# THE POTENTIAL CONTRIBUTION OF MILK PROTEIN LOCI TO IMPROVEMENT OF DAIRY CATTLE.

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## SUMMARY

Milk protein genetic variants have been shown to be associated with milk production traits and manufacturing properties of milk. Selection for milk protein genotypes, therefore, might contribute to improvement of dairy cattle. Selection for $\kappa$-casein and $\beta$-lactoglobulin genotypes might be of potential interest because of their effect on milk production traits and cheese production, respectively. The aim of the present study was to quantify the effects of selection for $\kappa$-casein and $\beta$-lactoglobulin genotypes by using stochastic simulation of a closed adult MOET nucleus breeding scheme. After 11 generations of selection for $\kappa$-casein genotypes in addition to selection for phenotypic milk production information, selection response was increased by 0.114 $\sigma_p$ over the selection response by selection for phenotypic milk production information only. The annual genetic progress was increased by 4.8% in the first seven generations. The additional response obtained by including $\beta$-lactoglobulin genotypes was 0.081 $\sigma_p$ and the annual genetic progress was increased by 3.9% in the first seven generations. These results were obtained assuming the effects of milk protein genotypes to be known without error. If estimated effects are inaccurate the additional response of selecting for $\kappa$-casein or $\beta$-lactoglobulin genotypes is lower. Additional gain for both milk protein genes was due to increased selection intensity on the male side.

## INTRODUCTION

Several studies have shown that milk protein genetic variants are associated with milk production traits. Results indicate that $\kappa$-casein genotypes are associated with protein content and $\beta$-lactoglobulin genotypes are associated with fat content (reviewed by Bovenhuis et al., 1992). $\kappa$-Casein and $\beta$-lactoglobulin genotypes, therefore, can be used as a source of information to estimate breeding values for protein and fat content and use of this information can increase genetic progress in selection for milk production traits (e.g., Smith and Simpson, 1986).

There are also reports of associations between milk protein genetic variants and manufacturing properties of milk, especially for cheese production: $\kappa$-casein genetic variants are associated with renneting time of milk and $\beta$-lactoglobulin genetic variants are associated with casein number (reviewed by Grosclaude, 1988). For countries where a large fraction of the milk is manufactured into cheese, milk protein genotypes might be of value as additional selection criteria to improve the quality of milk for cheese production.

Developments in DNA technologies have made it possible to type animals for $\kappa$-casein and $\beta$-lactoglobulin genotypes at the DNA-level (e.g., Medrano, 1990), which allows genotyping of both males and females at a young age. In dairy cattle breeding, accuracy of breeding value estimation is low for young animals because phenotypic records on the animal or its progeny are not available. Knowledge of milk protein genotypes, therefore, will be of special use in MOET (Multiple Ovulation and Embryo Transfer) nucleus breeding schemes where selection is at a young age.

The aim of the present study was to quantify the potential effects of selection for $\kappa$-casein

and β-lactoglobulin genotypes by using stochastic simulation of a closed adult MOET nucleus breeding scheme.

## MATERIALS AND METHODS

For this study two effects of milk protein genotypes were distinguished: the effect of milk protein genotypes on milk production traits and on manufacturing properties of milk.

### Effects of milk protein genotypes on milk production traits

Associations between milk protein genotypes and milk production traits were reviewed by Bovenhuis et al. (1992). It was concluded that κ-casein affects milk protein content and β-lactoglobulin affects fat content of milk. These effects are due to either the gene itself or to (a) very closely linked gene(s). In this study, therefore, these effects will be treated as if they were effects of the milk protein gene itself. Effects of genes linked to milk protein genes as described by Bovenhuis and Weller (1994) will not be considered in the present study.

At present, selection of dairy cattle is generally not for single traits but for an index combining breeding values for different milk production traits. It is necessary therefore, to quantify the effects of milk protein loci on the selection index. In the Netherlands, selection is for a net-profit-index (INET), combining breeding values for milk, fat and protein yield (Dommerholt and Wilmink, 1986). The current economic weights used in the INET are -0.14 for milk yield, 1.39 for fat yield and 11.68 for protein yield (in Dutch Guilders per kg). When using the genetic and phenotypic variances and covariances as estimated by Van der Werf and De Boer (1987), it can be calculated that the additive genetic standard deviation ($\sigma_g$) of INET is 114.5 and the phenotypic standard deviation ($\sigma_p$) of INET is 198.2, resulting in a heritability of 0.33.

