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## Effect of Kappa-Casein and Beta-Lactoglobulin Loci on Milk Production Traits and Reproductive Performance of Holstein Cows

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### ABSTRACT

The effects of  $\kappa$ -casein ( $\kappa$ -CN) and  $\beta$ -lactoglobulin ( $\beta$ -LG) loci on milk production traits (milk, fat, protein, and lactose yield, fat, protein, and lactose content) and reproductive performance (gestation length, calving interval, age at first and second calving, number of services per conception) was estimated for 278 Holstein cows in the first 2 lactations. Genotypes of  $\kappa$ -CN and  $\beta$ -LG were determined by alkaline and acidic polyacrylamide gel electrophoresis. Milk production was recorded daily. Single-trait, mixed, linear models were used for the statistical analysis of the data. Results indicated that  $\kappa$ -CN genotypes affected significantly protein yield and content (genotype AB > genotype AA). A tendency for increased milk and fat yield of animals having AB  $\kappa$ -CN genotype was also found. Fat content and lactose yield and content were not affected. In the  $\beta$ -LG system, significant differences were detected for milk yield (AB > AA), fat yield (BB and AB > AA), fat content (BB > AA and AB), and lactose yield (AB > AA). A tendency for higher protein yield was also observed (AB > AA). The  $\beta$ -LG locus had no significant effect on protein and lactose content. No associations between polymorphisms at the  $\kappa$ -CN locus and reproductive performance were found. There was a tendency, however, for cows with AB genotype to have older age at first and second calving. In the  $\beta$ -LG system, cows with AA genotype had significantly shorter gestation length than did those with AB or BB genotype. No differences were detected between  $\beta$ -LG polymorphisms for the other reproductive traits.

**(Key words:** milk protein genotype, milk protein loci, milk production trait, reproductive performance)

### INTRODUCTION

The initial discovery of genetic polymorphism at the  $\beta$ -LG locus by Aschaffenburg and Drewry (1955) oc-

curred almost 50 yr ago, and the complete amino acid sequences of the genetic variants A and B of  $\kappa$ -CN were determined by Grosclaude et al. (1972) and Mercier et al. (1973). Since then, many studies have been carried out to determine the effect of  $\kappa$ -CN and  $\beta$ -LG genotypes on milk production traits.

Published results on the effect of  $\kappa$ -CN genotypes on milk production traits have been conflicting. Although a favorable effect of the B  $\kappa$ -CN variant has been found on protein yield (Ng-Kwai-Hang et al., 1984; Van Eenennaam and Medrano, 1991) and protein content (Gonyon et al., 1987; Ng-Kwai-Hang et al., 1990b; Van Eenennaam and Medrano, 1991; Bovenhuis et al., 1992; Van den Berg et al., 1992) in most cases, results have been contradictory concerning the associations between  $\kappa$ -CN polymorphism and other milk production traits. The B variant of  $\kappa$ -CN has been associated with higher (Ng-Kwai-Hang et al., 1986; Lin et al., 1989; Van Eenennaam and Medrano, 1991) and lower (Bovenhuis et al., 1992) milk yield, whereas other studies have indicated no effect (Arave et al., 1971; McLean et al., 1984; Ng-Kwai-Hang et al., 1984; Lin et al., 1986; Ng-Kwai-Hang et al., 1990b; Lundén et al., 1997).

Results of studies on the effect of  $\beta$ -LG genotypes on milk production traits have been rather more consistent. The AA genotype of  $\beta$ -LG has been shown to have a favorable effect on protein yield (Ng-Kwai-Hang et al., 1984; Aleandri et al., 1990; Bovenhuis et al., 1992), whereas positive effects of the BB genotype on fat content have been reported (McLean et al., 1984; Ng-Kwai-Hang et al., 1984, 1986; Aleandri et al., 1990; Bovenhuis et al., 1992; Hill, 1993). In studies of  $\beta$ -LG genotypic effects on milk yield, several authors have reported no significant associations (McLean et al., 1984; Ng-Kwai-Hang et al., 1984; Lin et al., 1986, 1989; Van Eenennaam and Medrano, 1991; Lundén et al., 1997; Ojala et al., 1997). However, reports exist where  $\beta$ -LG genotype AA (Aleandri et al., 1990; Bovenhuis et al., 1992), AB (Pupkova, 1980), or BB (Jairam and Nair, 1983) have been positively associated with milk yield.

