Relationships between milk protein composition, milk protein variants, and cow fertility traits in Dutch Holstein-Friesian cattle

R. M. Demeter,*†¹ K. Markiewicz,* J. A. M. van Arendonk,* and H. Bovenhuis*

*Animal Breeding and Genomics Centre, Wageningen University, 6700 AH, Wageningen, the Netherlands

†Business Economics Group, Wageningen University, 6706 KN, Wageningen, the Netherlands

ABSTRACT

Selective breeding can change milk protein composition to improve the manufacturing properties of milk. However, the effects of such breeding strategies on other economically important traits should be investigated before implementation. The objectives of this study were to examine the association between cow fertility traits and (1) milk protein composition and (2) milk protein variants (β -lactoglobulin, β -casein, κ -casein, and β - κ -casein) in commercial Dutch Holstein-Friesian cattle. Data on 1,644 first-lactation cows were analyzed by fitting linear mixed models. Greater relative concentration of α_{S1} -casein within total milk protein had a positive phenotypic relationship with nonreturn rates and calving rate after first insemination. Furthermore, results showed virtually no significant relationship between cow fertility and concentration of other milk proteins or milk protein variants. Results of this study can be used to assess the correlated effects of breeding for improved milk protein composition on reproduction, thereby allowing for better evaluation of breeding programs before implementation. Our findings suggest that selecting cows based on milk protein composition or milk protein variants for improved manufacturing properties would have no negative influence on reproductive performance.

Key words: dairy cattle, milk protein composition, milk protein variant, female fertility

INTRODUCTION

Recent research has shown opportunities to change the protein composition of bovine milk by selective breeding (Heck et al., 2009; Schopen et al., 2009). Changing protein composition is important because of its effect on the manufacturing properties of milk. For example, cheese manufacturing properties (cheese yield, milk coagulation time, and curd firmness) are

influenced by protein composition (e.g., Auldist et al., 2004; Wedholm et al., 2006). In several countries, a large proportion of raw milk produced is destined for cheese production. In the Netherlands, for example, over 50% of the milk is used for cheese manufacturing, and cheese exports represent more than half of the dairy industry's total export value (Demeter et al., 2009a). Therefore, improving milk protein composition for dairy processing by selective breeding can increase the economic outcome of the dairy industry.

Recently, several studies have shown large variation in the relative concentration of the major milk proteins (Bobe et al., 1998; Bordin et al., 2001; Heck et al., 2008). Furthermore, research has found that a considerable part of this variation is genetic (Bobe et al., 1999; Graml and Pirchner, 2003). In a recent study, Schopen et al. (2009) found moderate to high intraherd heritabilities for milk protein composition in Dutch Holstein-Friesian cattle, ranging from 0.25 to 0.80. These results suggest the possibility of changing milk protein composition by using conventional selection methods. Furthermore, cows can be selected for particular genetic variants of milk proteins. When genetic variants are associated with the composition of milk protein, information on the genotype can be used for selecting animals. Milk protein genes have been extensively studied in cattle, and large genetic variation has been identified (e.g., Farrell et al., 2004; Caroli et al., 2009) that offers opportunities for animal breeding.

Before selecting for a specific milk protein or genetic variant, however, effects on other economically important traits should be investigated. A relevant trait to consider is the reproductive performance of cows, given its high economic importance. The relationship between protein-related production traits (e.g., protein concentration or protein yield) and fertility has been extensively investigated (e.g., Dematawewa and Berger, 1998; Roman and Wilcox, 2000). However, knowledge on the relationship between cow fertility and protein composition and protein variants is limited and in some cases contradictory. Regarding the β -LG genotype, Lin et al. (1987) and Tsiaras et al. (2005) found a significant association with several fertility traits. Other studies

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¹Corresponding author: robert.demeter@wur.nl