Effects of milk protein genotypes on INET were estimated using a model in which all milk protein genes were analyzed simultaneously and using data and methods as described by Bovenhuis et al. (1992). The estimated effects of κ-casein and β-lactoglobulin genotypes are in Table 1. κ-Casein genotypes affected INET ($p<0.05$) but the effect of β-lactoglobulin genotypes was not significant.

By using a multigene model to estimate effects of milk protein genes on INET, milk protein genotypes are adjusted for effects of other milk protein genes. Because the casein genes are closely linked they might be in linkage disequilibrium, which will result in different estimated casein genotype effects for a single gene model than for a multigene model. This is the case for the Dutch Black and White dairy cattle population (Bovenhuis et al., 1992). By using estimates of a multigene model, therefore, this study assumed that selection was for both κ-casein and β-casein genotypes, where κ-casein B in combination with β-casein $A^1$, $A^2$ and $A^3$ is favourable and κ-casein B in combination with β-casein B is unfavourable.

### Effects of milk protein genotypes on manufacturing properties

Several studies have shown that milk protein genotypes are associated with manufacturing properties of milk (reviewed by Grosclaude, 1988). Most studies investigated effects of κ-casein or β-lactoglobulin genotypes. Although there are some indications that $\alpha_{s1}$-casein and β-casein genotypes also affect manufacturing properties, they were not included in the present study.

Van den Berg et al. (1992) studied the effect of κ-casein and β-lactoglobulin genotypes on the conversion of total nitrogen content of milk into cheese nitrogen, which is a measure for efficiency of cheese production. Results indicated that the κ-casein B allele is associated with a slightly higher efficiency then the κ-casein A allele: 72.4% for κ-casein AA versus 72.9%

Table 1. Estimates of effects of $\kappa$-casein and $\beta$-lactoglobulin genotypes on INET* when using the multigene model (as deviations from the AA genotype, in $\sigma_p$).

| Milk protein genotype | $\kappa$ - Casein | $\beta$ - Lactoglobulin |
|---|---|---|
| AA | 0.0 | 0.0 |
| AB | 0.103 | -0.027 |
| BB | 0.184 | -0.088 |
| Significance | p = 0.034 | p = 0.123 |

*INET = net-profit-index combining breeding values for milk, fat and protein yield.

for $\kappa$-casein BB. Although $\kappa$-casein B is associated with a higher casein number, this is not clearly reflected in an association with efficiency for cheese production, because the $\kappa$-casein B allele is also associated with higher losses of glycomacropeptide in the whey (Van den Berg et al., 1992). $\beta$-lactoglobulin genotypes have an effect on the conversion of milk nitrogen into cheese nitrogen: 71.0% for $\beta$-lactoglobulin AA versus 73.9% for $\beta$-lactoglobulin BB. This indicates that $\beta$-lactoglobulin BB is more efficient for cheese production than is $\beta$-lactoglobulin AA. Van den Berg et al. (1992) explained this effect by the association between the $\beta$-lactoglobulin alleles and casein number. The B allele of $\beta$-lactoglobulin is related to a higher casein content and a lower whey protein content (reviewed by Grosclaude, 1988). Overall, however, this resulted in no significant effect of $\beta$-lactoglobulin genotypes on protein content (reviewed by Grosclaude, 1988; Bovenhuis et al.,1992). In this study it will be assumed that the effect of $\beta$-lactoglobulin on the conversion of total nitrogen into cheese nitrogen is an effect of the $\beta$-lactoglobulin gene itself. The consistency between effects of $\beta$-lactoglobulin on casein number found in different studies justifies this assumption (reviewed by Grosclaude, 1988).

The economic value of the effect of milk protein genotypes on conversion of total nitrogen into cheese nitrogen was computed based on: an economic value of protein yield of 11.68, as used in INET; a cheese to whey price ratio of 172 to 1; an average 305-day production per cow of 210 kg protein; and assuming that 50% of the milk is manufactured into cheese (for a detailed description see Bovenhuis, 1992). The difference between the $\kappa$-casein AA and BB genotypes is 0.042 $\sigma_p$, whereas the difference between $\beta$-lactoglobulin AA and BB genotypes is 0.244 $\sigma_p$ (Table 2).

