Genetic parameters for α_{S1} -casein and α_{S2} -casein phosphorylation isoforms in Dutch Holstein Friesian

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ABSTRACT

Relative concentrations of α_{S1} -casein and α_{S2} -casein (α_{S1} -CN and α_{S2} -CN) phosphorylation isoforms vary considerably among milk of individual cows. We estimated heritabilities for α_{S2} -CN phosphorylation isoforms, determined by capillary zone electrophoresis from 1,857 morning milk samples, and genetic correlations among α_{S2} -CN phosphorylation isoforms in Dutch Holstein Friesian. To investigate if phosphorylation of α_{S1} -CN and α_{S2} -CN are due to the same genetic mechanism, we also estimated genetic correlations between α_{S1} -CN and α_{S2} -CN phosphorylation isoforms as well as the genetic correlations between the phosphorylation degrees (PD) of α_{S1} -CN and α_{S2} -CN defined as the proportion of isoforms with higher degrees of phosphorylation in total α_{S1} -CN and α_{S2} -CN, respectively. The intra-herd heritabilities for the relative concentrations of α_{S2} -CN phosphorylation isoforms were high and ranged from 0.54 for α_{S2} -CN-10P to 0.89 for α_{S2} -CN-12P. Furthermore, the high intra-herd heritabilities of α_{S1} -CN PD and α_{S2} -CN PD imply a strong genetic control of the phosphorylation process, which is independent of casein production. The genetic correlations between α_{S2} -CN phosphorylation isoforms are positive and moderate to high (0.33–0.90). Furthermore, the strong positive genetic correlation (0.94) between α_{S1} -CN PD and α_{S2} -CN PD suggests that the phosphorylation processes of α_{S1} -CN and α_{S2} -CN are related. This study shows the possibility of breeding for specific α_{S1} -CN and α_{S2} -CN phosphorylation isoforms, and relations between the phosphorylation degrees of α_{S1} -CN and α_{S2} -CN and technological properties of milk need to be further investigated to identify potential benefits for the dairy industry.

Key words: milk protein composition, genetic correlation, posttranslational modification, capillary zone electrophoresis

INTRODUCTION

Phosphorylation of caseins is a crucial posttranslational modification that allows caseins to interact with calcium phosphate in the assembly of colloidal structures known as casein micelles. The formation and stability of micelles depend largely on the presence of κ -CN on the micelle surface and its glycosylation, as well as the interactions between calcium phosphate nanoclusters and phosphoserine residues of α_{S1} -CN, α_{S2} -CN, and β -CN (De Kruif and Holt, 2003; De Kruif et al., 2012). Although caseins are all phosphoproteins, α_{S1} -CN and α_{S2} -CN are more highly phosphorylated and possess multiple phosphoserine clusters, whereas β -CN possesses only one, and κ -CN does not possess any. This suggests that α_{S1} -CN and α_{S2} -CN might be more relevant for stabilizing internal micellar structure (Dalglish and Corredig, 2012; Huppertz et al., 2017).

In bovine milk, α_{S1} -CN accounts for about 35% of the total casein and has 2 common phosphorylation isoforms [i.e., α_{S1} -CN-8P as the predominant form and α_{S1} -CN-9P (where P indicates phosphate group attached; Holland and Boland, 2014)]; α_{S2} -CN accounts for about 10% of the total casein and is present with various degrees of phosphorylation ranging from 10P to 14P and occasionally with 9P or 15P (Fang et al., 2016). Milk containing a greater proportion of α_{S1} -CN and α_{S2} -CN isoforms with lower degrees of phosphorylation (e.g., α_{S1} -CN-8P, α_{S2} -CN-10P, and α_{S2} -CN-11P) has been suggested to possess better coagulation properties and to be hydrolyzed more efficiently by chymosin (Frederiksen et al., 2011; Jensen et al., 2012; Bijl et al., 2014b). Furthermore, relative concentrations of α_{S1} -CN and α_{S2} -CN phosphorylation isoforms in milk vary considerably between individual cows (Bijl et al., 2014a; Fang et al., 2016). Therefore, it is of great interest to

Received August 3, 2017.

Accepted October 10, 2017.

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investigate if genetic variation in individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms exists.

To date, only a few studies reported heritabilities for relative concentrations of α_{S1} -CN-8P and α_{S1} -CN-9P in Dutch Holstein Friesian (Bijl et al., 2014a), Danish Holstein and Jersey (Buitenhuis et al., 2016), and French Montbéliarde (Fang et al., 2017). Only one study in French Montbéliarde reported the heritabilities for relative concentrations of α_{S2} -CN-10P to α_{S2} -CN-14P (Fang et al., 2017). Additionally, Bijl et al. (2014a) reported a surprisingly low genetic correlation between α_{S1} -CN-8P and α_{S1} -CN-9P concentration. Heck et al. (2008) and Fang et al. (2016) reported that the proportion of α_{S1} -CN and α_{S2} -CN isoforms with lower degrees of phosphorylation phenotypically correlated negatively with the proportion of α_{S1} -CN and α_{S2} -CN isoforms with higher degrees of phosphorylation. However, genetic correlations among α_{S2} -CN phosphorylation isoforms as well as genetic correlations between α_{S1} -CN and α_{S2} -CN isoforms have not been reported.

Within the framework of the Dutch milk genomic initiative, detailed milk protein composition was determined by capillary zone electrophoresis (CZE) for approximately 2,000 cows. However, peaks assigned to α_{S2} -CN-10P and α_{S2} -CN-12P were based on predicted migration time without the use of standards of individual isoforms for confirmation, and α_{S2} -CN-13P and α_{S2} -CN-14P were not identified (Heck et al., 2008). The aim of this study was first to identify the peaks corresponding to individual α_{S2} -CN phosphorylation isoforms on CZE electropherograms for quantifying isoforms in the Dutch milk genomics initiative population, and second to estimate heritabilities for α_{S2} -CN phosphorylation isoforms and genetic correlations among α_{S2} -CN phosphorylation isoforms. To investigate if phosphorylation of α_{S1} -CN and α_{S2} -CN is due to the same genetic mechanism, we also estimated genetic correlations between α_{S1} -CN and α_{S2} -CN phosphorylation isoforms as well as the genetic correlations between the phosphorylation degrees (PD) of α_{S1} -CN and α_{S2} -CN defined as the proportion of isoforms with higher degrees of phosphorylation in total α_{S1} -CN and α_{S2} -CN, respectively. We also investigated the relations of α_{S1} -CN and α_{S2} -CN phosphorylation isoforms with milk protein composition, phosphorus content, and with routinely collected milk production traits.

