Polymorphisms of the calpain and calpastatin genes in two populations of colombian creole sheep

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ABSTRACT

Objective. Characterize the genetic polymorphism type SNPs in the calpain (CAPN) and calpastatin (CAST) genes of Colombian creole hair sheep (OPC). Materials and methods. In 300 individuals belonging to two OPC subpopulations from the departments of Sucre (SC) and Valle del Cauca (VC) were genotyped by PCR-RFLP (MspI) for the CAST locus and by PCR-SSCP for the CAPN locus. The allelic and genotypic frequencies, the observed (Ho) and expected heterozygosity (He), the fixation index (F) and the deviations from the Hardy-Weinberg equilibrium (HWE) were calculated and a molecular analysis of variance to estimate the values of FST, FIS and FST. Results. In the CAST locus, the MM genotype was the most frequent (83.9±1.1%), followed by the other genotypes (MN: 15.5±1.1, NN: 6.0±0.0%) and the allelic frequency of M (91.7±0.4%) exceeded that of N (8.3±0.4%). For the CAPN locus the heterozygous genotype (48.2±0.7%) was the most frequent, the other genotypes presented frequencies TT:44.7±1.9 and CC:7.0±1.4%. The T allele reached a frequency of 68.8±1.5% (C: 31.3±1.5%). Similar percentages of allelic and genotypic frequencies were found in the subpopulations. The He was less than the Ho in both loci, with negative values of F and deviations of HWE only in CAPN. All the variation found was due to differences within the individuals, with non-significant values (p>0.05) of FST, FIS, and FIS (0.002, -0.093 and -0.095, respectively). Conclusions. The loci studied has high variability, these results can be used for future gene-assisted selection plans to increase OPC productivity.

Keywords: Allele frequency, CAST gene, CAPN gene, genetic resources, (Source: CAB).

RESUMEN

Objetivo. El propósito del presente estudio consiste caracterizar el polimorfismo genético tipo SNPs en los genes calpaina (CAPN) y calpastatina (CAST) en el ovino de pelo criollo colombiano (OPC). Materiales y métodos. 300 individuos pertenecientes a dos subpoblaciones de OPC de los departamentos de Sucre (SC) y Valle del Cauca (VC) fueron genotipados por PCR-RFLP (MspI) para el locus CAST y por PCR-SSCP para el locus CAPN. Se calcularon las frecuencias genotípicas, alélicas, la heterocigocidad observada (Ho) y esperada (He), el índice de fijación (F), los desvíos del equilibrio de Hardy-Weinberg (EHW) y un análisis de varianza molecular para estimar los valores de FST, FIS y FIS. Resultados. En el locus CAST, el genotipo MM fue el más frecuente (83.9±1.1%), seguido por los otros genotipos (MN: 15.5±1.1; NN:6.0±0.0%) y la frecuencia alélica de M (91.7±0.4%) superó la del N (8.3±0.4%). Para el locus CAPN el genotipo heterocigoto (48.2±0.7%) fue el más frecuente; los otros genotipos presentaron frecuencias de TT:44.7±1.9 y CC:7.0±1.4%. El alelo T alcanzó una frecuencia de 68.8±1.5% (C:31.3±1.5%). Similaridades frecuencias alélicas y genotípicas se encontraron en las subpoblaciones. La He fue menor que la Ho en ambos loci, con valores negativos de F y desvíos de EHW solo en CAPN. Toda la variación encontrada fue debida a diferencias dentro de los individuos, con valores no significativos (p>0.05) de FST, FIS y FIS (0.002, -0.093 y -0.095, respectivamente). Conclusiones. Los loci estudiados tiene alta variabilidad, estos resultados pueden ser utilizados para futuros planes de selección asistida por genes para aumentar la productividad del OPC.

