Analysis of restriction fragment length polymorphism in the kappa-casein gene related to weight expected progeny difference in Nellore cattle


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Abstract

Restriction fragment length polymorphism (RFLP) has been detected at the bovine kappa-casein locus. The polymorphism has been analyzed for its effects in cattle production, mostly for milk traits and even for maternal effect on pre-weaning weights. We used polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) to genotype 408 Nellore animals for the non-silent mutation (Thr/Ile136 and Asp/Ala148) that characterizes the A and B variants of the polymorphism and compared expected progeny difference (EPD) for a maternal effect on 120 and 210 days weights and direct EPD for 120, 210, 450 and 550 day weight between AA and AB animals. The EPD values were obtained from the University of São Paulo (Brazil) Nellore Cattle Breeding Program, which evaluated 266,272 animals in 2001. Analysis of Variance was used to compare weight expected progeny differences (EPDs) between animals genotyped as AA and AB. The A allele frequency was 0.911. Although the AA animals had higher weight EPDs than AB animals the differences were not statistically significant (p > 0.05).

Key words: bovine, weight, kappa-casein, genetic marker.

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Introduction

The use of DNA polymorphic markers allows the determination of individual genotypes at many loci and provides information on population parameters such as allele frequencies as well as improving selection by marker assisted selection. Medrano and Aguilar-Cordova (1990) identified a restriction fragment length polymorphism (RFLP) at the kappa-casein (CSN3) bovine locus and detected two alleles (A and B) which, according to Lin et al. (1992), differed by two amino acid substitutions, tyrosine/isoleucine at position 136 and asparagine/alanine at position 143 of the kappa-casein protein.

The known participation of kappa-casein in the composition of milk protein (Bordim et al., 2001) justifies investigations correlating this polymorphism with milk production and composition. Although Haenlein et al. (1987) found no differences in milk traits between kappa-casein genotypes there have been many analyses of the kappa-casein CSN3 polymorphism or linked markers and their direct effects on milk production. For example, in Finnish Ayrshire cows the B allele has been associated with high protein content in the first lactation (Ikonen et al., 2001) while in Holstein-Frisian cows the same allele was found to affect protein composition without modifying the concentration of protein (Bobe et al., 1999). However, Ojala et al. (1997) demonstrated that the B allele alone did not have a positive effect on milk or protein production but, as compared to other haplotype combinations, the alpha, beta and kappa casein genotypes ABA1A2BB increased protein content and resulted in an increase milk yield of 86 kg for the first lactation. Bovenhuis et al. (1992) reported that dairy cattle with the BB genotypes produced less milk in kilograms but that the milk produced had 0.8% more protein and 5.28 kg less fat in the first lactation than AA animals, these authors having reviewed the association of CSN3 polymorphisms with kilograms of milk produced per lactation, percentage fat and protein and kilograms of fat and protein per lactation demonstrated a divergence regarding genotypes and their association with these traits. Quantitative trait loci screening in bovine chromosome 6, where the casein genes are located, have identified markers related to milk fat and milk protein yield (Kühn et al., 1999) and percentage protein, milk yield and fat yield (Velmala et al., 1999).
The CSN3 polymorphism has also been studied in association with the weight of beef cattle. Moody et al. (1996) analyzed the relation of the CSN3 polymorphism with direct and maternal expected progeny difference (EPD) for growth in Hereford cattle and suggested that the alleles explained 15% of the variation in EPD for birth weight and 8% of the maternal EPD for 180-days weight gain from birth to weaning. In Nellore cattle, Faria et al. (1999) investigated the relationship between the CSN3 polymorphism and calve-weight at 205 days and found no difference between AA and AB animals. Tambasco et al. (2003) have genotyped crossbred cattle (Aberdeen Angus x Nellore, Canchim x Nellore, Simmental x Nellore) and found no differences in the average daily weight-gain from birth to weaning and average daily weight-gain from weaning to yearling for AA, AB and BB genotypes.

Material and Methods

Population

We genotyped and analyzed 408 Bos indicus (Nellore) male and female cattle born into eight Brazilian herds between 1996 and 2001. The animals were the offspring of 69 sires but no family design was used in the analysis. All the animals are included in the 2001 Nellore Cattle Breeding Program (NCBP) of the University of São Paulo (São Paulo state, Brazil) that predicted the breeding value for 266,272 calves, cows and bulls from herds distributed over 12 Brazilian states.