MATERIALS AND METHODS

Animals

Test-day morning milk samples were collected from approximately 2,000 primiparous Dutch Holstein Frie-

sian cows as part of the Dutch Milk Genomic Initiative. Cows were located on 398 herds in the Netherlands, and at least 3 cows per herd were sampled. The pedigree of the cows was supplied by cattle improvement organization CRV (Arnhem, the Netherlands). Detailed description of the experimental design is provided in Schopen et al. (2009).

In this study, we analyzed milk production traits and milk protein composition from 1,857 milk samples collected in winter.

Phenotypes

Milk Production Traits. Protein, fat, and lactose percentages were determined by infrared spectroscopy using a MilkoScan FT 6000 (Foss Electric, Hillerød, Denmark) at the milk control station laboratory (Qlip, Zutphen, the Netherlands). Phosphorus concentration was determined by inductively coupled plasma-atomic emission spectrometry (Vista Axial, Varian, Australia) of whole milk as described in van Hulzen et al. (2009). Test-day morning milk yield was available for 1,721 cows and was obtained from CRV. Yields of protein, fat, lactose, and phosphorus were calculated by multiplying the respective content traits by the observed milk yield.

Milk Protein Composition. The relative concentrations (% wt/wt) of individual milk proteins and their isoforms were determined by CZE by Heck et al. (2008). The κ -CN was measured as only nonglycosylated and monophosphorylated form. The α_{S1} -CN phosphorylation isoforms were identified by using α_S -CN standards as described by Heck et al. (2008). The relative concentration of total α_{S1} -CN was the sum of relative concentrations of α_{S1} -CN-8P and α_{S1} -CN-9P and also the peak that migrates just before the α_{S1} -CN-8P peak and is predicted to be α_{S1} -CN-7P according to Heck et al. (2008). Standards for the individual isoforms were not available for α_{S2} -CN isoforms. Therefore, to identify peaks corresponding to individual α_{S2} -CN phosphorylation isoforms on electropherograms from CZE analysis, we purified α_{S2} -CN phosphorylation isoforms from milk samples. For this purpose, 10 fresh milk samples were analyzed by liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) for screening their α_{S2} -CN phosphorylation profiles using the method of Fang et al. (2016). Fractions containing individual α_{S2} -CN phosphorylation isoforms were separated and collected by LC with the same method using milk that contained highest concentrations of α_{S2} -CN-10P to α_{S2} -CN-14P, respectively. Collected fractions were analyzed by LC/ESI-MS to verify the components. The fractions were then analyzed by CZE using the method of Heck

et al. (2008) to confirm the peaks corresponding to individual α_{S2} -CN phosphorylation isoforms in CZE electropherograms. Yields (g) of individual milk proteins and their isoforms were calculated by multiplying relative concentrations (% wt/wt) by protein yield (g).

Relative concentrations of the α_{S1} -CN and α_{S2} -CN phosphorylation isoforms are the result of 2 distinct processes: the production of α_{S1} -CN and α_{S2} -CN, and the posttranslational modification of both caseins. To characterize the phosphorylation process that is independent of casein production, we defined the phosphorylation degrees (PD) of α_{S1} -CN and α_{S2} -CN as the proportion of isoforms with higher degrees of phosphorylation, which were calculated as

$$\alpha_{S1}\text{-CN PD} = \left(\frac{\alpha_{S1}\text{-CN-9P}}{\alpha_{S1}\text{-CN-8P} + \alpha_{S1}\text{-CN-9P}} \right) \times 100\%, \quad [1]$$

$$\alpha_{S2}\text{-CN PD} = \left(\frac{\alpha_{S2}\text{-CN-12P}}{\alpha_{S2}\text{-CN-10P} + \alpha_{S2}\text{-CN-11P} + \alpha_{S2}\text{-CN-12P}} \right) \times 100\%; \quad [2]$$

α_{S2} -CN-13P and α_{S2} -CN-14P were not included in Equation [2] due to low reproducibility of their quantification.

Output of Casein Phosphorus. To investigate if the phosphorus availability would be a limiting factor for phosphorylation of all caseins when more caseins are produced, we also estimated the phenotypic and genetic correlations between the total amount of phosphate groups attached to the caseins in milk (i.e., output of casein phosphorus, $\mathbf{P}_{cas}$), and the milk protein and milk phosphorus yields. To derive $\mathbf{P}_{cas}$, we first calculated the molar concentration ($\mathbf{C}_{molar}$) of each casein (α_{S1} -CN, α_{S2} -CN, κ -CN, and β -CN) as its concentration in milk [g/L; as protein percentage $\times 10 \times$ (% wt/wt)] divided by its respective molecular weight (Da). As κ -CN carried 1 phosphate group, and β -CN carried 5 in our milk samples, $\mathbf{C}_{molar}$ of phosphate groups attached to κ -CN and β -CN were approximated by multiplying $\mathbf{C}_{molar}$ of κ -CN and β -CN by 1 and 5, respectively. The $\mathbf{C}_{molar}$ of phosphate groups attached to α_{S1} -CN was the sum of $\mathbf{C}_{molar}$ of α_{S1} -CN-8P multiplied by 8 and $\mathbf{C}_{molar}$ of α_{S1} -CN-9P multiplied by 9. The $\mathbf{C}_{molar}$ of phosphate groups attached to α_{S2} -CN was the sum of $\mathbf{C}_{molar}$ of α_{S2} -CN-10P multiplied by 10, $\mathbf{C}_{molar}$ of α_{S2} -CN-11P multiplied by 11, and $\mathbf{C}_{molar}$ of α_{S2} -CN-12P multiplied by 12. Therefore, the total $\mathbf{C}_{molar}$ of the phosphate groups attached to the caseins was the sum of $\mathbf{C}_{molar}$ of the phosphate groups attached to the individual caseins. Subsequently, $\mathbf{P}_{cas}$ was approximated by multiplying the total $\mathbf{C}_{molar}$ of phosphate groups attached to the caseins by milk yield.