Contrary to the numerous studies on effects of milk protein genetic polymorphism on milk production traits, only a few research reports have been published

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regarding their association with reproductive performance. Results from the study of Hargrove et al. (1980) indicated no significant associations between  $\kappa$ -CN and  $\beta$ -LG genotypes with reproductive performance. Jairam and Nair (1983) reported that heifers with the genetic combination of the  $\kappa$ -CN AB genotype and the  $\beta$ -LG AB genotype had lower age at first calving than the other combinations, whereas Ronda and Perez-Beato (1983) did not find significant associations. Lin et al. (1987) showed that cows with AB  $\beta$ -LG genotype had longer gestation length than did those with AA or BB genotypes, whereas age at first calving tended to be lower for animals with AB  $\beta$ -LG genotype. The same study found that  $\kappa$ -CN genotypes had no effect on any of the reproductive traits studied. The genotypic effect of milk proteins on days to first insemination, days open, and number of services per conception was reported by Ng-Kwai-Hang et al. (1990a) but no significant associations were found.

To the best of our knowledge, no single study has investigated effects of polymorphism at the  $\kappa$ -CN and  $\beta$ -LB loci on milk production traits and reproductive performance. If milk protein genotypes are to be used as a selection criterion in cattle breeding programs to improve milk production traits, it is necessary to confirm that these genotypes are not adversely associated with reproductive performance. Furthermore, results regarding the effects of genetic polymorphism of  $\kappa$ -CN and  $\beta$ -LG on milk production traits have been conflicting, possibly due to different management and feeding regimens, population size and breed, as well as availability and accuracy of data in the various studies. This raises the need for a study that overcomes the potential sources of variability. There are few studies where animals are raised in a single herd under similar management conditions, in contrast to studies that involve many different herds where field conditions may introduce considerable variation.

The objectives of this study were to determine the effects of  $\kappa$ -CN and  $\beta$ -LG genotypes on milk production and reproductive performance traits in a large commercial dairy farm of Holstein cows.

## MATERIALS AND METHODS

### Population Description

The study was carried out from September 1, 1998 to December 31, 2001 in a large commercial farm located in Northern Greece. The herd consisted of 350 Holstein heifers that had been imported from France and The Netherlands in advanced pregnancy (sixth to seventh month of gestation). The present study included all animals of the herd that were being milked

at the time of sampling for the determination of  $\kappa$ -CN and  $\beta$ -LG genotypes ( $n = 278$ ).

The animals were housed in 2 free-stall barns. Windows and electric ventilators provided temperature and air control. The animals were fed a TMR that included corn silage, clover hay, straw hay, whole cottonseeds, soybean meal, corn grain, citrus pulp by-product, molasses, minerals, and vitamins.

Cows were milked twice daily in milking parlors equipped with electronic devices that automatically recorded the quantity of milk produced by individual animals.

The reproductive tract of each animal was examined by rectal palpation, routinely, within 29 to 35 d postpartum to check for normal uterine involution and ovarian structures. The goal for the interval from calving to first AI was 50 d. Cows were observed for signs of estrus twice daily for 30 min at 1000 and at 2030 h, as well as during the morning and afternoon milking. Additionally, animal mobility was used as an aid for the detection of estrus. Walking activity was constantly monitored electronically with the use of transmitters attached to the collar of the cow. This information was then integrated into an automatic telemetric system. Cows first observed in estrus were inseminated 12 h later. Cows that did not exhibit estrus within 80 to 100 d postpartum were included in a prostaglandin or combined gonadotropin-prostaglandin synchronization program. Pregnancy was diagnosed by return to estrus after insemination, by measuring progesterone concentration in milk on d 20, and by rectal palpation between d 40 and 54.

### Data Collection and Analysis

Milk yield of each animal was recorded daily. Milk samples were analyzed monthly for fat, protein, and lactose content with a MilkoScan 255 (Foss, Hillerød, Denmark) milk analyzer. Calibration of samples was done using standard methods for fat, protein, and lactose. All cows had complete lactations and total lactation milk, fat, and protein yield was calculated from the daily records.

Detailed information was available for all reproductive events and was used to determine the traits of reproductive performance for each cow, including gestation length, calving interval, age at first and second calving, and number of services per conception.