Laboratory analyses

Blood samples were collected in sterile tubes containing EDTA anticoagulant and the DNA extracted according to the method of Sambrook et al. (1989). The kappa-casein CSN3 polymorphism genotypes were determined as described by Medrano and Aguilar-Cordova (1990) using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique and the Hinf I restriction enzyme.

Breeding value estimation

The breeding values of the 408 animals were obtained from the 2001 NCBP, the methods used to estimate the expected progeny difference (EPD) being briefly explained in the next paragraph.

The traits studied were the standardized weight (kg) at 120, 210, 450 and 550 days (coded W120, W210, W450 and W550). Weights were measured every three months from birth to the twenty-first month and cows were also weighted at calving and at weaning. The standardized weights were calculated based on the weights prior to and after the day on which the animals were weighed. Covariance components, heritabilities and breeding values were estimated using the multiple traits derivative-free restricted maximum likelihood (MTDFREML) software (Boldman et al., 1995) applying the animal model. The expected progeny differences (EPDs) were calculated as half the breeding value.

To analyze the W120 and W210 traits and estimate maternal EPD (MEPD120 and MEPD210) and direct EPD (DEPD120 and DEPD210) for these traits the model included data on the contemporary group (birth year and month, sex, heard and feeding data) and age class of cows at calving (< 36 to 47; 48 to 59; 60 to 70; 71 to 119; >119 months) as fixed effects. The direct effect of calf, cow, permanent environment of the cow and the associated error were considered as random effects. The DEPD for W450 and W550 (DEPD450 and DEPD550) were estimated with the same fixed effects described above and the animal direct effect and the error were considered to be random variables. Each trait was analyzed separately.

The inbreeding coefficient (F) for the animals was calculated from the genealogy data using the multiple traits derivative free numerator relationship matrix (MTDFNRM) function which forms part of the MTDFREML software (Boldman et al., 1995).

Statistical analyses

Genotypic and allele frequencies were calculated as described by Falconer and Mackay (1996) while MEPD and DEPD data for the different genotypes were subjected to analysis of variance (ANOVAR) using the general linear model (GLM) from the Statistical Analysis Software (SAS Institute Inc., 2000). The statistical model used was: \( Y_{ij} = \mu + G_i + e_{ij} \), where \( Y_{ij} \) is the EPD for each trait of the \( ij^{th} \) animal, \( \mu \) is the general mean of each trait, \( G_i \) is the fixed effect of the \( i^{th} \) CSN3 (Hinf I) genotype and \( e_{ij} \) is the random error effect associated to the \( ij^{th} \) observation.

Results

The genotype frequencies for 408 animals were 0.8235 for the AA genotype and 0.1764 for the AB genotype while the frequency of the A allele was 0.911. The frequencies were similar when the animals were separated by heard or by sire (data not shown). Homozygote animals had higher mean values of weight EPD values (Table 1) but
there were no significant differences (p > 0.05) in the EPD values between AA and AB k-casein genotypes (Table 2).

Discussion

The genotype frequencies presented here are in accordance with Kemenes et al. (1999) who found an A allele frequency of 0.91 for 63 unrelated Brazilian Nellore cattle. Our results also agree with those of Del Lama and Zago (1996), Faria et al. (1999) and Tambasco et al. (2000) who genotyped 64, 82 and 180 Brazilian Nellore animals, respectively. Kemenes et al. (1999) analyzed other zebu breeds and found that the A allele frequency was 0.93 for the Gyr breed and 0.92 for the Guzera breed, similar to the 0.91 found by us for Nellore cattle, indicating that there is a tendency towards a higher A allele frequency in Brazilian zebu breeds. The highest inbreeding coefficient (F) for our sample was 0.14, with 98.53% of animals having F < 0.1. This data, and the frequency observed by Kemenes et al. (1999), indicate that the higher frequency of the A allele in Brazilian zebu breeds has not been influenced by inbreeding effects.

As for most zebu breeds, excluding the Gyr breed, Nellore cattle have been selected for meat production. This intensive selection for weight gain could have indirectly influenced kappa-casein allele frequencies, as proposed by Del Lama and Zago (1996). However, further studies should be carried out with zebu animals selected for meat and milk production in order to compare the allele frequencies.