Statistical Analyses

To estimate variance components and genetic parameters, the following animal model was used:

$$y_{klmn} = \mu + \beta_1 dim_{klmn} + \beta_2 e^{-0.05 \times dim_{klmn}} + \beta_3 ca_{klmn} + \beta_4 ca_{klmn}^2 + season_k + scode_l + animal_m + herd_n + e_{klmn}, \quad [3]$$

where y_{klmn} is the observation of the trait of interest; μ is the overall mean of the trait; $\beta_{1,2}$ are the regression coefficients for dim_{klmn} ; $\beta_{3,4}$ are the regression coefficients for ca_{klmn} ; dim_{klmn} is a covariate describing the effect of days in lactation, modeled with a Wilmink curve (Wilmink, 1987); ca_{klmn} is a covariate describing the effect of age at first calving; $season_k$ is the fixed effect with 3 classes of calving season (June–August 2004, September–November 2004, and December 2004–February 2005); $scode_l$ is the fixed effect accounting for possible differences in genetic level between proven bull daughters and young bull daughters; $animal_m$ is the random additive genetic effect assumed to be distributed as $N(\mathbf{0}, \mathbf{A}\sigma_a^2)$, where $\mathbf{A}$ is the additive genetic relationships matrix consisting of 26,300 animals, and σ_a^2 is the additive genetic variance; $herd_n$ is the random herd effect assumed to be distributed as $N(\mathbf{0}, \mathbf{I}\sigma_{herd}^2)$, where $\mathbf{I}$ is the identity matrix, and σ_{herd}^2 is the herd variance; e_{klmn} is the random residual effect assumed to be distributed as $N(\mathbf{0}, \mathbf{I}\sigma_e^2)$, where $\mathbf{I}$ is the identity matrix, and σ_e^2 is the residual variance. The intra-herd heritability was defined as

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}.$$

The proportion of phenotypic variance explained by herd was defined as

$$h_{herd} = \frac{\sigma_{herd}^2}{\sigma_{herd}^2 + \sigma_a^2 + \sigma_e^2}.$$

Phenotypic and genetic correlations between traits were estimated by bivariate analyses using model [3]. All statistical analyses were performed using ASReml (Gilmour et al., 2009).

RESULTS AND DISCUSSION

Characterization of α_{S2} -CN Phosphorylation Profile by CZE

In this study, we first characterized the α_{S2} -CN phosphorylation profile in CZE electropherograms. To

Figure 1. Electropherograms of 1 milk sample and 3 purified fractions of α_{S2} -CN phosphorylation isoforms showing α_{S1} -CN and α_{S2} -CN phosphorylation isoforms. (A) Purified fraction containing α_{S2} -CN-10P (peak 1) and α_{S2} -CN-11P (peak 2), where P indicates phosphate group attached. (B) Purified fraction containing α_{S2} -CN-11P, α_{S2} -CN-12P (peak 3), and α_{S2} -CN-13P (peak 4). (C) Purified fraction containing α_{S2} -CN-11P and α_{S2} -CN-14P (peak 5).

identify the peaks corresponding to individual α_{S2} -CN phosphorylation isoforms in a milk sample on the CZE electropherograms, the CZE electropherograms of the purified and identified fractions of individual α_{S2} -CN phosphorylation isoforms were aligned with the CZE electropherogram of a milk sample (Figure 1). The peaks corresponding to α_{S2} -CN-10P, α_{S2} -11P, and α_{S2} -12P matched the migration times predicted by Heck et al. (2008). Therefore, the reproducibilities of their measurements, calculated as the coefficient of variation from repeated measurements of a reference milk sample, were 15.7% for α_{S2} -CN-10P, 5.7% for α_{S2} -CN-11P, and 5.8 for α_{S2} -CN-12P (Heck et al., 2008). The peak between the α_{S2} -CN-11P peak and the α_{S2} -CN-12P peak was suggested to be products of casein hydrolysis by Recio et al. (1997). The peak corresponding to α_{S2} -CN-13P was difficult to quantify accurately using CZE due to co-migration with other components in milk, which resulted in poor reproducibility of its quantification. The peak corresponding to α_{S2} -CN-14P overlapped with the α_{S1} -CN-8P peak. Therefore, we only estimated the relative concentrations of α_{S2} -CN-10P, α_{S2} -11P, and α_{S2} -12P based on the applied CZE method. Furthermore, α_{S2} -CN-14P accounts for about 0.4% of total protein in milk according to Fang et al. (2016). Therefore, we do not expect the measurement of α_{S1} -CN-8P to be

strongly affected by the overlap with α_{S2} -CN-14P. Relative concentrations of α_{S1} -CN-8P and α_{S1} -CN-9P had been determined previously based on the same CZE electropherograms (Heck et al., 2008).

Genetic Parameters

Table 1 shows the descriptive statistics, heritabilities, and proportions of variance explained by herd for α_{S1} -CN and α_{S2} -CN phosphorylation isoforms and for the phosphorylation degrees of α_{S1} -CN (α_{S1} -CN PD) and α_{S2} -CN (α_{S2} -CN PD). To disentangle the casein production and the phosphorylation process, we defined the phosphorylation degree as the proportion of isoforms with higher degrees of phosphorylation in the sum of α_{S1} -CN-8P and α_{S1} -CN-9P and in the sum of α_{S2} -CN-10P, α_{S2} -CN-11P, and α_{S2} -CN-12P, respectively. The most abundant α_{S2} -CN phosphorylation isoform was α_{S2} -CN-11P. The average α_{S1} -CN PD was around 26%, meaning that 26% of α_{S1} -CN is phosphorylated at the highest level (i.e., 9P), and the average α_{S2} -CN PD was around 34%, meaning that 34% of α_{S2} -CN is phosphorylated at the highest level among the detected α_{S2} -CN isoforms (i.e., 12P in the current study). The average relative concentrations of α_{S1} -CN-8P, α_{S1} -CN-9P, α_{S2} -CN-10P, α_{S2} -CN-11P, and α_{S2} -CN-12P, and

Table 1. Mean, SD, phenotypic variance¹ (σ_p^2), intra-herd heritability estimates (h^2), and proportion of phenotypic variance explained by herd (h_{herd}) for relative concentrations of individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms and for the phosphorylation degrees (PD)² of α_{S1} -CN and α_{S2} -CN measured on test-day morning milk samples from 1,857 Dutch Holstein Friesian cows (SE in parentheses)

