

# The Effects of Milk Protein Polymorphisms on Milk Components and Cheese-Producing Ability

R. ALEANDRI L. G. BUTTAZZONI and J. C. SCHNEIDER

Associazione Italiana Allevatori

Via G. Tomassetti 9

00161 Rome, Italy

A. CAROLI

Università di Milano

Milano, Italy

R. DAVOLI

Università di Bologna

Reggio Emilia, Italy

## ABSTRACT

A Total of 2005 first lactation Holstein-Friesian cows with known 305-d lactation yield for milk, fat, and protein were available. For each cow, genotypes for  $\alpha_{s1}$ -casein,  $\beta$ -casein,  $\kappa$ -casein, and  $\beta$ -lactoglobulin were known. It appears that the milk protein variants  $\alpha_{s1}$ -casein,  $\beta$ -casein, and  $\kappa$ -casein may not be segregating independently. Effects of genetic variants of milk proteins on estimated individual Parmesan cheese yields were investigated. The relationships of the genetic variants of milk proteins to total lactation milk yield, fat yield, protein yield, fat percentage, and protein percentage were also investigated. Least squares analysis of the data indicated that  $\alpha_{s1}$ -casein genotype significantly influenced milk yield, fat yield, and protein yield with the highest yields obtained for the genotype *BB*. Cheese yield on a fixed amount of milk and fat percentage were significantly related to  $\beta$ -lactoglobulin genotype with the highest estimates obtained for *BB*. Protein percentage was influenced by  $\alpha_{s1}$ -casein and  $\kappa$ -casein, with the genotypes *BC* and *BB*, respectively, having the highest percentages. Significantly higher lactation cheese yields were estimated with  $\alpha_{s1}$ -casein genotype *BB*. Using the prediction equation

to estimate cheese yield (on data from another study), it was found that differences in Parmesan cheese yield from milk of either  $\kappa$ -casein genotype *AA* or *BB* were greater than expected based on composition. Differences in salted curd yield from another study using milk of either  $\beta$ -lactoglobulin genotype *AA* or *BB* were also greater than expected.

(Key words: milk protein polymorphisms, milk components, cheese yield)

## INTRODUCTION

The properties of milk that are important for cheese making are currently considered to be those of composition. Thus, any environmental or genetic factors affecting milk composition would also be expected to affect its capacity to produce cheese. The relationships between milk protein polymorphisms and milk composition have been well-documented (27, 30). Because the type of casein (CN) and  $\beta$ -lactoglobulin ( $\beta$ -LG) are controlled by autosomal genes, it may be important to verify the existence of genotypes that give better results in terms of cheese production.

It has generally been agreed that  $\beta$ -LG genotype *BB* is associated with a higher casein percentage in milk (5, 15) and  $\beta$ -LG *AA* has been associated with increased whey protein fraction (5, 25). Some authors (24, 25) have found an association of  $\beta$ -LG genotype *AA* with higher protein percentage and protein yield, but others (21, 23) found no association. One study (19) reported that  $\beta$ -LG *AA* was associated with higher curd firmness but another (31) found the

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*BB* genotype to be best.  $\beta$ -Lactoglobulin *BB* was associated with higher theoretical cheese yield (18) and higher Parmesan cheese yield (23).

Homozygous A genotypes of  $\beta$ -CN ( $A_1$ ,  $A_2$ , or  $A_3$ ) have been found to be associated with higher fat percentage, fat yield, and protein yield but not with protein percentage in first lactation cows (24). A later study, conducted over many lactations, found the  $\beta$ -CN genotype  $A_1B$  to be related with a higher protein percentage (25). The  $\beta$ -CN genotype, after adjustment for amount of  $\beta$ -CN, did not significantly influence curd firmness, as measured by the formagraph (19), but a later study (26) found  $\beta$ -CN genotype did significantly affect curd firmness, although the best genotype was not reported and very different enzymes were used. Both studies concluded that the amount of  $\beta$ -CN influenced curd firmness. The  $\beta$ -CN genotype  $A_1A_1$  was significantly associated with both theoretical and actual cheese yield in a trial in which only  $\beta$ -CN genotypes  $A_1A_1$  and  $A_1A_2$  had been considered (18).

Some studies found that  $\kappa$ -casein genotype *BB* is associated with higher protein percentage (1, 24), but another study (17) found that the *BB* genotype was only associated with higher casein percentage without an increase in protein percentage. The  $\kappa$ -CN *BB* genotype has also been associated with protein yield (24). Amount of  $\kappa$ -CN was significantly related to curd firmness as measured by the width of the formagraph at the end of 30 min (19). Schaar (30) found that the *AA* genotype took significantly longer to achieve a graph width of 30 mm using the formagraph, and Mariani et al. (14) found  $\kappa$ -CN *BB* to be better for curd firmness with the same instrument. The  $\kappa$ -CN *BB* produced more cheese under laboratory conditions (18). Mariani et al. (14) and Morini et al. (22) found that milk with  $\kappa$ -CN genotype *BB* yielded about 10% more Parmesan cheese.

Aleandri et al. (1) and Ng-Kwai-Hang et al. (25) found  $\alpha_{s1}$ -casein significantly influenced protein percentage. Ng-Kwai-Hang et al. (24), studying first lactation cows, found only a significant influence on protein yield, with the *BB* genotype being best. Kroeker et al. (13) found that the  $\alpha_{s1}$ -CN phenotype did not affect the casein fraction.

The existence of different physical properties for the genotypes of the milk protein vari-

ants presents the possibility that cheese yield could be improved beyond what can be explained by their association with milk composition. Differences in calcium caseinate micelle stability (6), micelle stability to rennin (7), and heat stability (8) have been reported for the genotypes of  $\alpha_{s1}$ -,  $\beta$ -, and  $\kappa$ -CN as well as  $\beta$ -LG. One study (18) examined Cheddar-type cheese yield (made in the laboratory) from milks of different milk protein genotypes while adjusting for the amounts of these proteins as well as other components of milk composition. This study found that milk with  $\beta$ -CN  $A_1A_1$ ,  $\kappa$ -CN *BB*, and  $\beta$ -LG *BB* had higher cheese yields. Other studies have examined Parmesan cheese yield from milk of different genotypes for  $\kappa$ -CN (14) and  $\beta$ -LG (23), balancing the genotypes of the other milk protein variants. These studies found that  $\kappa$ -CN *BB* and  $\beta$ -LG *BB* were associated with higher cheese yields.

The existence of linkage between the loci of the milk protein variants must be taken into consideration when selecting for a given genotype. Unusually high frequencies have been found for the allele combinations *BBB* and  $CA_3A$  of  $\alpha_{s1}$ -,  $\beta$ -, and  $\kappa$ -CN, respectively (11). Another study (12) found no occurrences of  $\alpha_{s1}$ -CN allele *C* with  $\beta$ -CN allele *B* in three breeds of cattle. A study that crossed bulls with genotype  $\alpha_{s1}$ -CN *BC* and  $\beta$ -CN *AB* with cows homozygous at these loci found that only the combinations  $\alpha_{s1}$ -CN *B* and  $\beta$ -CN *B*, and  $\alpha_{s1}$ -CN *C* and  $\beta$ -CN *A* were transmitted to the offspring (10). Because this study also found that 6 out of 400 genotyped cows chosen from the population were  $\alpha_{s1}$ -CN *BC*  $\beta$ -CN *BB*, close linkage between these loci is assumed. Links have also been found between the *J* system (a blood group marker) and  $\beta$ -LG by Mather (20), who presents arguments for the combination of the caseins into a "super gene" and the need to consider interactions in evaluating genotypes.