(Hargrove et al., 1980; Ruottinen et al., 2004), however, did not detect any significant relationship between β -LG variants and reproductive traits. Regarding the β -CN genotype, Hargrove et al. (1980) reported a significant relationship with a single fertility trait (probability of conception), whereas Lin et al. (1987) reported no effect. Regarding the κ -CN genotype, studies consistently found no significant association with fertility traits (Hargrove et al., 1980; Lin et al., 1987; Tsiaras et al., 2005). Ruottinen et al. (2004) examined the effects of β - κ -CN haplotypes on reproduction and reported a significant effect on a single trait (days to first service). Further analyses on the effects of β - κ -CN haplotypes are certainly warranted. To our knowledge, no studies have investigated the relationship between milk protein composition and cow fertility. The objectives of this study were to examine the association between (1) the relative concentrations of major milk proteins; (2) the genetic variants of β -LG, β -CN, and κ -CN; and (3) the β - κ -CN haplotypes and fertility traits in commercial Holstein-Friesian dairy cattle in the Netherlands.

MATERIALS AND METHODS

Animals

This study is part of the Dutch Milk Genomics Initiative, which focuses on the genetic background of detailed milk composition (Schennink et al., 2007; Heck et al., 2009; Schopen et al., 2009). Data on 1,644 first-lactation cows distributed among 390 Dutch commercial herds were used. The farms were invited to participate based mainly on pedigree according to the database of the cooperative cattle improvement organization CRV (Arnhem, the Netherlands). Records on pedigree, yield, and reproduction were also provided by CRV. The cows descended from one of either 5 proven sires (762 cows), 50 test sires (721 cows), or 15 additional proven sires (161 cows). Offspring from the last group of bulls were added to ensure sampling of at least 3 cows per herd. Each cow was at least 87.5% Holstein-Friesian. Further information on the animals used in this study is provided by Stoop et al. (2007).

Fertility Traits

From the records on reproductive performance provided by CRV, the following fertility traits were derived for all 1,644 cows: interval (d) between the first and second calving (**CIN**), interval (d) between calving and the first insemination (**DFS**), number of inseminations (**NINS**), nonreturn rate (0/1) for insemination 28 d after the first insemination (**NR28**), nonreturn rate (0/1) for insemination 56 d after the first insemination

(**NR56**), nonreturn rate (0/1) for insemination 90 d after first insemination (**NR90**), rate (0/1) of calving after the first insemination (**CFI**), and rate (0/1) of calving after any inseminations (**CR**). Records for CIN were considered missing if an animal did not have a known next calving date or if the CIN was less than 300 d or greater than 800 d. Records for DFS were set to missing if the DFS was less than 20 d or greater than 300 d. The NINS was set to missing if its value was 0 or greater than 10. These criteria were applied to exclude unrealistic, unrepresentative, or erroneous records under Dutch conditions (Calus et al., 2005). Descriptive statistics on the fertility traits are provided in Table 1. The average CIN was 411 d, and the mean DFS was approximately 90 d. Cows had about 2.1 services on average, and approximately 10% of the cows did not have a second calving. Mean nonreturn rates ranged from 52 to 72% for the different time intervals.

Protein Concentrations

Morning milk samples, one from each cow, were collected from each cow between February and March 2005. The samples were preserved with 0.03% wt/wt sodium azide until analysis. The cows were milked twice a day, and each cow was between 66 and 282 d in lactation. The milk samples were used to estimate the relative concentrations of the 6 major milk proteins: α -LA, β -LG, α_{S1} -CN, α_{S2} -CN, β -CN, and κ -CN for all 1,644 cows by capillary zone electrophoresis as described by Heck et al. (2008) and Heck et al. (2009). Table 1 shows the descriptive statistics of the relative concentration of major milk proteins and milk protein composition. The largest protein fraction was α_{S1} -CN, with an average relative concentration of 33.69% wt/wt, followed by β -CN and α_{S2} -CN, with average relative concentrations of 27.19 and 10.34% wt/wt. Furthermore, the mean proportion of total caseins within total milk proteins (**CN-index**) was 87%. These figures are similar to those reported by Heck et al. (2008) and Schopen et al. (2009), who analyzed slightly different subsets of the same Dutch Holstein-Friesian population.