Trait (% wt/wt)	Mean	SD	σ_p^2	h^2	h_{herd}
α_{S1} -CN ³	33.64	1.66	2.80	0.52 (0.11)	0.11 (0.02)
α_{S1} -CN-8P ³	21.26	1.13	1.32	0.48 (0.10)	0.12 (0.02)
α_{S1} -CN-9P ³	7.42	1.07	1.18	0.76 (0.12)	0.08 (0.02)
α_{S2} -CN	6.67	0.95	0.98	0.94 (0.12)	0.08 (0.02)
α_{S2} -CN-10P	0.99	0.39	0.16	0.54 (0.11)	0.10 (0.02)
α_{S2} -CN-11P	3.44	0.57	0.33	0.89 (0.12)	0.08 (0.02)
α_{S2} -CN-12P	2.24	0.22	0.05	0.71 (0.12)	0.07 (0.02)
Phosphorylation degree					
α_{S1} -CN PD ²	25.79	2.72	7.66	0.78 (0.12)	0.08 (0.02)
α_{S2} -CN PD ²	34.01	4.24	18.18	0.64 (0.11)	0.09 (0.02)

¹Phenotypic variance after adjusting for systematic effects.

² α_{S1} -CN PD = α_{S1} -CN-9P / (α_{S1} -CN-8P + α_{S1} -CN-9P) $\times$ 100; α_{S2} -CN PD = α_{S2} -CN-12P / (α_{S2} -CN-10P + α_{S2} -CN-11P + α_{S2} -CN-12P) $\times$ 100. P = phosphate group attached.

³Adapted from Bijl et al. (2014a).

the average α_{S1} -CN PD are in the range of the values reported for French Montbéliarde (Fang et al., 2017). The α_{S2} -CN-13P and α_{S2} -CN-14P were not included in the current study, but they account for about 24% of the total α_{S2} -CN concentration in French Montbéliarde (Fang et al., 2016). Because α_{S2} -CN-13P and α_{S2} -CN-14P were not included, α_{S2} -CN PD is defined differently in the current study and therefore cannot be compared directly with results for French Montbéliarde (Fang et al., 2016). The average α_{S2} -CN PD is similar in both studies when using the definition of the current study to calculate the α_{S2} -CN PD in French Montbéliarde.

The intra-herd heritabilities were high to very high for relative concentrations of individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms (0.48 to 0.89), for α_{S1} -CN PD (0.78), and for α_{S2} -CN PD (0.64). Differences between herds contributed approximately 10% to the phenotypic variation in relative concentrations of individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms, α_{S1} -CN PD, and α_{S2} -CN PD. Our results suggest that housing, management, and nutritional differences between farms have little effect on α_{S1} -CN and α_{S2} -CN phosphorylation under Dutch farming conditions. The high intra-herd heritabilities of individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms suggest an important role of genetic factors in Dutch Holstein Friesian. Furthermore, the high intra-herd heritabilities of α_{S1} -CN (0.52), α_{S2} -CN (0.94), α_{S1} -CN PD, and α_{S2} -CN PD imply a strong genetic control not only of the α_{S1} -CN and α_{S2} -CN synthesis but also of the phosphorylation process. It should be noted that we reported lower α_{S2} -CN concentration and higher intra-herd heritability of α_{S2} -CN compared with values reported by Schopen et al. (2009), who analyzed approximately the same set of

samples. In the current study, we accounted only the α_{S2} -CN isoforms that we identified on CZE electropherograms as described, whereas Schopen et al. (2009) also accounted multiple peaks around the α_{S2} -CN-11P peak. Therefore, α_{S2} -CN concentration is defined differently in the current study, which explains the differences between the 2 studies.

Few studies reported on the genetic background of individual α_{S2} -CN phosphorylation isoforms. Only a single study has reported genetic parameters for the relative concentrations of individual α_{S2} -CN phosphorylation isoforms and for α_{S2} -CN PD measured by LC/ESI-MS method in the milk of French Montbéliarde cows (Fang et al., 2017). The data did not allow Fang et al. (2017) to adjust for differences between herds. To allow comparison with that study, we also estimated inter-herd heritabilities for the current study. The inter-herd heritabilities for all the traits in this study (0.41 to 0.82) were higher than the inter-herd heritabilities from the French Montbéliarde (0.07 to 0.37; Fang et al., 2017). Discrepancies between both studies might be due to genetic differences between the breeds (Dutch Holstein Friesian vs. French Montbéliarde), limited sample size in the study of Fang et al. (2017), which resulted in high standard errors of the estimates, and the use of different analytical methods (CZE vs. LC/ESI-MS). For measurement of α_{S1} -CN isoforms and α_{S1} -CN PD, the difference between CZE and LC/ESI-MS was trivial when we compared measurements from the same 10 samples analyzed with both methods (data not shown). Therefore, differences between estimated genetic parameters for these traits might be mainly due to breed differences, e.g., allele frequencies might differ between breeds. The β -LG genotypes affect α_{S1} -CN-8P

concentration, and the *DGAT1* K232A polymorphism affects α_{S1} -CN-9P concentration in Dutch Holstein Friesian (Bijl et al., 2014a). However, the roles of β -LG and *DGAT1* in regulating specific phosphorylation isoforms remain unclear. Nonetheless, such established associations might vary between breeds due to differences in allele frequencies. The frequency of β -LG A and B alleles were 0.52 and 0.48 in Dutch Holstein Friesian, respectively (Bijl et al., 2014a), whereas the frequencies of β -LG A and B alleles were 0.44 and 0.55 in French Montbéliarde, respectively (Fang et al., 2016). Furthermore, the frequency of the *DGAT1* A allele was 0.65 in Dutch Holstein Friesian (Bijl et al., 2014a), whereas *DGAT1* is nearly fixed at the A allele in French Montbéliarde (0.96, Gautier et al., 2007). The *DGAT1* K232A polymorphism accounts for about 13% of the additive genetic variance of α_{S1} -CN-9P concentration according to Bijl et al. (2014a). After adjusting for the additive genetic variance attributed to the *DGAT1* K232A polymorphism and accounting for differences in the statistical model (herd differences), the inter-herd heritability of α_{S1} -CN-9P concentration in Dutch Holstein Friesian (0.60) is in range with that in French Montbéliarde (0.56). The genetic background of α_{S2} -CN isoforms is currently unknown, but a similar explanation might apply to the discrepancy between genetic parameters for individual α_{S2} -CN isoforms.