All of the past work was concerned with improving the cheese to milk ratio. It seems of some importance to strike a balance between selection for individual cow's milk yield and the ability of this milk to produce cheese. This specific aspect can be investigated if the cheese yield potential of individual milk is known. It is the purpose of this study to estimate the effects

of different genotypes at milk protein loci on the cheese producing capacity of the individual cows, based on a Parmesan cheese yield prediction equation (2). Some consideration is also given to the difference between expected cheese yield based on our equation and actual cheese yield from other studies (14, 23) and to the potential linkage between loci.

## MATERIALS AND METHODS

### Milk Records

A total of 4225 registered Holstein-Friesian cows were typed for the milk protein variants of  $\alpha_{s1}$ -CN,  $\beta$ -CN,  $\kappa$ -CN, and  $\beta$ -LG between 1981 and 1985. Protein variants for each cow were determined by starch gel electrophoresis (3, 4) on milk samples treated with  $\text{NaN}_3$  and frozen. The electrophoretic patterns of six combinations of  $\alpha_{s1}$ -CN,  $\beta$ -CN,  $\kappa$ -CN, and  $\beta$ -LG are presented in Figure 1. The patterns are all  $\alpha_{s1}$ -CN B  $\beta$ -CN A and represent six of the nine possible genotype combinations of  $\kappa$ -CN and  $\beta$ -LG. The electrophoretic patterns of four of the six  $\beta$ -CN genotypes found in our study are presented in Figure 2.

Each cow was required to have a first lactation record of at least 305 d in milk and a valid genotype for all four protein variants. All levels of classification factors were required to have at least two observations. After such screening, 2005 official first lactation records in 84 herds were used. Of these, 1405 observations came from a study conducted in 1985 and the other 600 came from a study conducted in 1981. Because of the techniques used, no distinction was possible between the alleles  $A_1$ ,  $A_2$ , or  $A_3$  of  $\beta$ -CN in the older experiment, and thus in the combined data set, these alleles were simply coded as A for all records. The 1405 more recent observations (only 1383 usable due to some small subclasses) were also analyzed as a separate subset to take advantage of the more complete coding of the  $\beta$ -CN alleles. Officially recorded milk, protein, and fat yields were used.

### Prediction of Cheese Yield

The Parmesan cheese yield prediction equation developed by an earlier study (2) based on vats of milk (on average 1000 kg) used in the production of Parmesan cheese is:

$$\text{Cheese yield} = 28.3329 + .9877 \times \text{fat kg} + .0179 \times \text{protein kg}^2$$

This study also developed a prediction equation incorporating the curd firmness measure ( $E_{30}$ ) of the formagraph, but it was decided not to use this particular formula since an acceptable measure of this value for an entire lactation was not calculated.

The Parmesan cheese maker normally skims fat from the milk before processing and in the data set used to develop the prediction equation an average of 28% of the milk fat was removed. This percentage may, in practice, be considerably higher. The fat of each cow was reduced by 28% to approximate the treatment by the cheese maker. Because the prediction equation was developed to predict cheese yield from an entire vat of milk, it was not directly applicable to the individual cow lactations. This problem was overcome by calculating cheese

TABLE 1. Number of the genotypes for  $\kappa$ -casein ( $\kappa$ -CN),  $\alpha_{s1}$ -casein ( $\alpha_{s1}$ -CN),  $\beta$ -casein ( $\beta$ -CN), and  $\beta$ -lactoglobulin ( $\beta$ -LG).

| Protein                                     | Genotype | (no.) | (%)  |
|---|----------|-------|------|
|   |          |       |      |
| $\kappa$ -CN                                | AA       | 1094  | 64.5 |
|   | AB       | 821   | 40.9 |
|   | BB       | 90    | 4.5  |
| $\alpha_{s1}$ -CN                           | BB       | 1935  | 96.5 |
|   | BC       | 70    | 3.5  |
| $\beta$ -CN                                 | AA       | 1902  | 94.8 |
|   | AB       | 103   | 5.2  |
| $\beta$ -LG                                 | AA       | 343   | 17.1 |
|   | AB       | 957   | 47.7 |
|   | BB       | 705   | 35.1 |
| — Subset with all $\beta$ -casein alleles — |          |       |      |
| $\kappa$ -CN                                | AA       | 706   | 51.0 |
|   | AB       | 612   | 44.3 |
|   | BB       | 65    | 4.7  |
| $\alpha_{s1}$ -CN                           | BB       | 1326  | 95.9 |
|   | BC       | 57    | 4.1  |
| $\beta$ -CN                                 | $A_1A_1$ | 440   | 31.8 |
|   | $A_1A_2$ | 681   | 49.2 |
|   | $A_1B$   | 30    | 2.2  |
|   | $A_2A_2$ | 196   | 14.2 |
|   | $A_2A_3$ | 4     | .3   |
|   | $A_2B$   | 32    | 2.3  |
| $\beta$ -LG                                 | AA       | 227   | 16.4 |
|   | AB       | 667   | 48.2 |
|   | BB       | 489   | 35.4 |

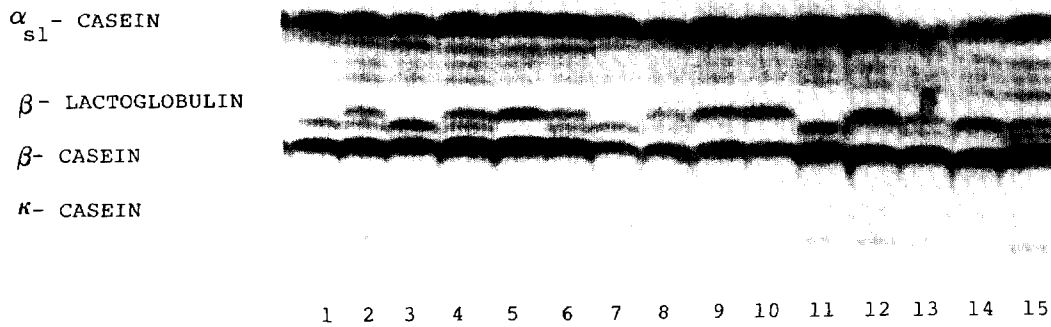


Figure 1. Starch-gel electrophoresis at alkaline pH (4). Samples are monomorphic for  $\alpha_{s1}$ -casein B and  $\beta$ -casein A alleles. Genotypes at  $\beta$ -lactoglobulin and  $\kappa$ -casein loci are, respectively: 1) BB/AA, 2) AB/AA, 3) BB/AB, 4) AB/AA, 5) AA/AB, 6) AB/AA, 7) BB/AA, 8) AB/AB, 9) AA/AB, 10) AA/AA, 11) BB/AA, 12) AA/AA, 13) AB/AA, 14) AA/AB, 15) AB/AA.