Genotypes

Blood samples for DNA isolation were collected between April and June 2005. Genetic variants of β -LG were determined by using a SNaPshot single-base primer extension assay (Applied Biosystems, Foster City, CA), analogous to Visker et al. (2010). The β -LG protein variants A and B were determined, and the genotypes were missing for 44 animals from the total 1,644 animals (AA: 513 cows, AB: 838 cows, and BB: 249 cows).

Table 1. Descriptive statistics of fertility traits and milk protein composition of 1,644 first-lactation Dutch Holstein-Friesian cows

Trait	Cows (n)	Mean	SD	Minimum	Maximum
Fertility traits ¹					
CIN	1,482	410.71	75.41	304	795
DFS	1,639	90.02	36.76	21	284
NINS	1,642	2.08	1.45	1	10
CR	1,644	0.90	0.29		
NR28	1,644	0.72	0.45		
NR56	1,644	0.60	0.49		
NR90	1,644	0.52	0.50		
CFI	1,644	0.44	0.49		
Proteins ²					
α-LA	1,644	2.45	0.31	1.02	3.67
β-LG	1,644	8.35	1.19	4.08	12.36
α _{S1} -CN	1,644	33.69	1.59	24.75	39.84
α _{S2} -CN	1,644	10.34	1.38	4.66	14.21
β-CN	1,644	27.19	1.57	20.70	33.37
κ-CN ³	1,644	4.01	0.58	2.29	6.12
CN-index ⁴	1,644	0.87	0.01	0.83	0.94

¹Fertility traits were interval (d) between first and second calving (CIN), interval (d) between calving and first insemination (DFS), number of inseminations (NINS), calving rate (CR), nonreturn rate for insemination 28 d after first service (NR28), nonreturn rate for insemination 56 d after first service (NR56), nonreturn rate for insemination 90 d after first service (NR90), and calving rate following the first insemination (CFI).

²Expressed as percentage of the specific protein in the total protein fraction (wt/wt), except for CN-index.

³Only κ-CN in the nonglycosylated mono-phosphorylated form.

⁴CN-index = (α_{S1}-CN + α_{S2}-CN + β-CN + κ-CN)/(α-LA + β-LG + α_{S1}-CN + α_{S2}-CN + β-CN + κ-CN).

Regarding genetic variants of β-CN, polymorphisms were genotyped using a SNaPshot single-base primer extension assay as described by Visker et al. (2010). Genotypes were used to determine β-CN protein variants A¹, A², A³, B, and I. Due to no available DNA sample or ambiguous genotyping, the genotypes for β-CN were missing for 65 animals from the total 1,644 animals (A¹A²: 455 cows, A²A²: 389 cows, A²I: 333 cows, A¹I: 165 cows, A¹A¹: 127 cows, II: 44 cows, A²B: 32 cows, A¹B: 17 cows, IB: 15 cows, and A²A³: 2 cows). Furthermore, the 2 records for β-CN variant A²A³ were excluded from further analyses.

Regarding variants of κ-CN protein, relevant polymorphisms were genotyped using the SNaPshot single-base primer extension method as described by Heck et al. (2009). Protein variants A, B, and E were determined for κ-CN. Genotypes for κ-CN were missing for 32 animals from the total 1,644 animals due to no available DNA sample or to ambiguous genotyping (AA: 642 cows, AB: 555 cows, AE: 179 cows, BB: 124 cows, BE: 97 cows, and EE: 15 cows).

Haplotypes

The β-κ-CN haplotypes were inferred with PHASE v.2.1.1 (Stephens et al., 2001) from β-CN and κ-CN protein variants as described by Visker et al. (2010). Seven β-κ-CN haplotypes were identified: A¹A, A¹B, A¹E, A²A, A²B, BB, and IB. Because no DNA sample

was available, the sample could not be genotyped unambiguously for β-CN or κ-CN, or the haplotype could not be inferred unambiguously, haplotypes were missing for 104 animals. Furthermore, the records for 2 copies of A¹B haplotype were set as missing because they were available for only 3 cows. An overview of the haplotype frequencies is provided in Table 2.

