casein. Previous studies described diverse results regarding the relationship between genotype and milk production. The results achieved by Sabour *et al.* (1996) and Hanusova *et al.* (2010) are in accordance with this study, with no significant differences identified between CSN2 genotypes and milk production. Molee *et al.* (2011) reported similar results: neither genotype of beta or kappa-casein had a favourable effect on milk production, with the exception of the A1A2 beta-casein genotype. Heck *et al.* (2009) found that the A1 allele led to decreased milk production. For kappa-casein, Çardak (2005) reported that AA homozygous Holstein cows produced the highest milk yield. Similarly, Matějíček *et al.* (2007) showed that the presence of the A allele in the kappa-casein genotypes led to increased milk production, as opposed to the B allele, which was associated with increased protein and solids concentrations in milk. In contrast, Rachagani and Dayal-Gupta (2008) found that the BB genotype had the most influence on milk production. Ikonen *et al.* (2001) reported that, in milk cows from a first calving, the A2 beta-casein haplotypes in combination with either kappa-casein allele (A or B) were associated with increased milk production and lower fat content.

The discrepancies between studies regarding which beta and kappa-casein genotypes are more conducive to increasing milk production could be explained by different genetic and environmental factors. The close linkage between casein gene loci could cause compound effects between genotypes or haplotypes and milk production (Çardak, 2005). Furthermore, the genetic makeup of each population and breed can significantly influence the effect of the casein genes on milk production, leading to different effects for the same haplotype depending on the population, as concluded by

Molee *et al.* (2011) and Värv *et al.* (2008). The differences and variations in the results from different studies could also be due to limited data, either because of the number of animals studied or an inadequate data collection methodology (Sabour *et al.*, 1996).

Additivity and dominance effects: Table 4 shows the additivity and dominance effects of beta and kappa-casein on milk production during the second lactation period, adjusted to 305 days. For both populations, the global additive effect of CSN2 was positive (172.22 and 597.86 kilograms), while the CSN3 global additive effect was negative (-841.47 and -167.00 kilograms). Additivity reflects the individual effects of an allele for a certain characteristic (Vázquez-Flores *et al.*, 2012). For the CSN2 gene, although positive additive values on milk production were detected for the A1 allele compared to the A2 allele, these increases represented less than 5% of total milk production during the lactation period. For the CSN3 gene, the additive effects of the A allele on the B allele were negative, with a low effect within total milk production (P>0.05). According to Kuehn *et al.* (2007), the deviation of heterozygous effects from the mid-point of the two homozygous genotypes is indicative of dominance. In the present study, the dominance effects were negative and similar for both genes in both populations (CSN2: -57.37 kg and -983.15 kg; CSN3: -1904.53 kg and -300.00 kg). This similarity indicated that there was no trend favouring a dominant allele.

Applying a regression model, similar estimates of allele effects were found for both beta and kappa-casein (P>0.05). This similarity indicated that there were no significant additivity or dominance effects of the CSN2 and CSN3 casein genes on milk production within the two populations.

Table 1. Number of animals and genotypic frequencies of CSN2 and CSN3

Gene	CSN2			CSN3			CSN2[1]		CSN3[2]	
Herd	A1A1	A1A2	A2A2	AA	AB	BB	A1	A2	A	B
Population 1	17	45	40	71	27	4	0.387	0.613	0.829	0.172
Population 2	17	52	31	65	29	6	0.430	0.570	0.795	0.205

[1]X^2, P=0.38; [2]X^2, P=0.38

Table 2. Genetic diversity of beta- and kappa-casein by population

Gene	CSN2		CSN3	
	Population 1	Population 2	Population 1	Population 2
Observed Heterozygosity	0.441	0.520	0.265	0.290
Nei's Expected Heterozygosity	0.475	0.490	0.284	0.326
Total heterozygosity	0.482	0.482	0.305	0.305
Fixation index (Fis)	0.070	-0.061	0.069	0.110
Genetic diversity (d)	0.477	0.493	0.286	0.328

Table 3. Average milk production in kilograms, adjusted to 305 days, per cow during the second lactation period relative to genotype and population

Population	A1A1	A1A2	A2A2	AA	AB	BB
Population 1	12651	12421	12306	12624	11561	14307
	(2473)	(2566)	(2917)	(2497)	(2910)	(3026)
Population 2	11391	9809	10195	10203	10070	10537
	(3232)	(2311)	(3487)	(3055)	(2665)	(2627)
Total	12039	11006	11371	11458	10788	12045
	(2892)	(2749)	(3330)	(3023)	(2859)	(3267)

( )= Standard deviation; P>0.05 between genotypes and populations.

Table 4. Additive and dominant effects in each population, expressed as kg of milk per 305-day lactation period

Gene	CSN2		CSN3	
	Additive Effect a = (A1A1 - A2A2)/2	Dominant Effect d = A1A2 - 0.5 (A1A1 + A2A2)	Additive Effect a = (AA - BB)/2	Dominant Effect d = AB - 0.5 (AA + BB)
Population 1	172.22	-57.37	-841.47	-1904.53
Population 2	597.86	-983.15	-167.00	-300.00
Total	334.00	-699.00	-293.50	-963.50
Regression Model (P>0.05)	Additivity (A2A2 effect)	Dominance (A1A2 effect)	Additivity (BB effect)	Dominance (AB effect)
	-240.48	-578.82	-197.99	-710.13

Conclusions: All of the polymorphisms for the CSN2 and CSN3 genes were identified using the PCR-RFLP technique in two Holstein cattle populations in Mexico. The genetic and genotypic frequencies of both casein genes were in equilibrium and were similar in both populations. Statistical analysis between genotypes and milk production, adjusted to 305 days, did not reveal any specific beta- or kappa-casein genotypes that had significantly favourable effects on milk production. The lack of significant additive effects of either casein gene on milk production is advantageous, given that either allele can be selected to improve other quantitative traits, including fat, protein, and/or solids content, without compromising milk production.

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