Correlations Among Phosphorylation Isoforms of α_{S1} -CN and α_{S2} -CN

Phenotypic correlations among individual α_S -CN phosphorylation isoforms were similar to the genetic ones (Table 2). Regarding genetic correlations, α_{S1} -CN-8P correlated weakly with the other α_S -CN phosphorylation isoforms (−0.10 to 0.17), suggesting that production of α_{S1} -CN-8P is rather independent from production of other α_S -CN phosphorylation isoforms. The phenotypic correlations (−0.31 to 0.20) of α_{S1} -CN-8P with other α_{S1} -CN and α_{S2} -CN phosphorylation isoforms in this study are in range with those (−0.12 to 0.43) reported by Fang et al. (2016) for French Montbéliarde. Both studies show weak to moderate correlations of α_{S1} -CN-8P with other isoforms, but the directions of the correlations are opposite. Taken together our results, results reported by Bijl et al. (2014a) and Fang et al. (2016), α_{S1} -CN-8P seems to be regulated differently than other α_S -CN phosphorylation isoforms. Regarding genetic correlations among α_{S2} -CN isoforms, α_{S2} -CN-12P correlated weakly with the other α_S -CN phosphorylation isoforms (−0.20 to 0.33) except moderately with α_{S2} -CN-11P (0.67); α_{S2} -CN-10P correlated strongly with α_{S2} -CN-11P (0.90).

The phenotypic correlation between α_{S2} -CN-10P and α_{S2} -CN-11P (0.60) is in line with the one reported for French Montbéliarde (0.62), whereas the phenotypic correlation between α_{S2} -CN-11P and α_{S2} -CN-12P (0.50) is considerably higher than the estimate for French Montbéliarde (−0.28; Fang et al., 2016). Discrepancies between the 2 studies could be explained by differences between studied breeds as mentioned above. Regarding genetic correlations among α_{S1} -CN and α_{S2} -CN isoforms, α_{S1} -CN-9P correlated strongly negatively with α_{S2} -CN-10P (−0.90) and α_{S2} -CN-11P (−0.80), suggesting an antagonistic regulation between α_{S1} -CN phosphorylated at a higher level and α_{S2} -CN phosphorylated at a lower level. The phenotypic correlations of α_{S1} -CN-9P with α_{S2} -CN-10P and α_{S2} -CN-11P in this study agree with those reported by Fang et al. (2016). Both studies show that the proportion of α_{S1} -CN isoforms with higher levels of phosphorylation (i.e., α_{S1} -CN-9P) correlate negatively with the proportion of α_{S2} -CN isoforms with lower levels of phosphorylation (i.e., α_{S2} -CN-10P and α_{S2} -CN-11P). Even though we could not estimate the correlations regarding α_{S2} -CN-13P and α_{S2} -CN-14P in the current study, our results partially support the hypothesis proposed by Fang et al. (2016) that either α_{S1} -CN and α_{S2} -CN isoforms phosphorylated at lower levels would be present in higher concentrations in milk than the ones phosphorylated at higher levels or vice versa based on the correlations of α_{S1} -CN-9P with the other α_S -CN isoforms. Furthermore, the strong positive genetic correlation (0.94) between α_{S1} -CN PD and α_{S2} -CN PD suggests that the phosphorylation degrees of α_{S1} -CN and α_{S2} -CN are related. This is also supported by strong correlations of different α_S -CN phosphorylation isoforms with both α_{S1} -CN PD and α_{S2} -CN PD.

Correlations of α_{S1} -CN and α_{S2} -CN Phosphorylation Isoforms with Major Milk Proteins

Phenotypic correlations among individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms and the major milk proteins were similar to the genetic ones (Table 3). Regarding genetic correlations, α_{S1} -CN correlated positively with both α_{S1} -CN phosphorylation isoforms, α_{S1} -CN PD and α_{S2} -CN PD (0.59 to 0.79), but negatively with α_{S2} -CN-10P (−0.57) and α_{S2} -CN-11P (−0.54). The α_{S2} -CN correlated positively with all α_{S2} -CN phosphorylation isoforms (0.74 to 0.99) but negatively with α_{S1} -CN-9P (−0.75), α_{S1} -CN PD (−0.77), and α_{S2} -CN PD (−0.74). The decrease of α_{S2} -CN PD while total α_{S2} -CN increases can be explained by an increase in the proportion of isoforms with lower degrees of phosphorylation (i.e., α_{S2} -CN-10P and α_{S2} -CN-11P). This

Table 2. Phenotypic (above diagonal) and genetic (below diagonal) correlations among individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms (% wt/wt) and the phosphorylation degrees (PD)¹ of α_{S1} -CN and α_{S2} -CN measured on test-day morning milk samples from 1,857 Dutch Holstein Friesian cows (SE in parentheses)

Trait (% wt/wt)	α_{S1} -CN-8P	α_{S1} -CN-9P	α_{S2} -CN-10P	α_{S2} -CN-11P	α_{S2} -CN-12P	α_{S1} -CN PD	α_{S2} -CN PD
α_{S1} -CN-8P		0.20 (0.03)	-0.31 (0.03)	-0.04 (0.03)	-0.00 (0.03)	-0.05 (0.03)	0.28 (0.03)
α_{S1} -CN-9P	0.17 (0.15)		-0.72 (0.02)	-0.69 (0.02)	0.00 (0.03)	0.96 (0.00)	0.75 (0.01)
α_{S2} -CN-10P	0.07 (0.17)	-0.90 (0.04)		0.60 (0.02)	0.06 (0.03)	-0.99 (0.02)	-0.82 (0.01)
α_{S2} -CN-11P	-0.06 (0.15)	-0.80 (0.05)	0.90 (0.04)		0.50 (0.03)	-0.80 (0.05)	-0.61 (0.02)
α_{S2} -CN-12P	-0.10 (0.16)	-0.20 (0.14)	0.33 (0.14)	0.67 (0.08)		-0.02 (0.04)	0.19 (0.03)
α_{S1} -CN PD ¹	-0.02 (0.16)	0.98 (0.01)	-0.68 (0.02)	-0.72 (0.02)	-0.02 (0.04)		0.71 (0.02)
α_{S2} -CN PD ¹	-0.02 (0.17)	0.91 (0.04)	-0.95 (0.03)	-0.80 (0.07)	-0.07 (0.16)	0.94 (0.03)	

¹ α_{S1} -CN PD = α_{S1} -CN-9P / (α_{S1} -CN-8P + α_{S1} -CN-9P) $\times$ 100; α_{S2} -CN PD = α_{S2} -CN-12P / (α_{S2} -CN-10P + α_{S2} -CN-11P + α_{S2} -CN-12P) $\times$ 100. P = phosphate group attached.

suggests that forming isoforms with lower degrees of phosphorylation is favored when production of α_{S2} -CN increases, whereas the opposite is observed when total α_{S1} -CN increases. Furthermore, Schopen et al. (2009) reported negative genetic correlation between α_{S1} -CN and α_{S2} -CN (-0.49). Here, we show that a higher α_{S1} -CN concentration is associated with lower α_{S2} -CN-10P and α_{S2} -CN-11P concentrations, and thus results in a higher degree of phosphorylation of α_{S2} -CN, whereas a higher α_{S2} -CN concentration is associated with lower α_{S1} -CN-9P concentration, and thus results in a lower degree of phosphorylation of α_{S1} -CN.