Statistical Analysis

To study the association between the relative concentrations of the 6 major milk proteins or the CN-index and the fertility traits, 2 modeling steps were performed. Because the protein compositions were measured on

Table 2. Number of cows according to the number of copies of the β-κ-CN haplotypes of 1,540 first-lactation Dutch Holstein-Friesian cows

Haplotype	Number of copies			Total
	0	1	2	
A ¹ A	1,114	361	65	1,540
A ¹ B	1,373	164	0	1,537 ¹
A ¹ E	1,264	261	15	1,540
A ² A	418	797	325	1,540
A ² B	1,430	105	5	1,540
BB	1,478	62	0	1,540
IB	992	504	44	1,540

¹The records for 2 copies of the A¹B haplotype were set as missing because they were available for only 3 cows.

milk samples taken at different lactation stages, the data on milk proteins and CN-index were first preadjusted for lactation stage. With the composition traits as dependent variables, univariate linear models were fitted (models not shown) using the GLM procedure in SAS 9.1 (SAS Institute Inc., Cary, NC) to model the effect of DIM (days between date of first calving and date of milk sample) modeled with a Wilmink curve (Wilmink, 1987). The composition traits adjusted for lactation stage were then used for further analyses in model [1]. Other effects in model [1] included the age at first calving, the season of calving, and the sire type (3 different sire groups: proven bulls, young bulls, or other proven bulls). Relationships between individuals were accounted for, and the total pedigree comprised 26,300 animals and was supplied by CRV. Variance components and fixed effects solutions were calculated using the ASReml software package (Gilmour et al., 2006). Model [1] is written as

$$y_{ijklmn} = \mu + b_1 \text{AFC}_i + b_2 \text{PCON}_j + \text{SEASON}_k \\ + \text{SCODE}_l + \text{HERD}_m + a_n + e_{ijklmn}, \quad [1]$$

where y is the dependent variable (fertility trait); μ is the general mean; b_1 is the regression coefficient associated with AFC, which is a fixed covariate describing the effect of age at first calving; b_2 is the regression coefficient associated with PCON, which is a fixed covariate accounting for the effect of the adjusted relative concentration of one of the 6 major milk proteins or the CN-index for lactation stage; SEASON is a fixed factor accounting for the effect of the calving season (June to August 2004, September to November 2004, or December 2004 to February 2005); SCODE is a fixed factor capturing the effect of the differences in genetic level between proven bull daughters and young bull daughters; HERD is a random factor accounting for the effect of groups of animals sampled in the same herd; a is a random factor accounting for the additive genetic effect of the given animal; and e is the random residual effect. Each fertility trait was analyzed separately, and each protein concentration and CN-index was also fitted separately (56 analyses in total).

To study the relationship between the milk protein variants and the fertility traits, a model similar to model [1] was used. Model [2] excluded the covariate PCON but included a fixed factor PVAR that captured the effect of the β -LG genotype (AA, AB, or BB), the β -CN genotype (A^1A^2 , A^2A^2 , A^2I , A^1I , A^1A^1 , II, A^2B , A^1B , or IB), the κ -CN genotype (AA, AB, AE, BB, BE, or EE), or the number of copies (0, 1, or 2) of the 7 β - κ -CN haplotypes (A^1A , A^1B , A^1E , A^2A , A^2B , BB, or IB). Each fertility trait was analyzed separately, and each

protein variant and haplotype was also fitted separately (80 analyses in total). Model [2] is written as

$$y_{iklmno} = \mu + b_1 \text{AFC}_i + \text{PVAR}_o + \text{SEASON}_k \\ + \text{SCODE}_l + \text{HERD}_m + a_n + e_{iklmno}. \quad [2]$$

Because the models above assumed normally distributed residuals, generalized linear mixed models were also fitted by using the GLIMMIX procedure in SAS 9.1 (Littell et al., 2006) for the count and binary variables. A Poisson distribution with log link function was assumed for NINS, and a binomial distribution with logit link function was assumed for CR, NR28, NR56, NR90, and CFI. By fitting generalized linear models, the same fixed effects were significant as when fitting the above linear models; therefore, we concluded that linear mixed models were robust enough to handle the nonnormally distributed fertility traits.

