Ecosistemas y Recursos Agropecuarios

Frequency and association of polymorphisms in CSN3 gene with milk yield and composition in Saanen goats

Frecuencia y asociación de polimorfismos en gene CSN3 con producción y composición de la leche de cabras Saanen

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ABSTRACT. K-casein (CSN3) gene is of particular interest in goat production due to the effect of its polymorphisms on milk quality and composition. The objective of this study was to assess the genotypic and allelic frequencies in CSN3 gene in the Saanen goat breed and its effect on milk production and some of its compositional traits. Allelic discrimination assays were designed to determine the variants (A, B or C) using qPCR and allelic discrimination method. Allelic frequencies for A, B and C were 0.23, 0.64 and 0.13, respectively. Association analysis indicated that the CSN3 was significantly associated only with protein percentage ($p = 0.006$). Results indicated that animals with AC, BB and CC genotypes showed higher milk protein percentage than those with genotype AB. This evidence suggests that genotypes favorable to this associated trait could be included into Saanen goat breeding strategies.
Key words: Allelic discrimination, caprine, kappa casein, milk quality

RESUMEN. El gen de la κ-caseína (CSN3) es de especial interés debido al efecto de sus polimorfismos sobre la calidad y composición de la leche. El objetivo de este estudio fue investigar las frecuencias genotípicas y alélicas del gen CSN3 en la raza caprina Saanen y su efecto sobre la producción de leche y algunas de sus características de composición. Se diseñaron diferentes ensayos de discriminación alélica para determinar las variantes (A, B y C) mediante qPCR. Las frecuencias alélicas para las variantes A, B y C fueron de 0.23, 0.64 y 0.13, respectivamente. El análisis de asociación indicó que el gen CSN3 esta asociado solamente al porcentaje de proteína en leche ($p = 0.006$). Los resultados mostraron que los animales con los genotipos AC, BB y CC producen leche con mayor porcentaje de proteína que aquellos con el genotipo AB. Esta evidencia sugiere que los genotipos favorables para la característica asociada podrían incluirse en estrategias de selección asistida por marcadores en cabras de la raza Saanen.
Palabras clave: Discriminación alélica, caprinos, kappa caseína, calidad de la leche

INTRODUCTION

Milk is a liquid food of easy access with plenty of nutrients such as carbohydrates, proteins, minerals and vitamins (Drewnowski and Fulgoni 2008). In ruminants, the four caseins (αs1, β, αs2 and κ) represent about 80 % of milk proteins (Ramunno *et al.* 2004), and κ-casein accounts for around 15 % of total caseins. κ-casein is one of the most important proteins joined physically to the functional role in micelle formation and stabilization, determined by its size and function (Rijnkels *et al.* 2003). In addition, κ-casein is different to other caseins due to its solubility in a wide interval of concentration of calcium ions which contain a c-terminal hydrophilic region (Yahyaoui *et al.* 2001). One of the most important characteristics of κ-casein is its influence on the size of milk mi-

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celles, which are smaller, which has an impact on the coagulation features of the milk, reflected in a stronger curd and retention of a major amount of substances increasing cheese yield (Ahmed 2011).

In Mexico and other countries, goat milk is mainly used for cheese production and confectionery. The economic worth of this product depends mostly on the amount of total proteins and fats in the milk (Montaldo and Manfredi 2002). Therefore, the variable composition which determines cheese making depends on casein variability and hence the genetic variants that can affect the properties of this milk product (Martin *et al.* 2002, Barillet 2007).

A total of 14 genetic variants have been identified in the κ-casein (CSN3) gene in different breeds of domestic goats (Jann *et al.* 2004, Prinzenberg *et al.* 2005); furthermore, other studies have shown that the number of alleles identified in the domestic goat is actually 16 (Yahyaoui *et al.* 2001, Angiolillo *et al.* 2002, Yahyaoui *et al.* 2003, Jann *et al.* 2004). From these variants, 13 are protein variants and 3 are silent mutations, with a total of 15 polymorphic sites in the exon 4 of the CSN3 gene (Kumar *et al.* 2009).

Different studies (Chiatti 2007, Caravaca *et al.* 2009, Caravaca *et al.* 2010) have shown that some variants have a significant effect on the production and nutritional composition of the milk. In a study conducted by Caravaca *et al.* (2009) in the Murciano-Granadina breed, it was found that the goats with the B allele show higher values of protein and casein percentages than the ones with the A allele. Therefore, their identification in breeding populations is important and fundamental for the implementation of assisted selection strategies complementing traditional methods of genetic improvement. The Saanen is one of the most popular goat breeds in the world, due to its great performance in milk production and milk composition traits (Scheepers *et al.* 2010). In Mexico, it is one of the breeds with major inventory. The objective of the present study is to estimate the genotypic and allelic frequencies of CSN3 gene variants and to evaluate their association with milk yield and composition in Saanen breed animals.