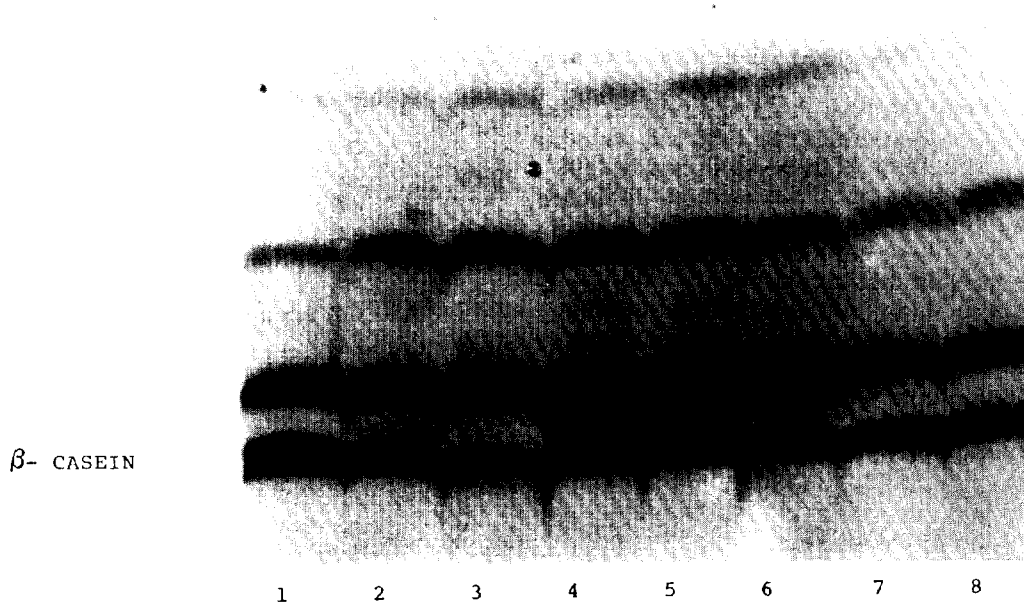


Figure 2. Starch-gel electrophoresis at acid pH (3). Genotypes at  $\beta$ -casein locus are, respectively: 1)  $A_2B$ , 2)  $A_1A_1$ , 3)  $A_2A_2$ , 4)  $A_1A_2$ , 5)  $A_1A_2$ , 6)  $A_1A_1$ , 7)  $A_1A_2$ , 8)  $A_1A_2$ .

yield based on 1000 kg of skim milk with the same protein percentage as recorded for the cow's lactation. Cheese yield calculated on 1000 kg skim cow's milk was considered one measure of potential cheese yield, which we called the "vat yield". The number of times a cow yielded 1000 kg of milk was determined by dividing her 305-d milk yield by 1000, and the calculated vat yield was multiplied by this number to come up with a total lactation cheese yield estimate. The formula was also applied in this way while holding fat constant at 1.8%, which is considered the minimum acceptable level for Parmesan cheese making (33). This measure has only a theoretical interest because of the variability of processing techniques among actual cheese plants (2). Individual Parmesan cheese yield for each cow was calculated in four different ways: 1) lactation cheese yield using the average skimming factor; 2) lactation cheese yield holding fat constant at 1.8%; 3) vat yield using the average skimming factor; and 4) vat yield holding fat constant at 1.8%.

Lactation and the vat cheese yields estimated using the skim milk represent the way the

Parmesan cheese would be made in the typical regions of Italy. The other estimates, based on a constant fat percentage, assume more rigorous processing control by the cheese maker and may better represent an "industrial" way of making cheese.

#### Statistical Analysis

Data were analyzed with a fixed linear model considering the classification factors of herd, season by year of calving, and the four protein variant genotypes. Seasons were defined as period of 3 mo each, beginning in January. The dependent variables were milk, fat yield, protein yield, fat percentage, protein percentage, and four estimated cheese yields as previously described. The model was:

$$y_{ijklmnp} = \mu + H_i + Y \times S_j + \alpha_{s1-CN_k} + \beta-CN_l + \kappa-CN_m + \beta-LG_n + e_{ijklmnp}$$

where:

TABLE 2. Partial sums of squares and significance of the sources of variation for total lactation milk yield, fat yield, and protein yield.

| Source <sup>1</sup>                     | df   | Milk ( $\times 10^4$ ) | Fat ( $\text{kg} \times 10^4$ ) | Protein ( $\text{kg} \times 10^4$ ) |
|---|------|------------------------|---------------------------------|-------------------------------------|
| Complete data set                       |      |                        |                                 |                                     |
| $\mu$                                   | 1    | 2656.93**              | 340.90**                        | 267.44**                            |
| H                                       | 83   | 795.04**               | 130.64**                        | 83.05**                             |
| S $\times$ Y                            | 48   | 245.35**               | 19.35**                         | 22.66**                             |
| $\alpha_{s1-CN}$                        | 1    | 5.41**                 | 1.55**                          | .29*                                |
| $\beta-CN$                              | 1    | 1.05                   | .20                             | .07                                 |
| $\kappa-CN$                             | 2    | 1.13                   | .02                             | .39†                                |
| $\beta-LG$                              | 2    | 3.68†                  | .24                             | .55*                                |
| Error                                   | 1867 | 1352.43                | 228.44                          | 125.82                              |
| R <sup>2</sup>                          | .44  | .40                    | .46                             |                                     |
| Subset with all $\beta$ -casein alleles |      |                        |                                 |                                     |
| $\mu$                                   | 1    | 2314.92**              | 278.34**                        | 229.31**                            |
| H                                       | 15   | 411.63**               | 55.54**                         | 41.44**                             |
| S $\times$ Y                            | 36   | 183.97**               | 14.33**                         | 15.79**                             |
| $\alpha_{s1-CN}$                        | 1    | 4.14*                  | 1.32**                          | .20†                                |
| $\beta-CN$                              | 5    | 1.65                   | .35                             | .13                                 |
| $\kappa-CN$                             | 2    | 2.58                   | .03                             | .53*                                |
| $\beta-LG$                              | 2    | 3.42                   | .15                             | .49*                                |
| Error                                   | 1321 | 1049.81                | 175.83                          | 92.47                               |
| R <sup>2</sup>                          |      | .38                    | .31                             | .41                                 |

<sup>1</sup> $\mu$  = Intercept, H = herd, S  $\times$  Y = season of calving and year, CN = casein, and LG = lactoglobulin.

\* $P < .05$ .

\*\* $P < .01$ .

† $P < .10$ .

TABLE 3. Least square estimates (LSE) of the effects of different genotypes and their standard errors for total lactation milk yield, total fat yield, and total protein yield.

| Component <sup>1</sup> | Genotype                      | Milk yield |     | Fat (kg) |      | Protein (kg) |      |
|------------------------|-------------------------------|------------|-----|----------|------|--------------|------|
|                        |                               | LSE        | SE  | LSE      | SE   | LSE          | SE   |
| $\alpha_{s1}$ -CN      | BB                            | 294        | 108 | 16       | 4.4  | 6.8          | 3.3  |
|                        | BC                            | 0          |     | 0        |      | 0            |      |
| $\beta$ -CN            | AA                            | 114        | 95  | 5        | 3.8  | 2.9          | 2.9  |
|                        | AB                            | 0          |     | 0        |      | 0            |      |
| $\kappa$ -CN           | AA                            | -47        | 99  | -1.3     | 4.1  | -5.6         | 3.0  |
|                        | AB                            | 5          | 98  | -.8      | 4.0  | -3.0         | 3.0  |
|                        | BB                            | 0          |     | 0        |      | 0            |      |
| $\beta$ -LG            | AA                            | 126        | 60  | -3.4     | 2.4  | 4.7          | 1.8  |
|                        | AB                            | 72         | 44  | -9       | 1.8  | 3.0          | 1.3  |
|                        | BB                            | 0          |     | 0        |      | 0            |      |
| $\alpha_{s1}$ -CN      | BB                            | 299        | 131 | 16.9     | 5.4  | 6.6          | 3.9  |
|                        | BC                            | 0          |     | 0        |      | 0            |      |
| $\beta$ -CN            | A <sub>1</sub> A <sub>1</sub> | 61         | 171 | 7.9      | 7.0  | 3.8          | 5.1  |
|                        | A <sub>1</sub> A <sub>2</sub> | 55         | 171 | 7.0      | 7.0  | 3.5          | 5.1  |
|                        | A <sub>1</sub> B              | -76        | 235 | 4.7      | 9.6  | 1.3          | 7.0  |
|                        | A <sub>2</sub> A <sub>2</sub> | 119        | 181 | 6.1      | 7.4  | 5.3          | 5.4  |
|                        | A <sub>2</sub> A <sub>3</sub> | 449        | 500 | 26.5     | 20.5 | 13.9         | 14.8 |
|                        | A <sub>2</sub> B              | 0          |     | 0        |      | 0            |      |
| $\kappa$ -CN           | AA                            | -65        | 124 | .3       | 5.1  | -5.9         | 3.7  |
|                        | AB                            | 31         | 121 | 1.3      | 5.0  | -1.8         | 3.6  |
|                        | BB                            | 0          |     | 0        |      | 0            |      |
| $\beta$ -LG            | AA                            | 155        | 75  | -2.2     | 3.1  | 5.9          | 2.2  |
|                        | AB                            | 59         | 55  | -2.3     | 2.2  | 2.5          | 1.6  |
|                        | BB                            | 0          |     | 0        |      | 0            |      |

<sup>1</sup>CN = Casein, LG = lactoglobulin.