Moody et al. (1996) demonstrated that A allele substitution had a positive effect on the EPD for birth weight, MEPD for birth weight, DEPD for 180-day weight-gain from birth to weaning and MEPD for 180-day weight-gain from birth to weaning (p < 0.01). The kappa-casein genotypes explained 15% of the EPD variability for birth weight and 8% of the MEPD for 180-day weight-gain from birth to weaning. Their hypothesis was that the differences in MEPD for CSN3 genotypes were probably due to differences in milk composition as well as milk yield. The AA genotype would provide better maternal conditions for the growth of calves and consequently higher pre-weaning weight. Our result are the first analysis of differences in weight EPD values between AA and AB kappa-casein CSN3 (Hinf I) genotypes in Brazilian Nellore cattle, but although the AA genotypes showed higher mean weight EPD values the differences found were not statistically significant, suggesting that this polymorphism does not influence the estimated breeding value for the growth trait.

Despite the fact that we analyzed EPD values only, the results agree with those of Faria et al. (1999) and Tambasco et al. (2003) which compared weight data sets. Faria et al. (1999) genotyped 82 cows for the A and B CSN3 polymorphisms and evaluated the effect of the different genotypes on the weaning weight of the progeny and found that the 205-day calf-weight in kg did not differ between AA and AB cows (p > 0.05). Tambasco et al. (2003) found that the CSN3 (Hinf I) genotypes had no effect on the average daily weight-gain in kg from birth to weaning and average daily weight-gain in kg from weaning to yearling in crossed calves resulting from Nellore females crossed with Aberdeen Angus, Canchim or Simmental bulls.

The situation as to how much the kappa-casein gene or a group of linked chromosome 6 genes contribute to milk traits is unclear. Depending on the breed and population investigated, the B and A alleles have been found to affect both milk yield and the components of the milk (Bovenhuis et al., 1992; Moody et al., 1990). However, both our results and previously published reports indicate that the kappa-

Table 1 - Weight (kg) expected progeny difference (EPD) values for a sample of 408 Brazilian Nellore cattle genotyped for the Kappa-casein CSN3 (Hinf I) polymorphism.

<table>
<thead>
<tr>
<th>Kappa-casein genotypes</th>
<th>Trait (kg)*</th>
<th>AA (n = 336)</th>
<th>AB (n = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean weight EPD (kg) ± standard deviation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maternal</td>
<td>MEPD_{120}</td>
<td>1.16 ± 0.86</td>
</tr>
<tr>
<td></td>
<td>MEPD_{210}</td>
<td>1.70 ± 1.26</td>
<td>1.55 ± 1.57</td>
</tr>
<tr>
<td>Direct</td>
<td>DEPD_{120}</td>
<td>2.72 ± 1.77</td>
<td>2.47 ± 1.84</td>
</tr>
<tr>
<td></td>
<td>DEPD_{210}</td>
<td>4.17 ± 2.57</td>
<td>3.87 ± 2.69</td>
</tr>
<tr>
<td></td>
<td>DEPD_{450}</td>
<td>6.09 ± 4.24</td>
<td>5.83 ± 4.33</td>
</tr>
<tr>
<td></td>
<td>DEPD_{550}</td>
<td>7.51 ± 5.04</td>
<td>6.41 ± 5.13</td>
</tr>
</tbody>
</table>

MEPD = Maternal EPD; DEPD = Direct EPD.

Table 2 - Analysis of variance mean square data and probability of significance between expected progeny difference (EPD) values for AA and AB k-casein CSN3 (Hinf I) genotypes of a sample of 408 Brazilian Nellore cattle.

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>MEPD_{120}</th>
<th>MEPD_{210}</th>
<th>DEPD_{120}</th>
<th>DEPD_{210}</th>
<th>DEPD_{450}</th>
<th>DEPD_{550}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td>1</td>
<td>0.709</td>
<td>1.53</td>
<td>4.364</td>
<td>6.402</td>
<td>6.781</td>
<td>77.164</td>
</tr>
<tr>
<td>Error</td>
<td>407</td>
<td>0.812</td>
<td>1.742</td>
<td>3.179</td>
<td>6.767</td>
<td>18.101</td>
<td>25.671</td>
</tr>
<tr>
<td>Pr &gt; F</td>
<td>0.35</td>
<td>0.35</td>
<td>0.24</td>
<td>0.33</td>
<td>0.54</td>
<td>0.08</td>
<td></td>
</tr>
</tbody>
</table>

MEPD = Maternal EPD; DEPD = Direct EPD.
casein CSV3 polymorphism has not influenced weight-gain in Brazilian Nellore herds, although the difference in the frequency of the A and B alleles means that it is difficult to make an accurate estimation of their influence on weight-gain in Brazilian Nellore cattle. It thus appears that experiments with structured families should be carried out to provide information on the effect of this polymorphism on weight breeding value in Brazilian Nellore cattle.

Acknowledgments

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