Genotypes for the  $\kappa$ -CN and  $\beta$ -LG were determined using polyacrylamide gel electrophoresis as described by Ng-Kwai-Hang et al. (1984). To eliminate errors due to sample identification, genotyping was performed on 2 occasions and the results obtained for individual cows were compared for consistency.

**Statistical Analysis**

Single-trait, mixed linear models were used to test the effects of  $\kappa$ -CN and  $\beta$ -LG genotypes on milk production and reproductive performance traits under study.

The effects of  $\kappa$ -CN and  $\beta$ -LG genotypes on total lactation milk, fat, protein, and lactose yield, and fat, protein, and lactose content were tested using model [1]; each trait was analyzed separately:

$$Y_{ijkl} = \text{mean} + \text{lact}_j + \text{ys}_k + b_1 \cdot \text{age} + b_2 \times \text{age}^2 \quad [1] \\ + \text{gen}_l + \text{cow}_i + e_{ijkl}$$

where Y = record of milk yield or fat, protein, lactose yield or content of the cow i, lact = fixed effect of lactation j (j = 1, 2), ys = fixed effect of year-season of calving k (k = 1, ..., 16), b<sub>1</sub>, b<sub>2</sub> = fixed linear and quadratic regression on age at calving (age), gen = fixed effect of the lth genotype for  $\kappa$ -CN (l = 1,2) or  $\beta$ -LG (l = 1, ..., 3), cow = random effect of cow i, with (co)variance matrix **A·G** (**A** = cow genetic relationship matrix), nested within the  $\kappa$ -CN or  $\beta$ -LG genotype, and e = random residual effect with (co)variance matrix **R**.

Calving years were 1998, 1999, 2000, and 2001. Calving season included 4 classes: December to February, March to May, June to August, and September to November. The  $\kappa$ -CN genotype included 2 classes: AA and AB. The  $\beta$ -LG genotype included 3 classes: AA, AB, and BB.

Model [2] was used to test the effects of  $\kappa$ -CN and  $\beta$ -LG genotypes on gestation length, calving interval, and number of services per conception; each trait was analyzed separately:

$$Y_{ijkl} = \text{mean} + \text{mlk}_j + \text{ys}_k + b_1 \cdot \text{age} + b_2 \cdot \text{age}^2 \quad [2] \\ + \text{gen}_l + \text{cow}_i + e_{ijkl}$$

where Y = record of gestation length, calving interval or number of services per conception of cow i, mlk = fixed effect of milk production level j (j = 1, ..., 10), and other effects as in model [1].

In model [2], milk yield was assigned to 10 categories, according to production level. The latter ranged from 5000 to 15,000 kg and individual categories were defined in 1000-kg intervals.

The effects of  $\kappa$ -CN and  $\beta$ -LG genotypes on age at first and second calving were tested with model [3]; each trait was analyzed separately:

$$Y_{ijkl} = \text{mean} + \text{ybco}_j + \text{gen}_l + \text{sire}_k + e_{ijkl} \quad [3]$$

where Y = age at first or second calving of cow i, ybco = fixed effect of birth year-birth season-country of origin

**Table 1.** Description of milk production and reproductive performance traits (n = 278 cows).

	First lactation		Second lactation	
	Mean	SD	Mean	SD
Milk production trait				
Milk yield, kg	8334	1599	7951	2124
Fat yield, kg	349	79	351	102
Fat, %	4.26	0.89	4.45	0.60
Protein yield, kg	271	44	259	69
Protein, %	3.28	0.26	3.28	0.24
Lactose yield, kg	406	73	383	103
Lactose, %	4.89	0.23	4.82	0.25
Reproductive trait				
Gestation length, d	278	5	280	6
Calving interval, <sup>1</sup> d	431	71	417	72
Age at calving, d	824	81	1300	150
Number of services per conception	4.34	2.92	3.26	2.33

<sup>1</sup>Interval refers to first to second and second to third calving interval, respectively.

combination j (j = 1, ..., 8) of cow i, sire = random effect of sire k of cow i, and other effects as in model [1].

In model [3], birth year was grouped into 2 classes: 1996 and 1997. Birth season was grouped in a similar way to calving season. Country of origin included 2 countries: France and The Netherlands. A sire effect was included instead of cow in this case, because each cow had a single observation.