$y_{ijklmnp}$  = the observation  $p$  of one of the nine dependent variables,

$\mu$  = the intercept,

$H_i$  = the herd  $i$  ( $i = 1, 2, 3, \dots, 84$ ),

$Y \times S_j$  = the year and season  $j$  of calving ( $j = 1, 2, \dots, 48$ ),

$\alpha_{s1}$ -CN <sub>$k$</sub>  = the  $\alpha_{s1}$ -CN genotype  $k$  ( $k = 1, 2$ ),

$\beta$ -CN <sub>$l$</sub>  = the  $\beta$ -CN genotype  $l$  ( $l = 1, 2$  or  $l = 1, 2, \dots, 6$ ),

$\kappa$ -CN <sub>$m$</sub>  = the  $\kappa$ -CN genotype  $m$  ( $m = 1, 2, 3$ ),

$\beta$ -LG <sub>$n$</sub>  = the  $\beta$ -LG genotype  $n$  ( $n = 1, 2, 3$ ), and

$e_{ijklmnp}$  = the residual error.

Models evaluating interactions among genotypes were also considered, but no interactions were significant. For this reason only the results of the main effect models are reported.

#### Comparison with Other Studies

The prediction equation was used to estimate cheese yield based on data from studies by Mariani et al. (14) and Morini et al. (23), which used milks differing only in the genotypes of one milk protein variant. Actual cheese yields from Mariani's trial are reported by Russo et al. (27). Because the study by Morini et al. (23) reported only salted curd yield, it was necessary to return to the original data set used to develop the equation for 6-mo aged cheese yield (2) and reestimate the coefficients for salted curd using the same model of analysis. The prediction equation was:

$$\begin{aligned} \text{Salted curd yield} = & 29.4221 + 1.0954 \\ & \times \text{fat (kg)} + .0162 \\ & \times \text{protein (kg)}^2 \end{aligned}$$

The coefficients were very similar to those reported for 6-mo aged cheese. The differences

between actual and predicted yields were calculated.

#### Consideration of Linkage

Allelic frequencies were determined by gene counting. The combination of the alleles on one specific chromosome is known as haplotype (11). Differences between the expected frequencies of the haplotypes and their actual frequencies could be an indication of linkage among loci. The actual frequencies of the haplotypes cannot always be seen from genotypes, and they were estimated by making all possible haplotype combinations for each cow's genotype of  $\alpha_{s1}$ -,  $\beta$ -, and  $\kappa$ -CN, eight for each cow (23). For example, a cow completely homozygous at all three loci would contribute eight identical haplotypes to the frequency determination. The potential haplotypes of all cows were then counted, and the frequency of each haplotype was determined by dividing its number by eight times the number of cows. The expected frequency of each possible haplotype was determined as if the alleles were segregating independently by multiplying the frequencies of the individual alleles in the data set.

The relationship between casein loci and the  $\beta$ -LG were studied by considering the allelic combinations  $\alpha_{s1}$ -,  $\beta$ -, and  $\kappa$ -CN as one gene and then determining the combination of this composite gene with  $\beta$ -LG. The expected results were determined by multiplying the frequency of each casein haplotype by the frequency of the  $\beta$ -LG allele. The expected and observed frequencies were compared using a continuity adjusted chi-square test (9).

## RESULTS AND DISCUSSION

#### Gene Frequencies

Table 1 contains the frequencies of each milk protein genotype for both the combined data set and the subset with all the  $\beta$ -CN alleles typed. The gene frequencies are very close to those reported in other studies (16, 28). In our study the most prevalent alleles were A for  $\kappa$ -CN, B for  $\alpha_{s1}$ -CN, A for  $\beta$ -CN ( $A_1$  for the subset), and B for  $\beta$ -LG. The C allele of  $\alpha_{s1}$ -CN is rare and appeared only as heterozygous BC. The rare A allele of  $\alpha_{s1}$ -CN (32) was not found. The majority of the  $A_2$  allele for  $\beta$ -CN was found in combination with the  $A_1$  allele.

TABLE 4. Partial sums of squares and significance of the sources of variation for fat percentage and protein percentage.

| Source                                  | df   | Fat %    | Protein % |
|---|------|----------|-----------|
| Complete data set                       |      |          |           |
| $\mu$                                   | 1    | 1242.9** | 966.6**   |
| H                                       | 83   | 92.0**   | 11.9**    |
| S $\times$ Y                            | 48   | 28.6**   | 3.9**     |
| $\alpha_{s1}$ -CN                       | 1    | .3       | .2*       |
| $\beta$ -CN                             | 1    | .1       | .0        |
| $\kappa$ -CN                            | 2    | .2       | .5**      |
| $\beta$ -LG                             | 2    | 2.9**    | .1        |
| Error                                   | 1867 | 334.6    | 67.7      |
| R <sup>2</sup>                          |      | .31      | .20       |
| Subset with all $\beta$ -casein alleles |      |          |           |
| $\mu$                                   | 1    | 877.9**  | 719.9**   |
| H                                       | 15   | 51.9**   | 6.1**     |
| S $\times$ Y                            | 36   | 23.5**   | 2.8**     |
| $\alpha_{s1}$ -CN                       | 1    | .3       | .2*       |
| $\beta$ -CN                             | 5    | .6       | .1        |
| $\kappa$ -CN                            | 2    | .4       | .3**      |
| $\beta$ -LG                             | 2    | 1.9**    | .1        |
| Error                                   | 1321 | 276.9    | 43.0      |
| R <sup>2</sup>                          |      | .23      | .19       |

<sup>1</sup> $\mu$  = Intercept, H = herd, S  $\times$  Y = season of calving and year, CN = casein, and LG = lactoglobulin.

\* $P < .05$ .

\*\* $P < .01$ .

The rare *B* allele of  $\beta$ -CN was observed only in heterozygotes. The majority of cows were heterozygous for  $\beta$ -LG.

#### Yield Traits

Selection for cheese yield may be approached in different ways. One is to improve the yield per cheese vat, in which case, it would be best to select for cows that produce milk that results in the best cheese to milk ratio. Another approach is to improve the total lactation cheese yield per cow in which case a balance must be achieved between milk yield and capacity of milk to produce cheese.