In the above models, the effects of protein concentrations, genotypes, and haplotypes on each fertility trait were tested separately in a series of sequential analyses. In each analysis, the null hypothesis had no effect on the fertility trait of interest. Because several fertility traits were analyzed, multiple hypotheses were tested. However, due to the correlations between those fertility traits, not all hypothesis tests were truly independent. To account for multiple testing, the analyses of (i) CIN, DFS, and NINS; (ii) CR and CFI; and (iii) NR28, NR56, and NR90 were considered as 3 independent hypothesis tests. Bonferroni correction based on the Šidák method (Šidák, 1967) was applied to account for multiple testing with regard to the 3 groups of fertility traits, analogous to Demeter et al. (2009b).

RESULTS AND DISCUSSION

Effects of Milk Protein Composition

To investigate the association between the relative concentration of major milk proteins and reproductive performance, the milk protein profiles from 1,644 first-lactation Dutch Holstein-Friesian cows were used (model [1]). The estimated effects and their significance are presented in Table 3. The relative concentration of milk protein α_{S1} -CN was found to have a relationship with several fertility traits. The results indicate that a greater relative concentration of α_{S1} -CN is associated with better female reproductive performance. More specifically, a 1% wt/wt increase in the relative α_{S1} -CN concentration is expected to result in a 1.9% greater nonreturn rate at 28 d, 2.7% greater nonreturn rate at 56 d, 2.9% greater nonreturn rate at 90 d, and 2.5% greater calving rates after the first service. As-

sociations between the relative α_{S1} -CN concentration and NR28, NR56, NR90, and CFI remained significant after Bonferroni correction. The relationship of the relative α_{S1} -CN concentration with other fertility traits was statistically nonsignificant. However, the estimated effects for CIN and NINS also suggest the positive relationship between greater relative α_{S1} -CN concentration and female reproductive performance. Moreover, to further investigate the relationship between the relative α_{S1} -CN concentration and cow fertility traits, model [1] with linear and quadratic effects for α_{S1} -CN was also tested. The results (not shown) indicated no significant quadratic effect, thereby confirming the linear relationship.

In addition to α_{S1} -CN, 2 other significant ($P < 0.05$) relationships were found. First, a greater relative concentration of α -LA was associated with a lesser (8.9%) nonreturn rate after 28 d, thereby indicating a possible antagonistic relation between α -LA concentration and cow fertility. Second, a greater relative concentration of β -CN was associated with a lesser (2.76 d) calving interval, thereby suggesting a positive effect of milk β -CN concentration on reproduction. However, these associations were found only for single fertility traits and became nonsignificant after correcting for multiple testing. Therefore, these findings were considered inconclusive. Regarding the other major milk proteins, no significant association was detected between β -LG, α_{S2} -CN, κ -CN, and CN-index and any of the fertility traits.

To our knowledge, this study is the first to report on the direct association between the relative concentrations of major milk proteins and fertility traits in cattle. Our findings indicate a positive relationship between greater relative α_{S1} -CN concentration and reproductive performance. On one hand, the relative concentration of α_{S1} -CN has positive genetic correlations with milk (0.52) and protein (0.29) yields (Schopen et al., 2009). These production traits are known to have an antagonistic relationship with fertility traits (e.g., Nebel and McGilliard, 1993; Dematawewa and Berger, 1998). Consequently, a greater relative α_{S1} -CN concentration would be expected to entail greater milk and protein yields, which in turn would be expected to negatively influence female fertility traits. The results of the present study, however, do not support this prior expectation because a positive association was found between the relative concentration of α_{S1} -CN protein and cow fertility traits.