In all cases, data were analyzed with ASREML (Gilmour et al., 1998) for variance component estimation and PEST (Groeneveld, 1990) for estimation of effect solutions.

It should be pointed out that the genotypic effects evaluated here pertain to an overall linkage background with other genes situated near the test loci. Linkage effects may be different across different cow families. The above models do not accommodate such differences. Within cow-family analysis with more detailed pedigree data, spanning more generations than those available for this study would be required to contrast each allele in specific linkage situations.

**RESULTS AND DISCUSSION**

Means and standard deviations of milk production and reproductive performance traits of animals under study are presented in Table 1. Estimates for production traits are presented for first and second lactations.

**$\kappa$ -CN and  $\beta$ -LG Genotype Frequencies**

Frequency distribution of genotypes for  $\kappa$ -CN and  $\beta$ -LG is shown in Table 2. In the  $\kappa$ -CN system, only the genotypes AA and AB were observed, with frequencies of 0.89 and 0.11, respectively. The absence of genotype



**Table 2.** Frequency distribution of  $\kappa$ -CN and  $\beta$ -LG genotypes.

Milk protein	Genotype	Cows (n)	Frequency (%)
$\kappa$ -CN	AA	246	88.5
	AB	32	11.5
$\beta$ -LG	AA	79	28.4
	AB	131	47.1
	BB	68	24.5

BB could be attributed to chance, combined with the low frequency of the B variant in the Holstein breed (Arave et al., 1971; Lin et al., 1986; Ng-Kwai-Hang et al., 1990b; Famula and Medrano, 1994). Based on our data, estimated allelic frequencies were 0.94 and 0.06 for A and B, respectively. Expected genotypic frequencies were then calculated and compared with observed frequencies; a  $\chi^2$  value of 1.04 was found, suggesting the locus was in Hardy–Weinberg equilibrium (testing at the 0.05 level of significance).

In the  $\beta$ -LG system, all 3 genotypes were present with frequencies ranging from 0.25 to 0.47. These frequencies were similar to those reported in the literature for Holstein populations (Ng-Kwai-Hang et al., 1986; Aleandri et al., 1990; Famula and Medrano, 1994). Estimated allelic frequencies were 0.52 and 0.48 for A and B, respectively. Comparison of expected and observed genotypic frequencies yielded a  $\chi^2$  value of 0.87, suggesting that this locus, too, was in Hardy–Weinberg equilibrium (testing at the 0.05 level of significance).

### Effect of $\kappa$ -CN and $\beta$ -LG Genotypes on Milk Production Traits

**$\kappa$ -CN locus.** Mean differences (linear contrasts of solutions), standard errors of difference, and significance levels ( $P$  value) for milk yield and for fat, protein, and lactose yield and content, for animals with  $\kappa$ -CN genotypes AA and AB are given in Table 3. Variance component estimates for milk production traits are also shown in Table 3.

Mean differences resulted from fitting linear contrasts to the solutions of the  $\kappa$ -CN effect, under the

hypothesis that the difference between 2 genotypes was zero. The value of 414 ( $\pm$  267) kg in Table 3 suggested that the AB genotype might be associated with higher milk yield than the AA genotype, but the difference found here was not statistically different from zero ( $P = 0.12$ ). This result is consistent with reports by Arave et al. (1971), Lin et al. (1986), and Ng-Kwai-Hang et al. (1990b). However, other researchers (Ng-Kwai-Hang et al., 1986; Lin et al., 1989; Van Eenennaam and Medrano, 1991) reported significant differences corroborating the superiority of the  $\kappa$ -CN AB genotype for milk yield.

Fat yield for the  $\kappa$ -CN AB genotype was higher than for the AA genotype by 24 ( $\pm$  14) kg but the difference was not statistically greater than zero ( $P = 0.10$ ). All previous studies reported nonsignificant effect of  $\kappa$ -CN on fat yield. Animals with the  $\kappa$ -CN genotype AA did not differ significantly from animals with the AB genotype in fat content ( $P = 0.75$ ). McLean et al. (1984), Ng-Kwai-Hang et al. (1984), Gonyon et al. (1987), Van Eenennaam and Medrano (1991), Bovenhuis et al. (1992), and Lundén et al. (1997) have reported nonsignificant differences in fat content between the AA and AB genotypes.