Better curd properties associated with $\kappa$-casein B, result in a higher resistance to mechanical forces during curd preparation and, therefore, in a lower amount of curd fines in the whey (Van den Berg et al., 1992). This effect of $\kappa$-casein genotypes is not included in the conversion of milk nitrogen into cheese nitrogen. The economic importance of this effect, however, is negligible (Van den Berg, 1992, personal communication). Further, several studies reported effects of $\kappa$-casein genetic variants on renneting time of milk (reviewed by Grosclaude, 1988). These effects to a great extent, however, could be compensated for by the addition of calcium chloride. The economic importance of the effect of $\kappa$-casein genotypes on renneting time, therefore, is negligible.

Besides effects of milk protein genetic variants on parameters related to cheese

Table 2. The relative value of $\kappa$-casein and $\beta$-lactoglobulin genotypes due to their effect on the conversion of total nitrogen content of milk into cheese nitrogen (in $\sigma_p$).

| Milk protein genotype | $\kappa$ - Casein | $\beta$ - Lactoglobulin |
|---|---|---|
| AA | 0.0 | 0.0 |
| AB | 0.015 | 0.154 |
| BB | 0.042 | 0.244 |

production, some studies investigated effects of milk protein genetic variants on heat stability of milk or concentrated milk (e.g., Schmidt and Koops, 1965; McLean et al., 1987; Van den Berg et al., 1992). Results, however, are not uniform. The reason for this might be that heat stability is strongly influenced by factors such as pH, mineral constituents, urea concentration, and methods and conditions of determination (Van den Berg et al., 1992). Effects of factors other than milk protein genotypes might also make it possible to standardise for possible milk protein genotype effects by adding for example sodium phosphate (Van den Berg et al., 1992). These were reasons for us to ignore possible effects of milk protein variants on heat stability in the present study.

**Breeding scheme**

In the previous sections, the effects of $\kappa$-casein and $\beta$-lactoglobulin genetic variants on milk production traits and manufacturing properties of milk were discussed. Based on this discussion we conclude that two effects of $\kappa$-casein and $\beta$-lactoglobulin are potentially interesting for dairy cattle breeding:

1) An effect of $\kappa$-casein genotypes on milk production traits (INET).

2) An effect of $\beta$-lactoglobulin genotypes on cheese production.

To quantify the potential effects of selection for $\kappa$-casein and $\beta$-lactoglobulin genotypes, a closed adult MOET (Multiple Ovulation and Embryo Transfer) nucleus breeding scheme was simulated stochastically. The founder population (generation 0) consisted of 64 donor cows and 16 sires that were unrelated and unselected. Each sire was mated at random to 4 donor cows, resulting in 8 progeny; 4 males and 4 females. Each subsequent generation, 64 donor cows and 16 sires were selected. Selection of males and females was after all 256 females had one phenotypic observation. To restrict inbreeding, the number of males selected per full sib group was limited to one. Selection response for a base situation, where selection is for estimated breeding values based on only phenotypic milk production information, without specific knowledge of milk protein genotypes, was compared with a situation where selection was for phenotypic milk production information and milk protein genotype information. The quantitative trait was simulated using the heritability of INET. In a situation were selection was also for milk protein genotypes, genotypes of males and females were assumed to be known at the time of selection. Furthermore, effects of $\kappa$-casein and $\beta$-lactoglobulin genotypes were assumed to be known without error. When selection was for $\beta$-lactoglobulin genotypes, the effect of $\beta$-lactoglobulin on cheese production was assumed to be unrelated to the index.

The frequency of the β-lactoglobulin B allele in the founder population was 0.54 (Bovenhuis and Van Arendonk, 1991). The frequency of the favourable κ-casein allele, i.e. κ-casein B in combination with β-casein $A^1$, $A^2$ and $A^3$ was assumed to be 0.11 (Bovenhuis et al., 1992). For a full description of the simulation model see De Boer and Van Arendonk (1994) and Bovenhuis (1992).

## RESULTS

Table 3 shows the difference in genetic level for a situation where selection is for a combination of phenotypic milk production and κ-casein or β-lactoglobulin genotype information and for the base situation where selection is based only on phenotypic milk production information. Results were obtained by averaging 350 replicates. With additional selection for κ-casein genotypes, the κ-casein B frequency was 0.97 in generation 11. Due to selection for only phenotypic milk production information, the κ-casein B frequency increased to 0.23 from 0.11 in the base situation. Additional selection for κ-casein B resulted in a 0.114 $\sigma_p$ higher genetic level in generation 11. For β-lactoglobulin, selection almost resulted in fixation of β-lactoglobulin B in generation 11. The genetic level in generation 11 was 0.081 $\sigma_p$ higher when selection was also for β-lactoglobulin B.