MATERIALS AND METHODS

Sample source

Hair follicles of 130 Saanen breed animals were collected from La Pastorcita farm located in Apaseo el Grande, Guanajuato, located geographically at 20° 33' 01,81" North latitude, 100° 41' 19,80" West longitude and at an elevation of 1771 m above sea level. The zootechnical purpose of this farm is the production of breeding animals and milk. The origin of genetic material (i.e. semen) is mainly France, Canada and the United States, whereas stud animals are exclusively from the United States.

Goats were maintained under intensive conditions and feeding of animals was based on alfalfa hay, corn silage and a commercial concentrate with an 18 % level of raw protein. The procedure for the recording of productive indicators consisted of the collection of milk samples by a specialized technician every 28 days, in two lactation periods. A Lactoscan MCC (Milkotronic Ltd) was used to determine the components of protein, fat, lactose and total solids. The animals were maintained in a bodily condition of 3.5 to 4.5 (scale from 1 for thin goats to 5 for heavy goats). Data was revised and only animals with complete kidding date records from 2014 to 2015 were included in the study.

DNA extraction and identification of variants in the CSN3 gene

DNA extraction was achieved using the GenElute (Genelute Mammalian Genomic Miniprep, Sigma-Aldrich, Cat. G1N350). All samples were genotyped using specific probes and primers to discriminate genotypes of the A, B and C variants of the CSN3 gene were designed (Table 1). Primers and probes for a allelic discrimination assay were synthetized, using the previously reported sequences of the CSN3 gene variants (GenBank Accessions: AF485339, AF485340 and AF485341). Each assay was analyzed by 96-well optic plaques using Applied Biosystems 7500 Real Time PCR System (Applied

Table 1. Primer and probe sequence design for CSN3 genotyping by allelic discrimination.

Locus	Forward	Variation discrimination	Reverse	Probe
CSN3	GCCATTCCACCAAAGAAAGATCAG	A x B	GGCTCAGCACTAGCAATGGTATT	**VIC**- ATGGCAGGGATTTCTGTT **FAM**- TGGCAGGGACTTCTGTT
	TGTAGATAATCCAGAAGCTTCCTCAGA	A x B	CTTGGGCTGTGTTGGTCTCA	**VIC**- ATGCACTCGCAATCG **FAM**- ATGCACTCACAATCG

BiosystemsTM) under the following conditions: one cycle of 2 min at 50 °C and 10 min at 95 °C, followed by 40 two-step cycles of 15 s at 92 °C and 1 min at 60 °C. For the genotyping of each genotype, the 7500 Real-Time PCR Products software package, version 2.0.5 (Applied BiosystemsTM) was used; each sample was verified through visual analysis to avoid false allele assignations.

Statistical analysis

An analysis of association of the genotypes of the variants identified in the CSN3 gene was performed for milk production per day (MPD, kg), protein (PR), fat (F), lactose (LC) and total solids (TS) percentages, at the 69th day of lactation. A general linear model was fitted including the fixed effects of year, month, genotype and goat age as linear covariates, and random effects of sire and error. No interaction effects among fixed traits were observed and were excluded from a final model. Animals with AA genotype were excluded from the analysis since no productive data was available for them. Means comparison was performed using a Tukey-Kramer adjustment test by PDIFF statement. All analyses were computed using SAS software v. 9.0 (SAS Institute, Cary, NC, USA).

RESULTS

The overall means for MPD, PR, F, LC and TS were 3.34 kg, 2.74, 5.89, 4.38 and 12.08 %, respectively. The genotypic and allelic frequencies of CSN3 are presented in Table 2. Three variants in the studied population of Saanen goats were identified, with B allele being the most frequent (0.64), followed by A (0.23) and C (0.13). Five genotypes were identified, with the most frequent genotype being BB (0.46), followed by AB (0.35) and CC (0.11), while the other two genotypes were found in very low frequencies.

Table 2. Genotypic and allelic frequencies of Kappa casein gene CSN3 gene variants.

Genotypes					Allelic variants		
AA	AB	AC	BB	CC	A	B	C
0.04	0.35	0.04	0.46	0.11	0.23	0.64	0.13

In relation to the genotype effect on the production traits and milk composition, CSN3 only showed a significant effect on the PR of the Saanen goats (p = 0.006). Results showed that the individuals with AC, BB and CC genotypes produce more protein in their milk (Table 3). However, the AC genotype doesn't show significant differences with the AB genotype. The least square means showed that the animal carriers of AC, BB and CC genotypes have similar levels of protein in their milk.

DISCUSSION

The CSN3 gene is characterized by the presence of seven allelic variants, of which A, B and C have been reported in the Saanen breed, with the A and B variants being more frequent (Yahyaoui *et al.* 2003, Chessa *et al.* 2003). Yahyaoui *et al.* (2001), in a study conducted in Spain in the Saanen breed, found similar allelic frequencies, with a higher frequency for the B allelic variant. Previous studies have associated this variant with the protein and casein contents in milk, and the goats which have the B allele show higher values in the content of these nutrients compared to those of other variants (Caravaca *et al.* 2009). Similar results were reported by Kummar *et al.* (2009) and Scheepers *et al.* (2010), who, after evaluating polymorphisms of the CSN3 gene in different goat breeds of Asia, Africa and Europe, found an allelic frequency to the

Table 3. Least square means ± standard errors of milk yield and compositional traits in Saanen goats by kappa casein genotype effect.