The  $\kappa$ -CN genotype affected protein yield ( $P = 0.02$ ) and content ( $P = 0.03$ ). Animals with the AB genotype produced 21 ( $\pm$  9) kg of milk protein more and had protein content higher by 0.08% ( $\pm$  0.04) compared with animals with the AA genotype. Increased protein yield and content associated with the AB  $\kappa$ -CN genotype have been found in other studies (Ng-Kwai-Hang et al., 1984; Gonyon et al., 1987; Ng-Kwai-Hang et al., 1990b; Van Eenennaam and Medrano, 1991; Bovenhuis et al., 1992).

Lactose yield ( $P = 0.22$ ) and content ( $P = 0.13$ ) were not significantly affected by the  $\kappa$ -CN genotype. Lundén et al. (1997) reported similar results in the only other available report on this association.

**$\beta$ -LG locus.** Table 4 summarizes estimates (mean differences, standard errors of differences, and signifi-

**Table 3.** Variance component estimates and effect of  $\kappa$ -CN genotypes on milk production traits: mean difference (linear contrast of solutions), standard error of difference (SED), and significance level ( $P$  value).

Milk production trait	Genetic SD	Residual SD	$\kappa$ -CN genotypes	Mean difference	SED	$P$ value
Milk, kg	1014	1200	AA-AB	-414	267	0.12
Fat, kg	50	69	AA-AB	-24	14	0.10
Fat, %	0.37	0.58	AA-AB	-0.04	0.11	0.75
Protein, kg	32	38	AA-AB	-21	9	0.02*
Protein, %	0.15	0.17	AA-AB	-0.08	0.04	0.03*
Lactose, kg	49	61	AA-AB	-16	13	0.22
Lactose, %	0.10	0.21	AA-AB	0.06	0.04	0.13

\* $P < 0.05$ .

**Table 4.** Variance component estimates and effect of  $\beta$ -LG genotypes on milk production traits: mean difference (linear contrast of solutions), standard error of difference (SED), and significance level ( $P$  value).

Milk production trait	Genetic SD	Residual SD	$\beta$ -LG genotypes	Mean difference	SED	$P$ -value
Milk, kg	1008	1191	AA-AB	-485	204	0.02*
			AA-BB	-309	234	0.19
			AB-BB	176	212	0.41
Fat, kg	50	69	AA-AB	-27	11	0.02*
			AA-BB	-33	12	0.01*
			AB-BB	-6	11	0.60
Fat, %	0.37	0.57	AA-AB	-0.10	0.09	0.26
			AA-BB	-0.28	0.10	0.01*
			AB-BB	-0.18	0.09	0.04*
Protein, kg	32	38	AA-AB	-12	7	0.06
			AA-BB	-10	8	0.19
			AB-BB	2	7	0.72
Protein, %	0.15	0.17	AA-AB	0.03	0.03	0.38
			AA-BB	-0.00	0.03	0.97
			AB-BB	-0.03	0.03	0.37
Lactose, kg	49	61	AA-AB	-23	10	0.02*
			AA-BB	-12	11	0.29
			AB-BB	11	11	0.29
Lactose, %	0.09	0.21	AA-AB	-0.01	0.03	0.65
			AA-BB	0.01	0.03	0.73
			AB-BB	0.02	0.03	0.40

\* $P < 0.05$ .

cance levels) of the  $\beta$ -LG genotypic effects on milk yield and fat, protein, and lactose yield and content, along with variance component estimates for these traits. Similarly to  $\kappa$ -CN, mean differences here also resulted from fitting pairwise linear contrasts to the solutions of the  $\beta$ -LG effect, under the hypothesis that the difference between any 2 genotypes was zero.

Cows with the AB genotype produced 485 ( $\pm$  204) kg of milk more than animals with the AA genotype ( $P = 0.02$ ), and 176 ( $\pm$  212) kg of milk more than those with the BB genotype ( $P = 0.41$ ). Our findings are in agreement with those of Pupkova (1980). The significantly higher milk yield in  $\beta$ -LG AB cows confirms the trends reported by Ron et al. (1994), although their results were not statistically significant. However, many researchers have not found any significant association between the  $\beta$ -LG locus and milk yield (Arave et al., 1971; McLean et al., 1984; Ng-Kwai-Hang et al., 1984; Lin et al., 1986; Van Eenennaam and Medrano, 1991; Bovenhuis et al., 1992; Lundén et al., 1997; Ojala et al., 1997).

