α-LACTALBUMIN POLYMORPHISM IN RELATION TO MILK LACTOSE

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SUMMARY

Here we report the first evidence that the point mutation located +15 relative to the transcription start point of the α-lactalbumin gene, previously reported to exist only in Holsteins, also is present in another dairy breed, the Swedish Red and White. Altogether 78 cows with weekly recordings on milk yield and composition were genotyped for the (+15) polymorphism as well as for the recently reported mutation 1689 basepairs 5' of the α-lactalbumin transcription start point. The two linked point mutations were analysed as a combined aggregate genotype. The results indicate a relationship between α-lactalbumin (-1689) polymorphism and concentration of lactose in milk, where cows with the (+15) BB in combination with (-1689) AA genotype had .08 percentage units more lactose per kg of milk (P<.014) than cows with the same (+15) genotype but with the AB genotype at the (-1689) mutation.

Keywords: α-lactalbumin, milk protein, genetic polymorphism, lactose concentration, cattle

INTRODUCTION

Increasing costs associated with the transportation of milk from the farm to the dairy plant and the lower processing value of milk with low dry matter content have brought forward the discussion regarding possibilities to reduce the water fraction in milk. Moreover, a relevant question is if the excessive water transport to the udder in high yielding cows means a stress to the cow as regards metabolism and fluid balance, thereby affecting the health of the cow.

The roles of α-lactalbumin and lactose in milk synthesis

Lactose is the major osmole in milk and as such thought to act as a regulator of milk volume by providing an osmotic gradient whereby water passively moves into the milk. Lactose synthase, the enzyme involved in lactose formation, is composed of two subunits, α-lactalbumin and galactosyl transferase. Although α-lactalbumin is active within the Golgi vessels of the milk secreting cells it is also found in the milk where it constitutes one of the major whey proteins. Of the various proteins in milk, α-lactalbumin is the most lactation specific. Its concentration in serum increases dramatically at the time of calving, being stable during the lactation, to drop in association to dry-off (Mao et al. 1991). Experiments in mice with inactivated copies of the α-lactalbumin gene have given definite proofs that α-lactalbumin is a prerequisite for lactose synthesis and that lactose has a major effect on milk volume (Stacey et al. 1995). In contrast to previous studies (Fitzgerald et al. 1970; McFadden et al. 1989; Grimble and Mansaray 1987), no clear relationship between contents in milk of α-lactalbumin and lactose was found.
The gene coding for bovine α-lactalbumin has been located to chromosome 5 (Threadgill and Womack 1990). Upstream of the coding part of the gene there are regions that are likely to affect gene expression, in part by hormonal influences. Genetic variation in this region of the α-lactalbumin gene has been reported at positions +15 (Bleck and Bremel 1993a) and -1689 (Voelker et al. 1997) relative to the transcription start point, which potentially influence the binding of RNA polymerase and various transcription factors whereby it may affect the expression of the gene. The point mutation at position +15 results in two alleles, A and B, and has been reported to be associated with milk production traits in the Holstein breed (Bleck and Bremel 1993b). Recent data by Voelker et al. (1997) indicate that the (+15) A allele is a result of a mutation that occurred in the A allele of the (-1689) polymorphism. The effects of this 'new' polymorphism on milk production traits are still not known. The objective of the present study was therefore to study the relationship between the α-lactalbumin (-1689) and (+15) polymorphisms and milk yield and composition.

MATERIALS AND METHODS

In total 268 cows of the Swedish Red and White breed (SRB), a breed with a large genetic influence from Finnish Ayrshire, on two experimental farms belonging to the Swedish University of Agricultural Sciences (SLU) were included in the study. The cows belonged to two selection lines in which sires with high and low breeding values for milk fat content, respectively, have been used. The bulls being used in the two lines have similar and high breeding values as regards total energy production from the milk which should result in a more concentrated milk being produced from the cows in the high fat line. Weekly analyses on milk composition as regards fat, protein, casein, and lactose are being performed.

To test if the α-lactalbumin (+15) polymorphism in Holstein cattle was present also in SRB, cows in the two selection lines were genotyped for the (+15) polymorphism, using the method described in Bleck and Bremel (1993a). If present, the purpose was to study its influence on milk composition and yields. In addition, we genotyped all the cows for the recently identified α-lactalbumin (-1689) mutation (Voelker et al. 1997) using a method based on allele discrimination by primer length (Lindersson et al. 1995).

Statistical analysis

The hypothesized effects of genetic polymorphisms of α-lactalbumin on milk production traits were analysed on a subsample of the genotyped cows by a linear model using the PROC GLM procedure of SAS (1986). Data on 78 cows with a total of 2930 observations for each of the traits kilograms of milk, energy corrected milk (ECM), fat, protein, and lactose and concentrations of fat protein and lactose were analysed. Fixed effects of α-lactalbumin genotype, selection line, number of generations of selection, stage of lactation, parity, sampling month, and cow nested within α-lactalbumin genotype and generation and line of selection were included in the analysis. Sires were not included in the model due to the limited number of daughters and genotypes within sire. When testing the effects of α-lactalbumin genotype the Type III MS value for the nested effect of cow was used as an error term.