On the other hand, the detected positive relationship is in agreement with the effect of the polymorphism in the α_{S1} -CN (*CSN1S1*) gene (which encodes the α_{S1} -CN protein) on reproductive performance. In US Holstein cattle, Hargrove et al. (1980) found that cows with the

Table 3. Association between the relative concentration of the 6 major milk proteins or the CN-index and the fertility traits¹ of 1,644 first-lactation Dutch Holstein-Friesian cows²

Protein	CIN (n = 1,482)	DFS (n = 1,639)	NINS (n = 1,642)	CR (n = 1,644)	NR28 (n = 1,644)	NR56 (n = 1,644)	NR90 (n = 1,644)	CFI (n = 1,644)
α -LA	0.746 $\pm$ 6.587	1.353 $\pm$ 3.053	0.029 $\pm$ 0.121	0.028 $\pm$ 0.024	-0.089 $\pm$ 0.037*	-0.042 $\pm$ 0.041	-0.031 $\pm$ 0.042	0.009 $\pm$ 0.041
β -LG	-0.925 $\pm$ 1.687	-0.102 $\pm$ 0.784	0.012 $\pm$ 0.032	0.006 $\pm$ 0.006	-0.005 $\pm$ 0.009	-0.010 $\pm$ 0.011	-0.010 $\pm$ 0.011	-0.005 $\pm$ 0.011
α_{S1} -CN	-0.531 $\pm$ 1.264	0.361 $\pm$ 0.586	-0.045 $\pm$ 0.023	-0.002 $\pm$ 0.005	0.019 $\pm$ 0.007**	0.027 $\pm$ 0.008**	0.029 $\pm$ 0.008**	0.025 $\pm$ 0.008**
α_{S2} -CN	0.741 $\pm$ 1.446	-0.772 $\pm$ 0.678	0.006 $\pm$ 0.027	0.005 $\pm$ 0.005	-0.004 $\pm$ 0.008	-0.007 $\pm$ 0.009	-0.011 $\pm$ 0.009	-0.009 $\pm$ 0.009
β -CN	-2.755 $\pm$ 1.265*	-0.839 $\pm$ 0.587	-0.006 $\pm$ 0.023	0.000 $\pm$ 0.005	-0.011 $\pm$ 0.007	-0.009 $\pm$ 0.008	0.002 $\pm$ 0.008	0.001 $\pm$ 0.008
κ -CN ³	0.548 $\pm$ 3.504	0.389 $\pm$ 1.632	-0.029 $\pm$ 0.065	-0.004 $\pm$ 0.013	-0.017 $\pm$ 0.020	-0.040 $\pm$ 0.022	-0.029 $\pm$ 0.022	-0.027 $\pm$ 0.022
CN-index ⁴	15.98 $\pm$ 144.8	-18.28 $\pm$ 67.08	-1.973 $\pm$ 2.713	-0.552 $\pm$ 0.534	0.854 $\pm$ 0.834	1.123 $\pm$ 0.906	1.284 $\pm$ 0.927	0.657 $\pm$ 0.918

¹Fertility traits were interval between first and second calving (CIN), interval between calving and first insemination (DFS), number of inseminations (NINS), calving rate (CR), nonreturn rate for insemination 28 d after first service (NR28), nonreturn rate for insemination 56 d after first service (NR56), nonreturn rate for insemination 90 d after first service (NR90), and calving rate following the first insemination (CFI).

²Values after $\pm$ signs are corresponding SE.

³Only κ -CN in the nonglycosylated mono-phosphorylated form.

⁴CN-index = $(\alpha_{S1}\text{-CN} + \alpha_{S2}\text{-CN} + \beta\text{-CN} + \kappa\text{-CN})/(\alpha\text{-LA} + \beta\text{-LG} + \alpha_{S1}\text{-CN} + \alpha_{S2}\text{-CN} + \beta\text{-CN} + \kappa\text{-CN})$.

*Significant ($P < 0.05$); **significant after Bonferroni correction.