Fat yield was significantly influenced by  $\beta$ -LG genotype. Cows with AB and BB genotypes for  $\beta$ -LG produced more fat by 27 ( $\pm$  11) and 33 ( $\pm$  12) kg, respectively, compared with  $\beta$ -LG AA cows, suggesting possible dominance of the B allele. Other researchers, however, have shown no significant association between fat yield and  $\beta$ -LG genotypes (Arave et al., 1971; McLean et al., 1984; Van Eenennaam and Medrano, 1991; Bovenhuis et al., 1992; Lundén et al., 1997; Ojala et al., 1997). Fat content was also affected by the  $\beta$ -LG

locus. The B allele was favorable, with BB homozygotes producing significantly more ( $0.28 \pm 0.10\%$ ,  $P = 0.01$ ) than AA cows. The heterozygote AB genotype was intermediate, implying an additive locus for this trait. The superiority of the  $\beta$ -LG B allele for fat content found here is in agreement with the findings of McLean et al. (1984), Ng-Kwai-Hang et al. (1984, 1986), Bovenhuis et al. (1992), and Hill (1993).

In this study, the  $\beta$ -LG locus had no significant association with protein yield and content, although the B allele tended to show a favorable effect. These results are in agreement with the findings of several previous studies (McLean et al., 1984; Lin et al., 1986; Van Eenennaam and Medrano, 1991; Lundén et al., 1997; Ojala et al., 1997; Bobe et al., 1999). On the contrary, Arave et al. (1971) and Bovenhuis et al. (1992) suggested that the AA genotype might be associated with higher production of protein.

The AB genotype of the  $\beta$ -LG locus was associated with significantly higher ( $P = 0.02$ ) production of lactose in milk compared with the AA genotype, probably reflecting the effect of the AB genotype on milk yield. Otherwise, no significant associations of  $\beta$ -LG genotypes with lactose yield and content were found, in agreement with the only other pertinent study (Lundén et al., 1997).

Because 3 distinct genotypes were identified at the  $\beta$ -LG locus, multiple pairwise comparisons had to be made. For this reason, any nonsignificant mean difference was confirmed by implementing the Bonferroni

**Table 5.** Variance component estimates and effect of  $\kappa$ -casein ( $\kappa$ -CN) genotypes on reproductive traits: mean difference (linear contrast of solutions), standard error of difference (SED), and significance level ( $P$  value).

Reproductive trait	Genetic SD	Residual SD	$\kappa$ -CN genotypes	Mean difference	SED	$P$ value
Gestation length, d	2	5	AA-AB	1	1	0.55
Calving interval, d	25	65	AA-AB	-17	13	0.20
Age at first calving, d	6	66	AA-AB	-21	13	0.10
Age at second calving, d	10	120	AA-AB	-54	31	0.08
Number of services per conception	0.70	2.66	AA-AB	-0.51	0.44	0.24

test. No discrepancies to the above mentioned results were found.

### Effect of $\kappa$ -CN and $\beta$ -LG Genotypes on Reproductive Performance

**$\kappa$ -CN locus.** Table 5 presents the mean differences (linear contrasts of solutions), standard errors of difference, and significance levels of  $\kappa$ -CN genotypic effects on gestation length, calving interval, age at first and second calving, and number of services per conception. Variance component estimates for reproductive performance traits are included in Table 5.

No association was found between  $\kappa$ -CN genotypes and gestation length ( $P = 0.56$ ). This result is consistent with the findings of Lin et al. (1987).

The effect of  $\kappa$ -CN genotype on calving interval was not significant ( $P = 0.20$ ). However, calving interval was 17 ( $\pm 13$ ) days longer for the AB compared with the AA genotype. This tendency might have been related to the (nonsignificantly) higher milk yield associated with the AB genotype (Table 3) and the unfavorable association between milk yield and calving interval. It should be noted, however, that level of milk production was accounted for in the model of analysis of calving interval (model [2]). No other studies on the effect of  $\kappa$ -CN genotypes on calving interval were found in the literature.