The relationships of the milk protein variants to individual milk components and yield traits will be presented first in order to compare them with results from earlier studies. Table 2 presents the analysis of variance for total lacta-

tion milk yield, total fat yield, and total protein yield for both the complete data set and the subset. Milk yield, fat yield, and to a lesser degree, protein yield are significantly influenced by the genotype of  $\alpha_{s1}$ -CN. An earlier study by Aleandri et al. (1) found no influence of  $\alpha_{s1}$ -CN genotype on milk yield, but that study considered a shorter lactation length. A significant influence of  $\beta$ -LG on milk yield in the complete data set was also found. Examining the relative size of the estimates in Table 3, the  $\alpha_{s1}$ -CN genotype *BB* appears to yield almost 294 kg more milk and 16 kg more fat than  $\alpha_{s1}$ -CN *BC*. The  $\beta$ -LG genotype *AA* was estimated to yield up to 126 kg more milk per lactation than the *BB* genotype with the *AB* genotype having an intermediate value. This result is supported by the work of Ng-Kwai-Hang (24) on first lactation cows and a later study (25) on cows of all lactations. However, that author found a significant association of

TABLE 5. Least square estimates (LSE) of the effects of different genotypes and their standard errors on fat and protein percentage.

| Component <sup>1</sup> | Genotype                          | Fat %             |     |      |     | Protein %                                   |    |     |    |
|------------------------|-----------------------------------|-------------------|-----|------|-----|---|----|-----|----|
|                        |                                   | Complete data set |     |      |     | Subset set with all $\beta$ -casein alleles |    |     |    |
|                        |                                   | LSE               | SE  | LSE  | SE  | LSE   | SE | LSE | SE |
| $\alpha_{s1}$ -CN      | <i>BB</i>                         | .066              | .05 | -.05 | .02 |   |    |     |    |
|                        | <i>BC</i>                         | 0                 |     | 0    |     |   |    |     |    |
| $\beta$ -CN            | <i>AA</i>                         | .028              | .04 | -.01 | .02 |   |    |     |    |
|                        | <i>AB</i>                         | 0                 |     | 0    |     |   |    |     |    |
| $\kappa$ -CN           | <i>AA</i>                         | .002              | .04 | -.08 | .02 |   |    |     |    |
|                        | <i>AB</i>                         | -.02              | .04 | -.06 | .02 |   |    |     |    |
|                        | <i>BB</i>                         | 0                 |     | 0    |     |   |    |     |    |
| $\beta$ -LG            | <i>AA</i>                         | -.12              | .03 | .018 | .01 |   |    |     |    |
|                        | <i>BB</i>                         | -.04              | .02 | .015 | .01 |   |    |     |    |
|                        | <i>BB</i>                         | 0                 |     | 0    |     |   |    |     |    |
| $\alpha_{s1}$ -CN      | <i>BB</i>                         | .09               | .07 | -.06 | .03 |   |    |     |    |
|                        | <i>BC</i>                         | 0                 |     | 0    |     |   |    |     |    |
| $\beta$ -CN            | <i>A<sub>1</sub>A<sub>1</sub></i> | .12               | .09 | .04  | .03 |   |    |     |    |
|                        | <i>A<sub>1</sub>A<sub>2</sub></i> | .09               | .09 | .04  | .03 |   |    |     |    |
|                        | <i>A<sub>1</sub>B</i>             | .10               | .12 | .07  | .05 |   |    |     |    |
|                        | <i>A<sub>2</sub>A<sub>2</sub></i> | .07               | .09 | .03  | .04 |   |    |     |    |
|                        | <i>A<sub>2</sub>A<sub>3</sub></i> | .16               | .26 | -.01 | .10 |   |    |     |    |
|                        | <i>A<sub>2</sub>B</i>             | 0                 |     | 0    |     |   |    |     |    |
| $\kappa$ -CN           | <i>AA</i>                         | .04               | .06 | -.08 | .03 |   |    |     |    |
|                        | <i>AB</i>                         | .00               | .06 | -.06 | .02 |   |    |     |    |
|                        | <i>BB</i>                         | 0                 |     | 0    |     |   |    |     |    |
| $\beta$ -LG            | <i>AA</i>                         | -.12              | .03 | .02  | .02 |   |    |     |    |
|                        | <i>AB</i>                         | -.05              | .03 | .01  | .01 |   |    |     |    |
|                        | <i>BB</i>                         | 0                 |     | 0    |     |   |    |     |    |

<sup>1</sup>CN = Casein, LG = lactoglobulin.



homozygous  $\beta$ -CN A alleles with fat yield in first lactation cows and a significant association of  $\beta$ -CN and  $\kappa$ -CN with milk yield for cows of all lactations, results not confirmed by this study.

Protein yield was significantly associated with the genotypes for  $\alpha_{s1}$ -CN,  $\kappa$ -CN, and  $\beta$ -LG (Table 2). Judging by the relative size of the estimates in Table 3 the best genotypes are *BB*, *BB*, and *AA* for  $\alpha_{s1}$ -CN,  $\kappa$ -CN, and  $\beta$ -LG, respectively. It should be kept in mind that  $\beta$ -LG genotype *AA* is associated with increased whey protein percentage (5) and milk higher in whey protein would not improve cheese yield. The association of  $\alpha_{s1}$ -CN and  $\beta$ -LG with higher milk yield may also contribute to their association with higher protein yield.

#### Percentages

The results of the analysis of fat and protein percentage are presented in Table 4. Fat percentage was only significantly associated with the genotype of  $\beta$ -LG.  $\beta$ -LG *BB* had the largest

effect (Table 5) with an estimate .12% greater than that for *AA*. Protein percentage was significantly influenced by  $\alpha_{s1}$ -CN and  $\kappa$ -CN genotype with *BC* and *BB* best, respectively. The effects of  $\beta$ -LG on fat percentage and  $\kappa$ -CN for protein percentage are in agreement with both studies by Ng-kwai-Hang (24, 25).

#### Lactation Cheese Yield

Studying the relationships among milk protein genotypes and predicted cheese yield can only show the effects of those genotypes that are important for the milk components considered by the prediction equation itself. Therefore, this portion of the study can only consider the importance of milk protein genotypes for improving cheese yield based on their relationship to milk, fat, and protein yield.

Table 6 presents the results of the analysis of total lactation cheese yield. Both dependent variables represent the total cheese yield from the milk produced by a cow during her entire lactation. The lactation yield based on skim

TABLE 6. Partial sums of squares and significance of the sources of variation for potential lactation cheese yield with skim milk or at constant 1.8% fat.

| Source <sup>1</sup>                     | df   | Cheese yield ( $\times 10^4$ ) |                   |
|---|------|--------------------------------|-------------------|
|   |      | Skim milk                      | Constant fat      |
| Complete data set                       |      |                                |                   |
| $\mu$                                   | 1    | 1371.17**                      | 1094.00**         |
| H                                       | 83   | 421.74**                       | 331.52**          |
| S $\times$ Y                            | 48   | 92.58**                        | 94.69**           |
| $\alpha_{s1}$ -CN                       | 1    | 3.00**                         | 1.58*             |
| $\beta$ -CN                             | 1    | .57                            | .38               |
| $\kappa$ -CN                            | 2    | .90                            | 1.06              |
| $\beta$ -LG                             | 2    | .53                            | 1.83*             |
| Error                                   | 1867 | 607.55                         | 505.12            |
| R <sup>2</sup>                          |      | .46                            | .46               |
| Subset with all $\beta$ -casein alleles |      |                                |                   |
| $\mu$                                   | 1    | 1160.76**                      | 946.14**          |
| H                                       | 15   | 191.98**                       | 167.47**          |
| S $\times$ Y                            | 36   | 66.26**                        | 68.68**           |
| $\alpha_{s1}$ -CN                       | 1    | 2.33**                         | 1.15*             |
| $\beta$ -CN                             | 5    | .73                            | .57               |
| $\kappa$ -CN                            | 2    | 1.18                           | 1.61 <sup>†</sup> |
| $\beta$ -LG                             | 2    | .65                            | 1.76*             |
| Error                                   | 1321 | 453.55                         | 386.54            |
| R <sup>2</sup>                          |      | .39                            | .40               |

<sup>1</sup> $\mu$  = Intercept, H = herd, S  $\times$  Y = season of calving and year, CN = casein, and LG = lactoglobulin.