The genetic correlations between the relative concentrations of individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms and those of the other 4 major milk proteins (κ -CN, β -CN, α -LA, and β -LG) were moderate to weak (-0.40 to 0.28). It should be noted that the relative concentration of κ -CN reported in the currently study was measured as its nonglycosylated and mono-phosphorylated form. Phenotypic correlations were in

line with the ones reported by Fang et al. (2016) for French Montbéliarde, and the genetic correlations of α_{S1} -CN-8P concentration with the other 4 major milk proteins were in line with those reported by Gebreyesus et al. (2016) for Danish Holstein.

The genetic correlations among α_{S1} -CN PD, α_{S2} -CN PD, and the other 4 major milk proteins were weak (-0.33 to 0.26). These moderate to weak correlations of α_{S1} -CN and α_{S2} -CN isoforms, α_{S1} -CN PD, and α_{S2} -CN PD with the other 4 major milk proteins suggest that phosphorylation of α_{S1} -CN and α_{S2} -CN might be regulated differently from phosphorylation of β -CN and κ -CN. This is supported by the finding that β -CN might be phosphorylated in a different compartments of the Golgi apparatus in mice and rabbit than α -CN (Turner et al., 1993; Boisgard and Chanat, 2000). Phosphorylation of α -CN occurs in the Golgi cisternae during the transportation of milk proteins, whereas the phosphorylation of β -CN seems to occur later in the trans-Golgi network.

Table 3. Phenotypic (r_p) and genetic (r_g) correlations of individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms (%wt/wt) and the phosphorylation degrees (PD)¹ of α_{S1} -CN and α_{S2} -CN with the 6 major milk proteins (% wt/wt) measured on test-day morning milk samples from 1,857 Dutch Holstein Friesian cows (SE in parentheses)

Trait (% wt/wt)	α_{S1} -CN-8P		α_{S1} -CN-9P		α_{S2} -CN-10P		α_{S2} -CN-11P		α_{S2} -CN-12P		α_{S1} -CN PD		α_{S2} -CN PD	
	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g
α_{S1} -CN	0.75 (0.01)	0.73 (0.08)	0.74 (0.02)	0.79 (0.06)	-0.59 (0.02)	-0.57 (0.11)	-0.37 (0.03)	-0.54 (0.11)	0.09 (0.03)	-0.09 (0.16)	0.52 (0.02)	0.65 (0.09)	0.58 (0.02)	0.59 (0.10)
α_{S2} -CN	-0.28 (0.03)	-0.09 (0.15)	-0.63 (0.03)	-0.75 (0.06)	0.77 (0.01)	0.84 (0.05)	0.86 (0.01)	0.99 (0.01)	0.54 (0.02)	0.74 (0.07)	-0.61 (0.02)	-0.77 (0.06)	-0.68 (0.02)	-0.74 (0.07)
κ -CN ²	-0.21 (0.03)	-0.33 (0.15)	-0.42 (0.03)	-0.40 (0.12)	0.26 (0.03)	0.28 (0.15)	0.23 (0.03)	0.14 (0.14)	-0.18 (0.03)	-0.20 (0.14)	-0.37 (0.03)	-0.33 (0.13)	-0.38 (0.03)	-0.33 (0.13)
β -CN	0.12 (0.03)	-0.24 (0.19)	-0.03 (0.03)	0.21 (0.17)	-0.26 (0.03)	-0.36 (0.16)	-0.17 (0.03)	-0.31 (0.15)	-0.14 (0.03)	-0.25 (0.17)	0.00 (0.03)	0.24 (0.17)	0.16 (0.03)	0.26 (0.17)
α -LA	0.22 (0.03)	0.27 (0.15)	-0.05 (0.03)	0.11 (0.15)	-0.07 (0.03)	0.09 (0.16)	0.17 (0.03)	0.13 (0.14)	0.04 (0.03)	0.24 (0.14)	-0.10 (0.03)	0.04 (0.15)	-0.08 (0.03)	-0.03 (0.15)
β -LG	-0.12 (0.03)	-0.18 (0.15)	0.04 (0.04)	0.09 (0.14)	-0.14 (0.03)	-0.31 (0.14)	-0.22 (0.04)	-0.30 (0.12)	-0.19 (0.04)	-0.26 (0.13)	0.12 (0.04)	0.16 (0.13)	0.13 (0.03)	0.25 (0.13)

¹ α_{S1} -CN PD = α_{S1} -CN-9P / (α_{S1} -CN-8P + α_{S1} -CN-9P) $\times$ 100; α_{S2} -CN PD = α_{S2} -CN-12P / (α_{S2} -CN-10P + α_{S2} -CN-11P + α_{S2} -CN-12P) $\times$ 100. P = phosphate group attached.

² κ -CN was measured as nonglycosylated mono-phosphorylated form.