Table 4. Significance (*P*-values) of the association between β -LG, β -CN, κ -CN, and β - κ -CN variants and fertility traits¹ on first-lactation Dutch Holstein-Friesian cows

Variant	CIN	DFS	NINS	CR	NR28	NR56	NR90	CFI
Genotypes								
β -LG (n = 1,600)	0.957	0.424	0.442	0.587	0.161	0.233	0.133	0.099
β -CN (n = 1,577)	0.359	0.819	0.317	0.391	0.207	0.151	0.222	0.407
κ -CN (n = 1,612)	0.306	0.804	0.212	0.263	0.071	0.392	0.475	0.523
Haplotypes								
A ¹ A (n = 1,540)	0.718	0.779	0.817	0.788	0.421	0.100	0.221	0.513
A ¹ B (n = 1,537)	0.482	0.242	0.588	0.404	0.944	0.642	0.621	0.978
A ¹ E (n = 1,540)	0.130	0.855	0.126	0.395	0.720	0.711	0.459	0.332
A ² A (n = 1,540)	0.070	0.506	0.990	0.090	0.730	0.828	0.973	0.829
A ² B (n = 1,540)	0.188	0.296	0.243	0.319	0.678	0.946	0.807	0.905
BB (n = 1,540)	0.058	0.546	0.457	0.423	0.828	0.389	0.548	0.286
IB (n = 1,540)	0.733	0.887	0.120	0.698	0.128	0.017*	0.059	0.221

¹Fertility traits were interval between first and second calving (CIN), interval between calving and first insemination (DFS), number of inseminations (NINS), calving rate (CR), nonreturn rate for insemination 28 d after first service (NR28), nonreturn rate for insemination 56 d after first service (NR56), nonreturn rate for insemination 90 d after first service (NR90), and calving rate following the first insemination (CFI).

*Significant (*P* < 0.05).

α _{S1}-CN BC genotype had greater probability of conception relative to cows with the BB genotype. The BC genotype has also been found to be associated with greater α _{S1}-CN concentration than the BB genotype (McLean et al., 1984; Graml and Pirchner, 2003). That is, the genetic variant of the α _{S1}-CN protein that is associated with greater relative α _{S1}-CN concentration is also associated with better female reproductive performance. According to the study by Heck et al. (2009), α _{S1}-CN protein variants B and C can be detected in Dutch Holstein-Friesian cows; however, the frequency of the α _{S1}-CN C allele in the population is low (0.3%).

The relationship between concentration of major milk proteins and cow fertility traits is of importance, because the findings can be used to assess the correlated effects of breeding programs on economically important traits. Recent research has shown that selective breeding can be used to improve the milk protein composition of bovine milk. Studies have shown large variation in the relative concentration of the major milk proteins (Bobe et al., 1998; Bordin et al., 2001; Heck et al., 2008). Furthermore, a considerable part of this variation is genetic (Bobe et al., 1999; Graml and Pirchner, 2003). In a recent study, Schopen et al. (2009) found moderate to high intraherd heritabilities for milk protein composition in Dutch Holstein-Friesian cattle. Changing milk protein composition by selection is desirable because it can result in raw milk with improved manufacturing properties. For cheesemaking, raw milk with high concentrations of α _{S1}-CN, β -CN, and κ -CN and with high proportion of κ -CN within total CN is desirable (e.g., Wedholm et al., 2006). Results of the present study indicate that selective breeding to increase α _{S1}-CN concentration could improve cow fertility, whereas selection to increase other desirable protein fractions would not affect reproductive performance.

Effects of Milk Protein Variants

To study the effects of β -LG variants on fertility traits, a sample of 1,600 animals was analyzed (model [2]). As the *P*-values indicate (Table 4), no statistically significant relationship was found between β -LG genotype and cow fertility traits. Few studies have investigated the direct association between the β -LG genotypes and reproductive traits in cattle. On one hand, Lin et al. (1987) reported a significant effect on age at first conception, age at first calving, and gestation length. On the other hand, other studies (Hargrove et al., 1980; Ruottinen et al., 2004; Tsiaras et al., 2005) found no significant relationship between β -LG genotypes and several fertility traits. Therefore, the conclusions of the available studies are contradictory. This study failed to detect any significant association between β -LG variants and cow fertility traits, thereby further supporting the hypothesis of no association.