\* $P < .05$ .

\*\* $P < .01$ .

<sup>†</sup> $P < .10$ .

milk is only related to  $\alpha_{s1}$ -CN genotype. The  $\alpha_{s1}$ -CN genotype *BB* has the largest estimate with 21.9 kg more cheese per lactation than *BC* (Table 7). There do not appear to be any other studies on  $\alpha_{s1}$ -CN and cheese yield in terms of cheese to milk ratio, and no study has considered lactation cheese yield. Ng-Kwai-Hang (25) suggested  $\alpha_{s1}$ -CN *BC* as best for cheese yield, based in its relationship to protein percentage, but increasing protein percentage does not generally increase lactation protein yield. The positive effect of  $\alpha_{s1}$ -CN *BB* on cheese yield is related to the positive effect that this is shown to have on milk, fat, and protein yields.

Lactation cheese yield based on constant fat was significantly associated with  $\alpha_{s1}$ -CN and  $\beta$ -LG with the genotypes *BB* and *AA* being best, respectively (Table 7). These are the same genotypes that were related to milk and protein

yields. Choosing all the largest estimates for the four proteins from Table 7 (although some are not significant) the best combination of genotypes is the same as that for protein yield, as reported earlier. However, genotypes that improve lactation cheese yield may not necessarily improve cheese to milk ratio, because their influence is largely through the increase in milk yield.

#### Cheese to Milk Ratio

The analysis of the estimates of vat cheese yield, based on skim or constant fat percentage, are presented in Table 8. Because the amount of milk is fixed, the vat cheese yield actually represents cheese to milk ratio. The results of Table 8 indicate that only  $\beta$ -LG is significant for vat yield on skim milk. The  $\beta$ -LG genotype

TABLE 7. Least square estimates (LSE) of the effects of different genotypes and their standard errors for potential lactation cheese yield on skim milk or on constant 1.8% fat.

| Component <sup>1</sup>                      | Genotype                          | Cheese yield      |      |              |      |
|---|-----------------------------------|-------------------|------|--------------|------|
|   |                                   | Skim milk         |      | Constant fat |      |
|   |                                   | Complete data set |      |              |      |
|   |                                   | LSE               | SE   | LSE          | SE   |
| $\alpha_{s1}$ -CN                           | <i>BB</i>                         | 21.9              | 7.2  | 15.9         | 6.6  |
|   | <i>BC</i>                         | 0                 |      | 0            |      |
| $\beta$ -CN                                 | <i>AA</i>                         | 8.4               | 6.3  | 6.9          | 5.8  |
|   | <i>AB</i>                         | 0                 |      | 0            |      |
| $\kappa$ -CN                                | <i>AA</i>                         | -7.9              | 6.7  | -7.8         | 6.1  |
|   | <i>AB</i>                         | -3.9              | 6.6  | -3.2         | 6.0  |
|   | <i>BB</i>                         | 0                 |      | 0            |      |
| $\beta$ -LG                                 | <i>AA</i>                         | 4.1               | 4.0  | 8.8          | 3.6  |
|   | <i>AB</i>                         | 3.4               | 2.9  | 5.4          | 2.7  |
|   | <i>BB</i>                         | 0                 |      | 0            |      |
| — Subset with all $\beta$ -casein alleles — |                                   |                   |      |              |      |
| $\alpha_{s1}$ -CN                           | <i>BB</i>                         | 22.4              | 8.6  | 15.8         | 8.0  |
|   | <i>BC</i>                         | 0                 |      | 0            |      |
| $\beta$ -CN                                 | <i>A<sub>1</sub>A<sub>1</sub></i> | 10.5              | 11.2 | 6.0          | 10.4 |
|   | <i>A<sub>1</sub>A<sub>2</sub></i> | 9.6               | 11.2 | 5.6          | 10.4 |
|   | <i>A<sub>1</sub>B</i>             | 4.0               | 15.4 | -7           | 14.2 |
|   | <i>A<sub>2</sub>A<sub>2</sub></i> | 11.5              | 11.9 | 9.3          | 10.9 |
|   | <i>A<sub>2</sub>A<sub>3</sub></i> | 39.0              | 32.9 | 28.1         | 30.4 |
| $\kappa$ -CN                                | <i>A<sub>2</sub>B</i>             | 0                 |      | 0            |      |
|   | <i>AA</i>                         | -7.3              | 8.2  | -8.7         | 7.5  |
|   | <i>AB</i>                         | -9                | 6.6  | -1.3         | 7.4  |
| $\beta$ -LG                                 | <i>BB</i>                         | 0                 |      | 0            |      |
|   | <i>AA</i>                         | 6.8               | 4.9  | 11.1         | 4.6  |
|   | <i>AB</i>                         | 1.9               | 3.6  | 4.5          | 3.3  |
|   | <i>BB</i>                         | 0                 |      | 0            |      |

<sup>1</sup>CN = Casein, LG = lactoglobulin.

*BB* has an estimate (Table 9) .65 kg higher than *AA*. This result appears to agree with those of Marziali et al. (18) in which  $\beta$ -LG *BB* was significantly associated with theoretical yield, although it was not associated with cheese produced under laboratory conditions. However, in another trial, Morini et al. (23) found that  $\beta$ -LG *BB* yielded more Parmesan cheese.

Vat cheese based on constant fat is significantly associated with  $\alpha_{s1}$ - and  $\kappa$ -CN; genotypes *BC* and *BB* have the largest estimates (Table 9). A study (18) on actual cheese produced in the lab found  $\kappa$ -CN *BB* to be associated with actual but not theoretical cheese yield. Mariani et al. (14) and Morini et al. (22) found that milk with  $\kappa$ -CN genotype *BB* yielded 10% more Parmesan cheese. Ng-Kwai-Hang's suggestion (25) that  $\alpha_{s1}$ -CN *BC* should be best for vat cheese yield appears correct based on its association with higher protein percentage.

#### Comparison of Estimates with Actual Yield

The difference between actual parmesan cheese yield (aged 6 mo) from a study by Mariani et al. (14) and the expected yield based on our equation is presented in Table 10. The milks used by Mariani to make these two groups of cheese were different only for the genotype of  $\kappa$ -CN, other milk protein genotypes were roughly balanced. Because our estimate represents the cheese yield for a given milk composition, any greater than expected difference in cheese yield between the two kinds of milk could be considered as the  $\kappa$ -CN genotype effect. The difference in actual yield between the two groups of milk presented in Table 10 is almost three times what would have been expected based on the composition of the milk (.6 vs. .23). In this case, it appears that the protein of type  $\kappa$ -CN genotype *AA* may be less suitable for the production of cheese.