When selection was also for κ-casein genotypes, accuracy of selection was 0.6161 for females and 0.4457 for males. This is slightly higher than accuracies in the base situation: 0.6143 for females and 0.4425 for males. These differences are small and cannot explain observed differences in genetic progress. Additive genetic variance and inbreeding were also similar for the base situation and for the situation with additional selection for κ-casein genotypes. However, the realised selection intensities in males was increased when selection was also for κ-casein genotypes, whereas the realised selection intensity of females was not changed. Similar to results for κ-casein, the gain for β-lactoglobulin resulted from increased selection intensity of males. For both milk protein genes, the increase in male selection intensity depended on the gene frequency.

## DISCUSSION

Using information on κ-casein or β-lactoglobulin genotypes for selection has the potential to increase selection response in a MOET nucleus breeding scheme. For κ-casein, the gain was 0.114 $\sigma_p$. In the simulated breeding scheme, this corresponded with the genetic progress that could be achieved in 1 year (assuming selection after 90 days in lactation and hence a generation interval of 3 years). Most gain was in the first seven generations, during which an increased annual genetic progress of 4.8% was achieved when selection was additionally for κ-casein genotypes. The additional response for β-lactoglobulin was 0.081 $\sigma_p$, which corresponded to the genetic progress attainable in 0.7 year. In the first seven generations the annual genetic progress was increased by 3.9%.

For both κ-casein and β-lactoglobulin, it was assumed that genotype effects were known without error. In a real situation, however, effects of milk protein genotypes must be estimated from data or estimates are known from literature. In both situations genotype effects are estimates that may be inaccurate. To study the consequences of inaccurate estimates of genotype effects, a situation was simulated in which κ-casein genotype effects and breeding values were estimated simultaneously. Because in the early generations the amount of data are small, estimates of κ-casein genotype effects will be inaccurate in those generations. For this alternative, the additional gain that was obtained after 11 generations of selection for κ-casein genotypes was 0.043 $\sigma_p$. Part of the reduction, compared to 0.114

Table 3. The difference in genetic level (in $\sigma_p$) between a situation with additional selection for $\kappa$-casein or $\beta$-lactoglobulin genotypes and the base situation.

| Generation | $\kappa$-casein | $\beta$-lactoglobulin |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0.011 | 0.031 |
| 3 | 0.026 | 0.045 |
| 4 | 0.046 | 0.060 |
| 5 | 0.067 | 0.068 |
| 6 | 0.080 | 0.070 |
| 7 | 0.099 | 0.083 |
| 8 | 0.101 | 0.088 |
| 9 | 0.109 | 0.083 |
| 10 | 0.113 | 0.087 |
| 11 | 0.114 | 0.081 |

$\sigma_p$, could be ascribed to the fixation of the $\kappa$-casein gene at the unfavourable A allele in 60 of the 350 replicates. These results, however, indicate that gains obtained from selection for milk protein genes can be greatly reduced if estimates of the genotype effects are inaccurate.

The effects of selection for $\kappa$-casein or $\beta$-lactoglobulin genotypes were studied here for an adult MOET nucleus breeding scheme. Additional gain was due to increased selection intensity on the male side. To restrict inbreeding, in the base situation selection of males was restricted to one per full sib group. This male was chosen randomly because at the time of selection all male full sibs had the same estimated breeding value. When selection was also for $\kappa$-casein or $\beta$-lactoglobulin genotypes, estimated breeding values of full sibs differed if genotypes of full sibs differed. Therefore, the results of the present study depend upon restrictions set to inbreeding in the base situation and the availability of full sibs.

Gibson et al. (1990) and Pedersen (1991) examined selection for $\kappa$-casein genetic variants through deterministic simulation of a progeny testing scheme. Gibson et al. (1990) considered the situations where $\kappa$-casein genotypes affected only protein yield and where $\kappa$-casein genotypes had an additional effect on cheese yield, independent of their effect on protein yield. Pedersen (1991) assumed that $\kappa$-casein genotypes had an effect on the value of milk and not on milk yield. In the present study, it is assumed that $\kappa$-casein genotypes affect only milk production traits and that effects of $\kappa$-casein genotypes on manufacturing properties are of negligible economic importance. Gibson et al. (1990) examined a progeny testing scheme in which selection for milk protein genotypes did not increase selection intensity. Accuracy of selection was not substantially improved because information on 50 half sib daughters was available at the time of selection. Therefore, when $\kappa$-casein only had an effect on protein yield, their study indicated no advantage in genotyping sires. Where an additional effect of $\kappa$-casein genotypes on cheese yield was considered, typing of animals was beneficial in a progeny testing scheme, particularly when a large fraction of the milk was manufactured into cheese. As in the present study, Gibson et al. (1990) showed that inaccurate estimates of gene effects substantially reduced genetic response.