Table 4. Genetic correlations of individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms (% wt/wt) and the phosphorylation degrees (PD)¹ of α_{S1} -CN and α_{S2} -CN with milk production traits measured on test-day morning milk samples from 1,857 Dutch Holstein Friesian cows (SE in parentheses)

Trait (% wt/wt)	Percentage (%)			Yield (kg)			
	Protein	Fat	Lactose	Protein	Fat	Lactose	Milk
α_{S1} -CN-8P	-0.15 (0.17)	-0.20 (0.17)	0.02 (0.16)	0.13 (0.22)	-0.04 (0.19)	0.20 (0.18)	0.21 (0.19)
α_{S1} -CN-9P	-0.56 (0.11)	-0.59 (0.12)	0.19 (0.14)	-0.01 (0.21)	-0.29 (0.16)	0.36 (0.16)	0.32 (0.16)
α_{S2} -CN-10P	0.47 (0.13)	0.51 (0.14)	0.02 (0.16)	0.01 (0.22)	0.33 (0.15)	-0.30 (0.17)	-0.28 (0.18)
α_{S2} -CN-11P	0.37 (0.12)	0.34 (0.13)	-0.03 (0.14)	0.20 (0.19)	0.33 (0.15)	-0.05 (0.16)	-0.05 (0.16)
α_{S2} -CN-12P	-0.10 (0.15)	-0.20 (0.15)	0.18 (0.14)	0.53 (0.17)	0.35 (0.16)	0.52 (0.14)	0.48 (0.15)
α_{S1} -CN PD ¹	-0.52 (0.10)	-0.53 (0.12)	0.17 (0.14)	-0.07 (0.20)	-0.31 (0.16)	0.28 (0.16)	0.25 (0.17)
α_{S2} -CN PD ¹	-0.43 (0.14)	-0.45 (0.15)	0.24 (0.15)	0.16 (0.21)	-0.07 (0.19)	0.41 (0.16)	0.38 (0.17)

¹ α_{S1} -CN PD = α_{S1} -CN-9P / (α_{S1} -CN-8P + α_{S1} -CN-9P) $\times$ 100; α_{S2} -CN PD = α_{S2} -CN-12P / (α_{S2} -CN-10P + α_{S2} -CN-11P + α_{S2} -CN-12P) $\times$ 100. P = phosphate group attached.

Genetic Correlations of α_S -CN Phosphorylation Isoforms with Milk Production Traits and Phosphorus

Generally, genetic correlations between individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms (% wt/wt) and milk production traits were weak to moderate, ranging from -0.59 to 0.53 (Table 4). All α_{S1} -CN and α_{S2} -CN phosphorylation isoforms except α_{S2} -CN-12P were either uncorrelated or correlated weakly with yields of protein, fat, lactose, and milk (-0.30 to 0.36). The α_{S2} -CN-12P correlated moderately positive with yields of protein (0.53), lactose (0.52), and milk (0.48). These correlations suggest that cows producing larger quantities of protein, lactose, and milk produce milk protein with a higher proportion of α_{S2} -CN-12P. Moreover, cows producing milk with higher protein and fat percentages tend to have milk protein with a higher proportion of α_{S2} -CN-10P and a lower proportion of α_{S1} -CN-9P, which results in lower α_{S1} -CN PD and α_{S2} -CN PD as shown by the genetic correlations of α_{S1} -CN PD and α_{S2} -CN PD with the milk production traits (-0.53 to -0.43).

In the past decades, selection for higher milk yield has been the main focus of most dairy cattle breeding schemes (Miglior et al., 2005). Based on the weak to moderate correlations between milk yield and α_{S1} -CN and α_{S2} -CN phosphorylation isoforms, α_{S1} -CN and α_{S2} -CN phosphorylation profile might have changed as selection for milk yield is expected to result in an increase of α_{S1} -CN-9P and α_{S2} -CN-12P concentrations while having little effect on the rest of the isoforms. Some countries include protein and fat percentages in their breeding goal, such as France, Italy, and Switzerland (Miglior et al., 2005). Selection for higher protein and fat percentages will decrease α_{S1} -CN-9P concentration and increase α_{S2} -CN-10P and α_{S2} -CN-11P concentrations, which leads to lower α_{S1} -CN PD and α_{S2} -CN

PD. This is also observed when comparing the α_{S1} -CN and α_{S2} -CN phosphorylation profiles between Danish Holstein and Danish Jersey. Milk from Danish Jersey, which has higher protein and fat percentages compared with Danish Holstein, contains higher proportions of isoforms with lower degrees of phosphorylation (α_{S1} -CN-8P and α_{S2} -CN-11P; Buitenhuis et al., 2016). Furthermore, this agrees with the effects of *DGAT1* K232A genotypes; the *K* allele is associated with lower milk yield, higher protein and fat percentages, and lower α_{S1} -CN-9P concentration (Grisart et al., 2002; Winter et al., 2002; Bijl et al., 2014a).

The correlations of individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms with milk production traits also allow us to explore possible effects of selection on technological properties of milk. To date, a few studies have investigated the relations between relative concentrations of individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms and milk technological properties. For dairy products favoring efficient α_{S1} -CN hydrolysis by chymosin, milk containing higher α_{S1} -CN-8P concentration and lower α_{S1} -CN-9P concentration might be preferred (Bijl et al., 2014b). In addition, to improve milk coagulation properties, milk containing a greater proportion of α_{S1} -CN and α_{S2} -CN isoforms with lower degrees of phosphorylation (e.g., α_{S1} -CN-8P, α_{S2} -CN-10P, and α_{S2} -CN-11P) might be preferred (Frederiksen et al., 2011; Jensen et al., 2012; Ketto et al., 2017). Selecting for milk production traits shows little effect on α_{S1} -CN-8P concentration. However, selecting for higher protein and fat percentages would increase α_{S2} -CN-10P concentration and decrease α_{S1} -CN-9P concentration, which might be desirable for cheese production.

Limiting Factors of Casein Phosphorylation?

Phosphorylation of caseins is a process of phosphate addition to the polypeptide chains. Therefore, produc-

ing individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms is a function of both α_{S1} -CN and α_{S2} -CN synthesis and their subsequent phosphorylation. To investigate the biological relations between production of α_{S1} -CN and α_{S2} -CN isoforms, milk production, and phosphorus in milk, besides correlations with milk protein composition (Table 4), we also estimated the phenotypic and genetic correlations between yields of individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms, milk production traits, and phosphorus yield (Table 5). The phenotypic and genetic correlations between yields of individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms and the protein yield were moderate to high (0.39 to 0.96). These correlations are expected as all isoforms undergo common regulation as milk protein synthesis. Furthermore, the weak phenotypic and genetic correlations (−0.23 to 0.16) among α_{S1} -CN PD, α_{S2} -CN PD, and the yields of protein and phosphorus suggest that the phosphorylation is not an important rate-limiting step; when more α_{S1} -CN and α_{S2} -CN are produced, the proportion of isoforms with higher degrees of phosphorylation is hardly affected.