Age at first calving was only marginally affected by  $\kappa$ -CN genotypes (AB > AA by 21 ( $\pm 13$ ) days,  $P = 0.10$ ). On the contrary, Jairam and Nair (1983) reported that cows with AB  $\kappa$ -CN genotype had younger age at first calving than the other genotypes, whereas Ronda and Perez-Beato (1983) and Lin et al. (1987) did not find significant associations. The discrepancies could be explained by the fact that Jairam and Nair (1983) and Ronda and Perez-Beato (1983) used animals that were cross-bred, and the study of Lin et al. (1987) involved 3 different genetic groups.

Age at second calving was affected by the  $\kappa$ -CN locus in similar way as age at first calving (AB > AA by 54  $\pm$  31 d,  $P = 0.08$ ). No other studies examining the association between age at second calving and genetic

polymorphism of  $\kappa$ -CN have been found in the literature.

Animals having the AA  $\kappa$ -CN genotype needed 0.51  $\pm$  0.44 fewer services per conception compared with the AB genotype. The difference, however, was not significant ( $P = 0.24$ ), in agreement with the findings of Ng-Kwai-Hang et al. (1990a).

**$\beta$ -LG locus.** Mean differences (linear contrasts of solutions), standard errors of difference, and significance levels for the reproductive traits are given in Table 6.

A significant ( $P < 0.05$ )  $\beta$ -LG effect was observed on gestation length. Animals with AB and BB genotypes had longer gestation length by just less than 2 d compared with animals with AA genotype. Similar results have been previously reported (Lin et al., 1987). Although differences found here were significant, we do not expect them to have any economic impact on animal improvement.

In this study the  $\beta$ -LG locus had no significant effect on calving interval. No publications concerning the association of  $\beta$ -LG genotypes with calving interval were found in the literature.

Age at first calving was not statistically different for animals with different  $\beta$ -LG genotypes. These findings are in agreement with the results of Ronda and Perez-Beato (1983). Jairam and Nair (1983), however, concluded that heifers having the AB  $\beta$ -LG genotype in combination with AB  $\kappa$ -CN genotype had lower age at first calving, possibly suggesting epistatic associations between the 2 loci.

No significant associations were found between the genetic polymorphism of  $\beta$ -LG and age at second calving. No other studies on the effect of  $\beta$ -LG genotypes on this reproductive trait were found in the literature.

Furthermore, the effect of the  $\beta$ -LG locus on number of services per conception was found to be nonsignificant, consistent with the study of Ng-Kwai-Hang et al. (1990a) that involved 4819 Holstein cows.

Because 3 distinct genotypes were identified at the  $\beta$ -LG locus, multiple pairwise comparisons had to be made. For this reason, any nonsignificant mean differ-

**Table 6.** Variance component estimates and effect of  $\beta$ -LG genotypes on reproductive traits: mean difference (linear contrast of solutions), standard error of difference (SED), and significance level (*P* value).

Reproductive trait	Genetic SD	Residual SD	$\beta$ -LG genotypes	Mean difference	SED	<i>P</i> value
Gestation length, d	2	5	AA-AB	-2	1	0.01*
			AA-BB	-2	1	0.03*
			AB-BB	0	1	0.95
Calving interval, d	25	64	AA-AB	2	10	0.83
			AA-BB	6	12	0.60
			AB-BB	4	11	0.70
Age at first calving, d	7	66	AA-AB	-4	10	0.69
			AA-BB	7	11	0.55
			AB-BB	11	10	0.30
Age at second calving, d	11	121	AA-AB	3	24	0.91
			AA-BB	-5	27	0.87
			AB-BB	-8	24	0.76
Number of services per conception	0.69	2.58	AA-AB	0.02	0.33	0.95
			AA-BB	-0.39	0.38	0.31
			AB-BB	-0.41	0.35	0.24

\**P* < 0.05.

ence was also confirmed by implementing the Bonferroni test. No discrepancies to the above mentioned results were found.

## CONCLUSIONS

The results of the present study indicate that  $\kappa$ -CN and  $\beta$ -LG genotypes influence certain milk production traits. The AB  $\kappa$ -CN genotype had a positive effect on protein yield and content. The B variant of  $\beta$ -LG was associated with higher milk yield, and fat yield and content. Therefore, these genotypes appear to be obvious candidates for selection aiming at improving milk production traits.

Reproductive performance was virtually unaffected by  $\kappa$ -CN and  $\beta$ -LG genotypes. Results of this study suggest that selection for favorable milk protein genotypes should not be expected to influence the reproductive performance of dairy cattle.

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