In the present simulation study, effects of milk protein loci on INET were used to quantify

potential effects of selection for κ-casein or β-lactoglobulin genotypes. In other countries, other selection indices include different traits and/or different economic weights. As indicated previously, two effects of milk protein loci on milk production traits are evident; an effect of κ-casein genotypes on protein content and an effect of β-lactoglobulin genotypes on fat content. When selection is for an index. other effects which are less clear, are also of importance. The effects of κ-casein genotypes can be best summarised as: no effect on milk yield, no effect on fat content, a positive effect of the B-variant on protein content, no effect on fat yield and, although not significant in many studies, a tendency for the B variant to have a positive effect on protein yield; the latter agrees with the lack of an effect on milk yield and a positive effect on protein content. For β-lactoglobulin, most studies do not find a significant effect on milk yield, but the B variant tends to be associated with lower milk yields. Further, there is a positive effect of the B variant on fat content, a tendency of the B variant to have a negative effect on protein content and, although not significant in many studies, a tendency for the B-variant to have a positive effect on fat yield and a negative effect on protein yield (reviewed by Bovenhuis et al., 1992). Because in most countries selection favours protein, the effect of κ-casein B on most selection indices will be positive. β-Lactoglobulin B, however, tends to be associated with higher fat yields and lower protein yields. In general, this results in a negative effect of β-lactoglobulin B on indices favouring protein. In the present study, this effect was also observed (Table 1) but the effect was not significant and therefore ignored. If these apparent effects of β-lactoglobulin on milk production traits are true effects, then the β-lactoglobulin B variant will have a negative effect on selection indices used at present in most countries. The size of this effect will depend on the relative weights in the index.

In the present study, effects of milk protein genotypes on the conversion of milk nitrogen into cheese nitrogen were considered. This conversion factor represents differences in protein composition, i.e., for cheese production caseins are more important than whey proteins. For fluid milk, the casein/whey protein ratio is of little importance. Therefore, the fraction of milk that is manufactured into cheese is a main factor determining the economic value of β-lactoglobulin genotypes. In the present study, this fraction was ½. For countries where this fraction is ¼ or ¾ differences between β-lactoglobulin AA and BB genotypes were 0.122 $\sigma_p$ and 0.365 $\sigma_p$. At present, regulations in most countries do not allow for dairy factories to alter milk protein content or composition. A change in these regulations can greatly reduce the economic value of β-lactoglobulin genotypes because it would be possible to replace part of the caseins in fluid milk for whey proteins and to replace part of the whey proteins in "cheese milk" by caseins. Another example in which regulations affect the value of milk protein genotypes is where some countries do not allow the addition of calcium chloride to the milk. For these countries it might not be justified to neglect the effect of κ-casein genotypes on renneting time as was done in the present study.

Several studies indicated that manufacturing properties of milk are influenced by many environmental and genetic factors (reviewed by Graml et al., 1988). Heritability estimates for traits related to manufacturing properties vary between 0.20 and 0.60 (Oloffs et al., 1992, Hortien et al., 1992). To change manufacturing properties, these traits could be included in the breeding goal and in the selection index. Milk protein genes should be looked upon as genes affecting these traits. The advantage of selecting for milk protein genes directly is that they can be determined independent of milk production, i.e. at a young age and in both sexes.

To summarize the discussion we conclude that κ-casein genotype information can make a contribution to selection which is in favour of milk protein. β-Lactoglobulin genotype information can contribute to selection for protein composition that is favourable for cheese production. However, this selection is expected to have a slightly negative effect on indices favouring selection for protein. The economic value of the effects of milk protein genotypes

on manufacturing properties depend upon the technological possibilities of compensating for these effects in combination with local or international regulations that might not allow for the alteration of milk protein or mineral content or composition. To make fully use of milk protein genotype information in selection, information should be used to preselect animals in early life, within families.

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