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RESULTS AND DISCUSSION
The same two α-lactalbumin alleles (denoted A and B) of each of the (+15) and (-1689) mutations (Bleck and Bremel (1993a) and Voelker et al. (1997), respectively) were found in the SRB breed. This was verified by sequencing the PCR-products from one AA and one BB individual. As these animals could not be considered to be a random sample of the SRB population, the frequency estimates should mainly be used for comparisons between the two selection lines. As seen in Table 1, the (+15)A variant is less frequent in the low fat line than in the high fat line in both herds whereas the opposite is true for the (-1689)A allele. Because of linkage disequilibrium between alleles at the two point mutations they were analysed as a combined aggregate genotype. Due to the low frequency of the (+15)A allele (with a total absence of (+15)AA cows) among the SRB cows and only two cows with the (-1689)BB genotype, only two aggregate genotypes, (+15)BB in combination with either AA or AB of the (-1689) mutation, were included in the further analyses. Thus, the two (-1689) genotypes are contrasted against a common (+15) genotype.

Table 1. Gene frequencies of the A-variants of α-lactalbumin (+15) and (-1689) polymorphisms in two selection lines1 of the Swedish Red and White (SRB) breed

<table>
<thead>
<tr>
<th>Herd</th>
<th>n</th>
<th>High fat</th>
<th>Low fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(+15)</td>
<td>(-1689)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>frequency</td>
<td>frequency</td>
</tr>
<tr>
<td>SRB2</td>
<td>102</td>
<td>11.5 %</td>
<td>5.6 %</td>
</tr>
<tr>
<td>SRB3</td>
<td>165</td>
<td>16.3 %</td>
<td>13.2 %</td>
</tr>
</tbody>
</table>

1 Lines selected for high and low milk fat content, but with similar and high total energy production from the milk
2 Experimental herd at the department of Animal Breeding Genetics, SLU
3 Experimental herd at the department of Animal Nutrition and Management, SLU

In Table 2 least squares means for test-day yield and composition of milk are presented for each of the two analysed α-lactalbumin aggregate genotypes. No difference in production of ECM was found between the two selection lines (data not shown), which is in agreement with the selection objectives. Although any conclusions at this stage could only be tentative, these data indicate a relationship between α-lactalbumin (-1689) polymorphism and concentration of lactose in milk, where cows with the (+15)BB in combination with (-1689)AA genotype had .08 percentage units more lactose per kg of milk than cows with the same (+15) genotype but with the AB genotype at the (-1689) mutation. In contrast to the results obtained on Holstein cattle (Bleck and Bremel 1993b), no relationships with milk yield or yields and concentrations
of fat or protein were observed in the present study. Genotyping the breeding bulls that has been selected for use in the respective selection line will reveal if the higher frequency of the (-1689)A variant in the cows of the low fat line has paternal origin. Further analyses on an extended material will give improved estimates of the effects of genetic variation in the α-lactalbumin gene.

Table 2. Least squares means for test day yield and composition of milk in cows¹ with different α-lactalbumin aggregate genotype² from two selection lines³ of the Swedish Red and White (SRB) breed

<table>
<thead>
<tr>
<th>α-Lactalbumin aggregate genotype</th>
<th>BB:AA</th>
<th>BB:AB</th>
<th>(P_{(overall)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield, kg</td>
<td>24.6 ± .2</td>
<td>24.5 ± .2</td>
<td>.914</td>
</tr>
<tr>
<td>ECM, kg⁴</td>
<td>25.0 ± .2</td>
<td>24.9 ± .2</td>
<td>.972</td>
</tr>
<tr>
<td>Protein yield, kg</td>
<td>.81 ± .01</td>
<td>.80 ± .01</td>
<td>.772</td>
</tr>
<tr>
<td>Fat yield, kg</td>
<td>1.03 ± .01</td>
<td>1.04 ± .01</td>
<td>.732</td>
</tr>
<tr>
<td>Lactose yield, kg</td>
<td>1.12 ± .01</td>
<td>1.10 ± .01</td>
<td>.589</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.39 ± .01</td>
<td>3.37 ± .01</td>
<td>.961</td>
</tr>
<tr>
<td>Fat, %</td>
<td>4.28 ± .02</td>
<td>4.32 ± .03</td>
<td>.387</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.49 ± .01</td>
<td>4.41 ± .01</td>
<td>.014</td>
</tr>
</tbody>
</table>

¹ 53 BB:AA cows and 25 BB:AB cows with altogether 2930 observations for each of the analysed traits
² Aggregate genotype combining polymorphisms at positions +15 and -1689 relative to the transcription start point of the α-lactalbumin gene
³ Lines selected for high and low milk fat content, but with similar and high total energy production from the milk
⁴ Energy corrected milk

REFERENCES