Palabras clave: Frecuencias alélicas, gen CAST, gen CAPN, recurso genético (Fuente: CAB).
INTRODUCTION

The sheep production systems in Colombia are based on the Colombian Creole sheep breed (OPC; by its acronyms in Spanish). The OPC presents important adaptive characteristics to the tropical climate, such as heat tolerance, ectoparasitosis and the ability to consume pastures of low nutritional value (1). However, these systems have been considered as a secondary activity of livestock that is carried out in traditional and/or family systems, with low input requirements and in mixed production systems with other species (2,3), which entails to which problems in nutrition, reproduction, health, genetic progress, animal welfare, and business management; among others, are common and are reflected in low productive efficiency (1,4).

There are few breeding programs in the OPC, based on absorbent crosses with imported breeds to take advantage of the hybrid vigor (5). However, in order to increase the competitiveness of the sector, it is necessary to incorporate new technologies or management practices that generate competitive advantages through knowledge management systems that adapt to the conditions of primary production in a developing country like Colombia (2,4,6). This is how molecular tools are used in animal genetic improvement programs, through molecular marker-assisted selection. This practice improves accuracy and increases genetic progress, through the identification, mapping and analysis of polymorphism of the genes involved in the main metabolic pathways related to animal growth, the distribution of nutrients in different tissues and product quality (7). Calpain (CAPN) and calpastatin (CAST) can be considered as candidate genes with the potential to be used in selection programs since single nucleotide polymorphisms (SNPs) have been associated with improvement in meat quality (8).

The CAPN gene is associated with the degradation of postmortem myofibrillar proteins, causing softening of the meat; is located on chromosome seven and has 25 exons. The SNP g.24962426T> C located in exon ten has been the more studied (9). On the other hand, the CAST gene is the main inhibitor of the action of calpain slowing down the speed and the degree of postmortem softening of the meat (8). It is located in chromosome 5 of the sheep, the calpains system is made up of the genes of µ-calpain (CAPN1), m-calpain (CAPN2) and calpain 3 (CAPN3), in which numerous SNPs correlated with gene expression and meat tenderness have been identified in different species (9,10). Also, the calpains are involved in muscle growth at different stages of development and in the degradation of muscle proteins, influencing the loss of muscle mass (7,8,9).

Data analysis. The genotypic and allelic frequencies, the observed (Ho) and expected heterozygosity (He) and the fixation index (F) for each subpopulation and in total were calculated. The deviations from the Hardy-
Weinberg equilibrium (EHW) were estimated and the molecular analysis of variance was performed to estimate the values of $F_{ST}$, $F_{IS}$, and $F_{IT}$. The genotypic and allelic frequencies found between loci and subpopulation were compared using the Fisher test with a significance of 5%. All analyses were performed with the Arlequin programs ver 3.5.2.2 (13) and GENALEX ver 6.5 (14).

RESULTS

The two SNPs evaluated were polymorphic. The allelic and genotypic frequencies did not differ significantly ($p>0.05$) between subpopulations for both loci.

In the CAST locus, for the whole population, the MM genotype was the most frequent, followed by the other genotypes (Table 1). The MM genotype was more frequent in the SC subpopulation than in the VC subpopulation, although the opposite occurred in the MN genotype. While the NN genotype presented the same frequency in both subpopulations (6%). Regarding the allelic frequencies, the M allele exceeded the N allele in frequency (Table 1). The T allele was more frequent in the SC subpopulation and the N allele was the most frequent in the VC subpopulation.

For the CAPN locus, a greater frequency of the heterozygous genotype was found in the whole population (48.2±0.7%) and in the subpopulations SC (48.7%) and VC (47.6%). The CC genotype had the lowest frequency (48.2±0.7%) and in the subpopulations SC (48.7%) and VC (47.6%). The CC genotype had the lowest frequency (48.2±0.7%) and in the subpopulations SC (48.7%) and VC (47.6%). For the CAST locus (AF016006, AF016007, and AF016008.1) fragments digested by the MspI enzyme were found between exons 1C and 1D, where a nucleotide substitution of A by G occurs (15). In Dalagh sheep genotypes MM (36%), MN (38%) and NN (26%) were found with higher frequency of the M allele (55%) in both subpopulations studied and for the OPC the genotype MM and the M allele presented higher frequency (Table 1).