To study the relationship between β -CN variants and fertility traits, a sample of 1,577 animals was analyzed (model [2]). The high *P*-values reported in Table 4 indicate no statistically significant relationship. Only 2 earlier studies analyzing the association between β -CN variants and cow fertility traits have been reported. Hargrove et al. (1980) found that the β -CN genotype had a significant effect on the probability of conception. However, Lin et al. (1987) did not find any significant association. To our knowledge, this is the first study to report on the effects of the β -CN protein variants B and I on cow fertility traits. The B allele, which is known to be common for β -CN protein (Farrell et al., 2004), has been shown to have a frequency of approximately 2% in the Dutch Holstein-Friesian cows (Heck et al., 2009; Visker et al., 2010). The I allele was recently detected for the first time in the Dutch Holstein-Friesian

population by Visker et al. (2010), exhibiting 19.2% allele frequency. The present study did not detect any significant association between any β -CN variant and cow fertility traits.

To study the relationship between κ -CN genotypes and fertility traits, we analyzed a sample of 1,612 animals (model [2]) and found no statistically significant relationship (Table 4). This result is in line with that of other studies found in the literature. Earlier studies (Hargrove et al., 1980; Lin et al., 1987; Tsiaras et al., 2005) consistently reported no significant effect of the κ -CN locus on cow fertility. Therefore, the present results confirm those earlier conclusions. Furthermore, to our knowledge the present study is the first to report on the effects of κ -CN variant E on reproductive traits. The E allele has been recently detected in the Dutch Holstein-Friesian population, with an allele frequency of 9.2% (Heck et al., 2009). In the present analyses, no relationship between the E allele and fertility traits was detected.

To estimate the effects of β - κ -CN haplotypes on fertility traits, a sample of 1,540 animals was analyzed (model [2]). The observed haplotype frequencies are presented in Table 2. Our analyses did not detect a significant relationship between the β - κ -CN haplotypes and the fertility traits (Table 4), except for the association between IB haplotype and NR56 ($P = 0.017$). However, this latter association occurred for a single trait only and became nonsignificant after Bonferroni correction. Therefore, the results suggest that cow fertility is virtually unaffected by the β - κ -CN haplotype in Dutch Holstein-Friesian cattle. We found only a single study reporting on the association between β - κ -CN haplotypes and cow reproductive traits. Ruottinen et al. (2004) detected a significant effect on the number of days from calving to first service in Finnish Ayrshire cows; however, they concluded that the β - κ -CN haplotypes have virtually no effect on female reproduction. Our results agree with this conclusion.

Investigating the effects of genetic variants of milk proteins on economically important traits is important because this information can be used to evaluate gene-assisted selection strategies. The influence of protein genes on milk protein composition and cheesemaking properties has been thoroughly investigated, and the protein genes have been generally considered as candidates for improving milk protein composition (e.g., Caroli et al., 2009). The present study indicates that selection based on β -LG, β -CN, and κ -CN genotypes and β - κ -CN haplotypes would not affect female reproductive performance in Dutch Holstein-Friesian cattle.

CONCLUSIONS

The objective of this study was to examine the relationship between cow fertility traits and milk protein composition and milk protein variants in commercial Dutch Holstein-Friesian dairy cattle. A greater relative concentration of α_{S1} -casein within total milk protein had a positive phenotypic relationship with nonreturn rates for insemination 28, 56, and 90 d after first service and with calving rate after first insemination. Furthermore, results did not show any significant association between β -LG, β -CN, and κ -CN genotypes and β - κ -CN haplotypes and cow fertility traits. The findings of the present study suggest that a breeding program aiming to improve the manufacturing properties of milk by selecting animals based on milk protein composition or milk protein variants would have no negative influence on the reproductive performance of cows. Results of this study can be used to assess the (economic) effects of changing milk protein composition, which consequently allows for better evaluation of selective breeding programs before implementation.

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