TABLE 8. Partial sums of squares and significance of the sources of variation for potential cheese vat yield (kg of cheese out of 1000 kg of milk) with skim milk or at constant 1.8% fat.

| Source <sup>1</sup> | df   | Vat (kg)                                |              |
|---------------------|------|---|--------------|
|                     |      | Skim milk                               | Constant fat |
|                     |      | Complete data set                       |              |
| $\mu$               | 1    | 496,814.8**                             | 394,018.3**  |
| H                   | 83   | 6781.6**                                | 1526.9**     |
| S $\times$ Y        | 48   | 1982.4**                                | 463.1**      |
| $\alpha_{s1}$ -CN   | 1    | 2.2                                     | 26.6*        |
| $\beta$ -CN         | 1    | 1.7                                     | .2           |
| $\kappa$ -CN        | 2    | 66.1                                    | 77.0**       |
| $\beta$ -LG         | 2    | 88.9 <sup>†</sup>                       | 10.6         |
| Error               | 1867 | 32,767.6                                | 7835.5       |
| R <sup>2</sup>      |      | .24                                     | .22          |
|                     |      | Subset with all $\beta$ -casein alleles |              |
| $\mu$               | 1    | 364,625.8**                             | 296,050.4**  |
| H                   | 15   | 3402.8**                                | 773.7**      |
| S $\times$ Y        | 36   | 1770.3**                                | 360.4**      |
| $\alpha_{s1}$ -CN   | 1    | .9                                      | 25.8*        |
| $\beta$ -CN         | 5    | 62.6                                    | 10.3         |
| $\kappa$ -CN        | 2    | 26.8                                    | 48.6**       |
| $\beta$ -LG         | 2    | 45.4                                    | 11.3         |
| Error               | 1321 | 26,005.7                                | 5550.7       |
| R <sup>2</sup>      |      | .17                                     | .19          |

<sup>1</sup> $\mu$  = Intercept, H = herd, S  $\times$  Y = season of calving and year, CN = casein, and LG = lactoglobulin.

\* $P < .05$ .

\*\* $P < .01$ .

<sup>†</sup> $P < .10$ .

TABLE 9. Least square estimates (LSE) of the effects of different genotypes and their standard errors for potential cheese vat yield (kg of cheese out of 1000 kg milk) on skimmed milk or on constant 1.8% fat.

| Component <sup>1</sup>                      | Genotype                          | Vat yield         |     |              |      |
|---|-----------------------------------|-------------------|-----|--------------|------|
|   |                                   | Skim milk         |     | Constant fat |      |
|   |                                   | Complete data set |     |              |      |
|   |                                   | LSE               | SE  | LSE          | SE   |
| $\alpha_{s1}$ -CN                           | <i>BB</i>                         | -.19              | .5  | -.65         | .26  |
|   | <i>BC</i>                         | 0                 |     | 0            |      |
| $\beta$ -CN                                 | <i>AA</i>                         | .14               | .5  | -.05         | .23  |
|   | <i>AB</i>                         | 0                 |     | 0            |      |
| $\kappa$ -CN                                | <i>AA</i>                         | -.95              | .5  | -.96         | .24  |
|   | <i>AB</i>                         | -.87              | .5  | -.72         | .24  |
|   | <i>BB</i>                         | 0                 |     | 0            |      |
| $\beta$ -LG                                 | <i>AA</i>                         | -.65              | .3  | .19          | .14  |
|   | <i>AB</i>                         | -.15              | .2  | .14          | .11  |
|   | <i>BB</i>                         | 0                 |     | 0            |      |
| — Subset with all $\beta$ -casein alleles — |                                   |                   |     |              |      |
| $\alpha_{s1}$ -CN                           | <i>BB</i>                         | -.14              | .7  | -.75         | .30  |
|   | <i>BC</i>                         | 0                 |     | 0            |      |
| $\beta$ -CN                                 | <i>A<sub>1</sub>A<sub>1</sub></i> | 1.28              | .8  | .45          | .39  |
|   | <i>A<sub>1</sub>A<sub>2</sub></i> | 1.12              | .8  | .41          | .39  |
|   | <i>A<sub>1</sub>B</i>             | 1.48              | 1.2 | .75          | .54  |
|   | <i>A<sub>2</sub>A<sub>2</sub></i> | .85               | .9  | .37          | .42  |
|   | <i>A<sub>2</sub>A<sub>3</sub></i> | .94               | 2.5 | -.24         | 1.15 |
|   | <i>A<sub>2</sub>B</i>             | 0                 |     | 0            |      |
| $\kappa$ -CN                                | <i>AA</i>                         | -.65              | .6  | -.92         | .29  |
|   | <i>AB</i>                         | -.70              | .6  | -.70         | .28  |
|   | <i>BB</i>                         | 0                 |     | 0            |      |
| $\beta$ -LG                                 | <i>AA</i>                         | -.58              | .4  | .25          | .17  |
|   | <i>AB</i>                         | -.21              | .3  | .17          | .13  |
|   | <i>BB</i>                         | 0                 |     | 0            |      |

<sup>1</sup>CN = Casein, LG = lactoglobulin.

Table 11 presents the results of a similar approach applied to salted curd yield rates reported in a study by Morini et al. (23). In this case, the controlled milk protein variant was  $\beta$ -LG, and two batches of cheese were produced. In the first batch (made between July and September), the difference in actual yield between genotypes was greater than expected. In the second batch, the milk of genotype *AA* was expected to yield more cheese, but the actual yield of genotype *BB* was slightly higher. It would appear, in this case, that the milk protein of  $\beta$ -LG genotype *BB* is better for cheese production, perhaps because  $\beta$ -LG genotype *AA* is associated with a higher percentage of whey protein (5).

However, these discrepancies could be due to the inherent chemical differences between the genotypes reported in other studies (6, 7, 8).

Further studies are necessary to determine if any clear explanation can be found for a "protein variant" effect on Parmesan cheese yield beyond differences in milk composition.

#### Potential Linkage

Table 12 presents expected and observed frequencies of the allelic combinations for  $\alpha_{s1}$ -,  $\beta$ -, and  $\kappa$ -CN. The continuity adjusted chi-square test indicated that overall the observed frequencies differed significantly from the expected ( $P < .001$ ). This difference does not represent conclusive proof of the existence of linkage, since it could be due to several causes, such as early culling of cows of a particular genotype combination, but the possibility of linkage cannot be ruled out. A study by King et al. (12) found no occurrence of  $\alpha_{s1}$ -CN *C* with

TABLE 10. Comparison between actual 6-mo aged Parmesan cheese<sup>1</sup> cheese to milk ratio using milk of either casein variant  $\kappa$ -AA or  $\kappa$ -BB with our prediction of cheese to milk ratio based on fat and protein kilograms.

| $\kappa$ -Casein type | Milk (kg) | Fat % | Protein % | Actual cheese | Predicted cheese | Difference |
|-----------------------|-----------|-------|-----------|---------------|------------------|------------|
| BB                    | 351.8     | 2.32  | 3.28      | 7.07          | 7.05             | +0.02      |
| AA                    | 340.1     | 2.41  | 3.00      | 6.47          | 6.82             | -.35       |
| Difference            |           |       |           | .60           | .23              |            |

<sup>1</sup>Taken from Mariani et al. (14) and 6-mo aged cheese yields reported by Russo et al. (25).

$\beta$ -CN B, and a study by Grosclaude et al. (10) concluded that  $\alpha_{s1}$ -CN and  $\beta$ -CN were closely linked with the most frequent allele combinations being  $\alpha_{s1}$ -CN B  $\beta$ -CN B and  $\alpha_{s1}$ -CN C  $\beta$ -CN A. It appears that the alleles of  $\alpha_{s1}$ -CN and  $\kappa$ -CN occur together as expected based on their gene frequencies, making it plausible that these loci are linked through  $\beta$ -CN. Because  $\beta$ -CN does not seem to have any influence on milk composition, the potential that linkage could interfere with selection for optimal combinations of favorable genotypes, such as  $\alpha_{s1}$ -CN BB with  $\kappa$ -CN BB, is not likely. This combination, which gives cows with higher milk yields and higher protein percentage, occurs in about 5% of the cows (Table 13). The combination of  $\beta$ -LG with the composite gene of  $\alpha_{s1}$ -,  $\beta$ -, and  $\kappa$ -CN did not differ significantly from expected making the existence of linkage between  $\beta$ -LG and these loci unlikely. Optimal combinations such as  $\kappa$ -CN BB with  $\beta$ -lactoglobulin BB, for milk rich in protein and fat, should therefore be easily obtained.