This is consistent with the frequencies found by Santos et al (12) in three of the four breeds studied in Brazil; among them Pantaneira (MM: 58%, MN: 23%, NN: 19%, M: 70%), Suffolk (MM: 80%, MN: 16%, NN: 4%, M: 88%) and the Ile de France (MM: 82%, MN: 12%, NN: 6%, M: 88%) but not with what was found in the Bergamaco breed (MM: 28%, MN: 34%, NN: 38%, M: 45%). In Afshari Iranian sheep the M and N alleles are reported here; the others genotypes had frequencies of 57 and 33% for MM and NN, respectively.

The analysis of molecular variance showed that 100% of the variation is due to differences within individuals, with non-significant values ($p>0.05$) of $F_{ST}$, $F_{IS}$ and $F_{IT}$ of 0.002, -0.093 and -0.095, respectively.

DISCUSSION

The values of $H_o$ and $H_e$ averages were similar with a slight excess of heterozygotes not significant; on the other hand, in the CAST locus this excess induced a negative value of $F$ and significant deviations of the EHW ($p<0.05$). The $H_o$ and $H_e$ values for the CAST locus were higher in the VC subpopulation and in the CAST locus in the VC subpopulation. Only the value of $F$ was positive in the subpopulation SC for the CAST locus (0.004) the other values were negative. The locus-to-locus analysis indicated that all were in EHW.

Table 1. Genotypic and allelic frequencies at the CAST and CAPN loci in the OPC breed

<table>
<thead>
<tr>
<th>Populations</th>
<th>Locus CAST</th>
<th>Allele frequencies</th>
<th>Genotypic frequencies</th>
<th>Locus CAPN</th>
<th>Allele frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genotypic frequencies</td>
<td>M</td>
<td>N</td>
<td>TT</td>
<td>TC</td>
</tr>
<tr>
<td></td>
<td>MM</td>
<td>MN</td>
<td>NN</td>
<td>M</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>84.7</td>
<td>14.7</td>
<td>0.6</td>
<td>92.0</td>
<td>±3.0</td>
</tr>
<tr>
<td>VC</td>
<td>83.2</td>
<td>16.2</td>
<td>0.6</td>
<td>91.4</td>
<td>±2.0</td>
</tr>
<tr>
<td>Total</td>
<td>83.9</td>
<td>15.5</td>
<td>6.0</td>
<td>91.7</td>
<td>±0.4</td>
</tr>
</tbody>
</table>

Table 2. $H_o$, $H_e$, $F$ values and deviations of the EHW at the loci studied in the OPC breed.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Locus CAST</th>
<th>Allele frequencies</th>
<th>Genotypic frequencies</th>
<th>Locus CAPN</th>
<th>Allele frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$H_o$</td>
<td>$H_e$</td>
<td>$F$</td>
<td>HWE</td>
<td>$H_o$</td>
</tr>
<tr>
<td>SC</td>
<td>0.147</td>
<td>0.147</td>
<td>0.004</td>
<td>0.002</td>
<td>0.487</td>
</tr>
<tr>
<td>VC</td>
<td>0.161</td>
<td>0.157</td>
<td>-0.028</td>
<td>0.147</td>
<td>0.479</td>
</tr>
<tr>
<td>Mean</td>
<td>0.154</td>
<td>0.152</td>
<td>-0.012</td>
<td>0.07</td>
<td>0.483±0.004</td>
</tr>
</tbody>
</table>

SC: Sucre. VC: Valle del Cauca. $H_o$: observed heterozygosity. $H_e$: expected heterozygosity. $F$: fixation index. HWE: Hardy-Weinberg equilibrium (qui-square value). *$p<0.05$. 