Item	Genotypes			
	AB (n = 46)	AC (n = 5)	BB (n = 60)	CC (n = 14)
MPD (kg)	3.25 ±0.37	2.78 ±0.62	2.79 ±0.36	2.73 ±0.47
PP	2.79 ± 0.11^{b}	2.97 ± 0.19^{ab}	2.97 ± 0.11^{a}	3.16 ± 0.14^{a}
FP	2.65 ±8.09	3.76 ±13.17	5.74 ±7.69	3.96 ±10.05
LP	4.54 ±0.08	4.66 ±0.13	4.45 ±0.08	4.55 ±0.10
TS	13.51 ±0.99	14.25 ±1.61	13.16 ±0.94	13.05±1.22

MPD = Milk production per day; PP = Protein percentage, FP = Fat percentage, LP = Lactose percentage; TS = Total solids. ^{a,b} Means with different superscripts are significantly different ($p < 0.01$).

B allele of 0.66 and 1.00, respectively.

On the other hand, the A allele frequency was similar to that reported by Reale *et al.* (2005) a in research conducted in a Jónica goat population of southern Italy, with a frequency of 0.25. Similarly, higher frequencies of the A allele with 0.92, 0.94, 0.92, 0.75 and 0.93 have been reported in Saanen, Camosciata, Verzaschese, Orobica and Frisa breeds, respectively (Chiatti *et al.* 2005) in Italy. Probably the higher proportion of the A allele can be explained by the genetic flow provided by the selection of carrier animals directly or indirectly, which could be segregating this allele to the new generations. Regarding the C allele its incidence in this study was low (0.13), which is similar to that reported by Yahyaoui *et al.* (2001, 2003) in the Murciano, Granadina, Canaria, French Saanen and Italian Teramana breeds, among others. Landi *et al.* (2011) mentioned that in future generations this mutation could disappear because it is found in very low frequencies.

The BB genotype has been associated with a higher content of proteins and casein (Chiatti *et al.* 2005, 2007). The favorable effect of the BB genotype in the CSN3 gene on protein could be linked to the amino acid differences in the mature protein, which possibly affects the biological properties of κ-casein (Meisel 2005) and its biochemical interactions with the other fractions in the casein micelles (Lucey *et al.* 2003). It has also been suggested that the genetic variation in CSN3 implies that the mature protein could be associated with other polymorphisms in the non-coding sequences (promoter), which could be mutations that cause different expressions among some alleles, such as two main alleles in the CSN3 gene in bovine cattle (Martin *et al.* 2002). Existing evidence has shown that when a null mutation was introduced in CSN3 and caused the destabilization of the casein micelles, there was a failure in the lactation process (Shekar *et al.* 2006).

In the present assessment, the BB genotype confirmed its association with a higher percentage of protein. However, its effect was lower than that reported by Caravaca *et al.* (2010) who, after evaluating the effect of CSN1S1 and CSN3 genotypes on the coagulation properties of the milk in Murciano-Granadina goats in Spain, reported an average 3.61 % effect of this variant on PB. Furthermore, Caravaca *et al.* (2010) found a similar effect as the present research, with an average 2.94 % effect on the BB genotype (Chiatti *et al.* 2007), in an Orobica population in Italy.

Some implications in the evidence found here are directly related to the productive purpose of the studied farm and the purebred breeding animal production as brood stock. All the animals that carry AC, BB and CC genotypes might contribute with favorable performance in protein percentage, which will be reflected by the improvement of industry cheese production. The interest in the use of CSN3 genes associated with milk production and composition traits has been strongly related to the potential of improving the global efficiency in different sectors of the dairy products industry, due to the increasing scientific evidence of association of genetic variants with the improvement of the physicochemical properties of the milk and cheese quality (Martin *et al.* 2002, Ng-Kwai-Hang 2006).

The identification of variants significantly associated with milk composition traits can be proposed to be implemented in marker-assisted selection strategies. Genotyping information can be used to increase the variant frequency positively associated with a trait, through the selection of animals which carry the copies of the favorable variant. However, it is important to consider that the results of association could be validated in larger populations and verified in local populations, since purported associations with economically important traits can change according to the selection history of the studied populations (Parra-Bracamonte *et al.* 2007).

CONCLUSIONS

The B variant genotype of the CSN3 gene is associated with the milk protein percentage of Saanen goats. The validation of this evidence in commercial populations could support strategies to improve the protein in milk and contribute to the industry with better cheese production. The segregation of more frequent and favorable alleles which are associated with a particular trait on the nutritional quality of the milk could be a main objective for improving the economic value of the Saanen breed in Mexico.

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