### Conclusions

Our results confirm the association of  $\kappa$ -CN genotype BB with higher protein percent-

age and the association of  $\beta$ -LG BB with higher fat percentage as well as  $\beta$ -LG AA with protein yield. The use of milk protein polymorphisms is attractive because it may be possible to select in such a way that not only does not compromise yield but also provides proteins more suitable for cheese production. One attractive combination is  $\kappa$ -CN genotype BB with  $\beta$ -LG genotype BB. The  $\kappa$ -CN genotype is important for two reasons. First,  $\kappa$ -CN BB is associated with higher protein content of milk; second, differences in cheese yield from milk differing only for  $\kappa$ -CN BB or AA appear to be larger than expected and support the superior quality of BB. Genotype BB of  $\beta$ -LG is important because of its association with higher fat percentage, which can improve cheese yield. The AA genotype of  $\beta$ -LG should be avoided because its association with higher crude protein is due to an increase in the whey protein fraction, which is not likely to improve cheese yield. This appears confirmed by trials that found differences in actual yield between  $\beta$ -LG genotype BB and AA beyond that expected with higher yields for  $\beta$ -LG BB. The BB genotype of  $\alpha_{s1}$ -CN should be considered because of its relationship to higher milk, protein, and fat

TABLE 11. Comparison between actual salted curd to milk ratio from the production of Parmesan cheese<sup>1</sup> using milk of either casein variant  $\beta$ -lactoglobulin (LG) AA or BB with our prediction of salted curd to milk ratio based on fat and protein kg.

| $\beta$ -LG Type | Milk (kg) | Fat % | Protein % | Actual curd | Predicted curd | Difference |
|------------------|-----------|-------|-----------|-------------|----------------|------------|
| B <sup>2</sup>   | 547.0     | 2.36  | 3.16      | 7.35        | 7.14           | +0.21      |
| A <sup>2</sup>   | 547.0     | 2.38  | 3.13      | 7.17        | 7.13           | +0.04      |
| Difference       |           |       |           | .18         | .01            |            |
| B <sup>3</sup>   | 520.2     | 2.53  | 3.27      | 8.03        | 7.44           | +0.59      |
| A <sup>3</sup>   | 520.2     | 2.59  | 3.37      | 7.98        | 7.62           | +0.36      |
| Difference       |           |       |           | .05         | -.18           |            |

<sup>1</sup>Taken from Morini et al. (23).

<sup>2</sup>Cheese made between July 7 and September 16, 1981.

<sup>3</sup>Cheese made between September 19 and October 31, 1981.

TABLE 12. Observed and expected frequencies of the allelic combinations (haplotypes) for  $\alpha_{s1}$ -casein,  $\beta$ -casein, and  $\kappa$ -casein (based on 3063 animals genotyped in 1985).

| Combinations      | Observed frequency <sup>1</sup> | Expected frequency <sup>2</sup> |
|-------------------|---------------------------------|---------------------------------|
| BA <sub>1</sub> A | .3900                           | .4087                           |
| BA <sub>1</sub> B | .1723                           | .1481                           |
| BA <sub>2</sub> A | .3186                           | .2926                           |
| BA <sub>2</sub> B | .0753                           | .1060                           |
| BA <sub>3</sub> A | .0008                           | .0007                           |
| BA <sub>3</sub> B | .0001                           | .0003                           |
| BBA               | .0106                           | .0187                           |
| BBB               | .0143                           | .0068                           |
| CA <sub>1</sub> A | .0035                           | .0075                           |
| CA <sub>2</sub> A | .0012                           | .0027                           |
| CA <sub>1</sub> B | .0101                           | .0054                           |
| CA <sub>2</sub> B | .0022                           | .0019                           |
| CA <sub>3</sub> A | .0006                           | .00001                          |
| CA <sub>3</sub> B | .0                              | .0003                           |
| CBA               | .0003                           | .00001                          |
| CBB               | .0003                           | .000005                         |

<sup>1</sup>Frequencies determined on all possible combination of alleles from the genotype of each animal.

<sup>2</sup>Frequency determined from combination of the individual  $\alpha_{s1}$ -,  $\beta$ -, and  $\kappa$ -casein alleles.

yield, but since approximately 96% of all cows are already of this genotype this should not present a problem. The available  $\beta$ -CN genotypes do not appear to be associated with any of the traits considered, but there is a need for further experiments on possible direct effects of the genotypes at this locus on actual cheese: milk ratio. Given that there is no interaction among the genotypes and that linkage should not interfere with selection for the desired combinations, it appears best to consider several loci simultaneously to obtain animals whose milk is optimal for cheese production. There is potential that the milk of these animals may produce more cheese than can currently be expected based solely on the content of crude protein and fat in the milk. The consideration of optimal genotypes together with the expected cheese yield of a cow based on the equation could be an efficient way to improve cheese yield.

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TABLE 13. Observed numbers of the 26 existing genotype combinations for  $\alpha_{s1}$ -casein,  $\beta$ -casein, and  $\kappa$ -casein (based on 3063 animals genotyped in 1985).

| Combinations  | (No.) | (%)   |
|---|-------|-------|
| BBA <sub>1</sub> A <sub>1</sub> AA                  | 365   | 11.92 |
| BBA <sub>1</sub> ant <sub>1</sub> A <sub>1</sub> AB | 497   | 16.23 |
| BBA <sub>1</sub> A <sub>1</sub> BB                  | 90    | 2.94  |
| BBA <sub>1</sub> A <sub>2</sub> AA                  | 821   | 26.80 |
| BBA <sub>1</sub> A <sub>2</sub> AB                  | 580   | 18.94 |
| BBA <sub>1</sub> A <sub>2</sub> BB                  | 29    | .95   |
| BBA <sub>1</sub> BAA                                | 4     | .13   |
| BBA <sub>1</sub> BAB                                | 52    | 1.70  |
| BBA <sub>1</sub> BBB                                | 26    | .85   |
| BBA <sub>2</sub> A <sub>2</sub> AA                  | 329   | 10.74 |
| BBA <sub>2</sub> A <sub>2</sub> AB                  | 86    | 2.81  |
| BBA <sub>2</sub> A <sub>2</sub> BB                  | 4     | .13   |
| BBA <sub>2</sub> A <sub>3</sub> AA                  | 1     | .03   |
| BBA <sub>2</sub> A <sub>3</sub> AB                  | 1     | .03   |
| BBA <sub>2</sub> BAA                                | 3     | .10   |
| BBA <sub>2</sub> BAB                                | 60    | 1.96  |
| BBA <sub>2</sub> BBB                                | 4     | .13   |
| BCA <sub>1</sub> A <sub>1</sub> AB                  | 1     | .03   |
| BCA <sub>1</sub> A <sub>2</sub> AA                  | 31    | 1.01  |
| BCA <sub>1</sub> A <sub>2</sub> AB                  | 21    | .69   |
| BCA <sub>1</sub> A <sub>2</sub> BB                  | 3     | .10   |
| BCA <sub>2</sub> A <sub>2</sub> AA                  | 32    | 1.05  |
| BCA <sub>2</sub> A <sub>2</sub> AB                  | 8     | .26   |
| BCA <sub>2</sub> A <sub>2</sub> BB                  | 1     | .03   |
| BCA <sub>2</sub> A <sub>3</sub> AA                  | 7     | .23   |
| BCA <sub>2</sub> BAB                                | 7     | .23   |

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