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On the other hand, in the Zel breed, the allelic frequencies were 85.5% for M and 14.5% for N (17), frequencies for genotypes MM (75%) and NN (4%) lower than that found in the OPC. In autochthonous sheep of Pakistan Balkhi, Kajli and Beetal the frequencies of the M allele were 88, 86 and 100%, respectively, with the most frequent MM genotype (Balkhi: 76%, Kajli: 74%, Beetal: 100%) and absence of the NN genotype in the Balkhi and Beetal breeds and with low frequency (2%) in Kajli (18). The frequency of the N allele in the Kurdi race was higher (12%) than that found in the OPC with the absence of the NN genotype and higher MM frequency (76%) (19). It is noteworthy that the M allele of this locus has been associated with high weights at birth (13).

For the CAST locus, the PCR-RFLP and PCR-SSCP techniques were compared as alternatives for genotyping. It was determined that the A and C alleles obtained by SSCP could be digested with the MspI enzyme, also, that the AA, AC and CC were similar to the MM genotype determined by RFLP, similarly the MN genotype is similar with the AB and BC genotypes (by SSCP) and finally, the NN genotype agrees with the BB genotype (11, 17). In another locus of the CAST gene (exons 10, 11 and intron 10) in 80 animals of the OPC breed, the allelic frequencies found were 93.7% for A and 6.3% for B, with genotype frequencies of 88.75, 10 and 1.25% for AA, AB and BB respectively (20).

The value of He at the CAST locus was lower than that found in the Dalagh (11) (49%) and Zel (16) (25%), Pantaneira (42%), Suffolk (21%) and Ile de France (21%) and Bergamacia (50%) (12) Balkhi (21%) and Kajli (24%) (18) breeds, but higher than that reported in OPC (11%) (20) and the Kurdi sheep (7.9%) (19). Similarly, in the present investigation a non-significant excess of heterozygotes was found, as was EHW. This indicates that no selection has been made in favor of any of the alleles of this locus, or the absence of genetic improvement programs assisted by molecular tools. On the other hand, Molano (20) in the same racial group studied here, presents a non-significant heterozygous deficit and absence of the theoretical proportions of the EHW. Contrary results are presented in Dalagh sheep (11) and Zel (17) the Pantaneira, Ile de France and Bergamacia (12) where the authors found heterozygous deficit with significant deviations of EHW (Table 2). For the CAPN locus (AF309634.1) a variation located in the Dalagh sheep (11) and Zel (12) the Pantaneira, Ile de France and Bergamacia (12) where the authors found heterozygous deficit with significant deviations of EHW (Table 2).

The expected heterozygosity found in this study for the CAPN locus is higher than that reported in the breeds Bandur (29.5%) (24), Zel (26%) (17) and Kurdi (7%) (19), but lower than that reported by Molano (20) in OPC (49%). On the other hand, a non-significant excess of intra-population heterozygotes was found at this locus, but when analyzing the entire study population (Table 2), this excess was significant with deviations from the EHW (p<0.05). This could be attributed to the lack of inbreeding due to the existence of gene flow, as well as the rapid crossing of migrating females and males with the individuals residing in the subpopulations. In contrast, heterozygous deficits without deviations of EHW occur in OPC (20), and in the Zel breed, this deficit caused deviations of EHW (p<0.05) (17). Likewise, in the Kurdi and Bandur breeds, the excess of heterozygotes found did not cause significant deviations of EHW (19, 24).

The analysis of molecular variance showed that the variation found is only due to differences within individuals, which is confirmed by the lowest value of $F_{CT}$ found (0.002, p=0.653), which indicates a low population structure with gene flow among the subpopulations studied (5). In addition, the values of negative and not significant $F_{ST}$ and $F_{IS}$ (p > 0.05) contrast with what was reported in OPC ($F_{ST}=0.183$) (20), which could be explained by the different origin of the animals evaluated as well as differences in the management of production systems with respect to their systems or crossing schemes.

Finally, the results of the present study showed that the loci studied have good variability that is necessary to conserve. Additionally, the allelic frequencies found are considerably high, so these results could be used in future gene-assisted selection plans, in order to increase the productivity of the sheep systems that use the OPC as a genetic base.

**Interest conflict**

The authors declare no conflicts of interest.

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