We also investigated if phosphorus availability is a limiting factor for phosphorylation of all caseins when the total amount of casein is higher, including the phosphoproteins β -CN and κ -CN in addition to α_{S1} -CN and α_{S2} -CN. A strong positive phenotypic (0.91) and genetic (0.75) correlation between the total amount of phosphate groups attached to the caseins (P_{cas}) and the total amount of phosphorus in milk suggests little competition between P_{cas} and other fractions of phosphorus (mainly inorganic phosphorus) for the total amount of phosphorus available in milk. Furthermore, our results are in line with those reported by Bijl et al. (2013), who showed that micellar phosphorus correlated positively with protein content and suggested that increase in total phosphorus mainly results from an increase in P_{cas} and micellar inorganic phosphorus. Results are supported by the fact that dietary phosphorus in cow feed usually exceeds the requirements of the recommendation in the Netherlands (Valk et al., 2002). It is currently unknown if phosphorus distribution in milk will differ if cows suffer from phosphorus deficiency.

CONCLUSIONS

The high intra-herd heritabilities for α_{S1} -CN and α_{S2} -CN phosphorylation isoforms and for the phosphorylation degrees of α_{S1} -CN and α_{S2} -CN suggest high relevance of genetic factors and the possibility of altering α_{S1} -CN and α_{S2} -CN phosphorylation profiles by selective breeding in Dutch Holstein Friesian cows. Furthermore, the strong positive genetic correlation between α_{S1} -CN PD and α_{S2} -CN PD suggests that the phosphorylation

Table 5. Phenotypic (r_p) and genetic (r_g) correlations of individual α_{S1} -CN and α_{S2} -CN phosphorylation isoforms expressed in yields (g), the phosphorylation degrees (PD)¹ of α_{S1} -CN and α_{S2} -CN and the total amount of phosphate groups attached to the caseins in milk (P_{cas}) with milk production traits and P yield measured on test-day morning milk samples from 1,721 Dutch Holstein Friesian cows (SE in parentheses)

Trait (g)	Protein			Fat			Lactose			Milk			P		
	r_p	r_g	r_g	r_p	r_g	r_g	r_p	r_g	r_g	r_p	r_g	r_g	r_p	r_g	r_g
α_{S1} -CN-8P	0.96 (0.00)	0.92 (0.04)	0.73 (0.01)	0.48 (0.16)	0.87 (0.01)	0.76 (0.09)	0.88 (0.01)	0.83 (0.07)	0.90 (0.01)	0.76 (0.10)					
α_{S1} -CN-9P	0.77 (0.01)	0.53 (0.15)	0.55 (0.02)	0.06 (0.19)	0.83 (0.01)	0.69 (0.09)	0.83 (0.01)	0.72 (0.09)	0.72 (0.02)	0.30 (0.20)					
α_{S2} -CN-10P	0.49 (0.02)	0.39 (0.20)	0.37 (0.03)	0.41 (0.16)	0.28 (0.03)	−0.06 (0.19)	0.30 (0.03)	−0.02 (0.19)	0.46 (0.02)	0.29 (0.21)					
α_{S2} -CN-11P	0.80 (0.01)	0.59 (0.13)	0.62 (0.02)	0.50 (0.14)	0.62 (0.02)	0.29 (0.16)	0.62 (0.02)	0.31 (0.16)	0.76 (0.01)	0.60 (0.14)					
α_{S2} -CN-12P	0.89 (0.01)	0.86 (0.06)	0.65 (0.02)	0.48 (0.14)	0.83 (0.01)	0.73 (0.08)	0.83 (0.01)	0.75 (0.08)	0.83 (0.01)	0.78 (0.09)					
α_{S1} -CN PD ¹	−0.16 (0.03)	−0.07 (0.20)	−0.17 (0.03)	−0.31 (0.16)	0.07 (0.03)	0.28 (0.16)	0.05 (0.13)	0.25 (0.17)	−0.17 (0.03)	−0.23 (0.20)					
α_{S2} -CN PD ¹	−0.14 (0.03)	0.16 (0.21)	−0.09 (0.03)	−0.07 (0.19)	0.10 (0.03)	0.41 (0.16)	0.07 (0.03)	0.38 (0.17)	−0.12 (0.03)	0.06 (0.22)					
P_{cas}^2	0.97 (0.00)	0.93 (0.03)	0.72 (0.01)	0.50 (0.16)	0.86 (0.01)	0.65 (0.12)	0.87 (0.01)	0.72 (0.10)	0.91 (0.01)	0.75 (0.11)					

¹ α_{S1} -CN PD = α_{S1} -CN-9P / (α_{S1} -CN-8P + α_{S1} -CN-9P) × 100; α_{S2} -CN PD = α_{S2} -CN-12P / (α_{S2} -CN-10P + α_{S2} -CN-11P + α_{S2} -CN-12P) × 100.

² P_{cas} = $\left[\frac{\text{concentration of individual casein fraction in milk (g/L; \% wt/wt} \times \text{protein percentage} \times 10)}{\text{molecular weight (Da) of respective individual casein fraction}} \right] \times \text{number of phosphate groups attached to the respective casein fraction}$ × milk yield.

degrees of α_{S1} -CN and α_{S2} -CN are related. Selecting for higher milk yield is expected to increase α_{S1} -CN-9P and α_{S2} -CN-12P concentrations. Selecting for higher protein and fat percentages is expected to increase α_{S2} -CN-10P concentration while decreasing α_{S1} -CN-9P concentration. Currently, knowledge regarding the effects of the phosphorylation degrees of α_{S1} -CN and α_{S2} -CN on technological properties of milk is limited and requires further investigation.

ACKNOWLEDGMENTS

This study is part of the Dutch Milk Genomics Initiative, funded by Wageningen University (Wageningen, the Netherlands), the Dutch Dairy Association NZO (Zoetermeer, the Netherlands), Cooperative Cattle Improvement Organization CRV (Arnhem, the Netherlands), and the Dutch Technology Foundation STW (Utrecht, the Netherlands). The first author benefited from an Erasmus-Mundus fellowship and a grant by APIS-GENE, within the framework of the European Graduate School in Animal Breeding and Genetics (EGS-ABG, Paris, France). We gratefully acknowledge Guy Miranda [National Institute for Agricultural Research (INRA), Animal Breeding and Integrative Biology (GABI)-équipe Lait, Génome & Santé (LGS), Jouy-en-Josas, France] and Guido Staring (NIZO, Ede, the Netherlands) for the excellent technical assistance of LC/ESI-MS and CZE analyses.

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