Effect of shortening or omitting the dry period of Holstein-Friesian cows on casein composition of milk

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ABSTRACT

The aim of this study was to evaluate the effect of shortening or omitting the dry period of dairy cows on milk casein composition. For this study, we analyzed milk samples of 90 cows with a dry period of 0, 30, or 60 d and either a glucogenic or a lipogenic ration in early lactation. Milk was sampled at 6 and 2 wk prepartum and at 2, 6, and 12 wk postpartum. Milk was analyzed for casein (CN) composition by capillary zone electrophoresis, and isoforms of κ -CN were measured by reversed phase-HPLC. Shortening the dry period from 60 to 30 d reduced the α_{S1} -CN fraction by 3.8% and increased the α_{S2} -CN fraction by 5.5%. In milk from cows with a 0-d dry period, the glycosylated κ -CN fraction in late lactation increased from 8 to 12% between 6 and 2 wk prepartum. After calving, the glycosylated κ -CN fraction in milk was higher for cows with a 0-d dry period (6.7%) compared with cows with a 60-d dry period (5.2%). The glycosylated κ -CN fraction at 2 wk postpartum was negatively correlated with milk yield, suggesting that glycosylation was related to reduced productivity of mammary epithelial cells. In early lactation, the β -CN fraction was reduced in milk of cows with a 0-d dry period. A lowered β -CN fraction was associated with high somatic cell count and greater parity, indicating that it was the result of proteolytic activity. In conclusion, casein composition changes that result from shortening the dry period from 60 to 30 d are not expected to affect processing characteristics of milk. Applying a 0-d dry period may affect processability of milk because of a higher glycosylated κ -CN fraction, and possibly because of higher proteolytic activity compared with a 60-d dry period.

Key words: milk casein, κ -casein glycosylation, capillary electrophoresis, reverse phase-HPLC, dry period

INTRODUCTION

A dry period of 6 to 8 wk is known to maximize milk yield in the subsequent lactation (Kuhn et al., 2005). Shortening the dry period reduces milk yield in the subsequent lactation but improves the energy balance and metabolic health of dairy cows in early lactation (Rastani et al., 2005; van Knegsel et al., 2014). A meta-analysis showed that milk production after calving decreases by 1.4 kg/d as a result of shortening the dry period (to 28–35 d) and by 5.9 kg/d as a result of omitting the dry period. At the same time, milk protein content increases when the dry period is shortened (0.06%) or omitted (0.25%; van Knegsel et al., 2013). By applying a 30-d or 0-d dry period, late-lactation milk is obtained very close to calving. This late-lactation milk (50–0 d prepartum) is related to a shortened renneting time and high gel strength after renneting (Remond et al., 1997), but casein composition of this milk has not been determined.

Caseins comprise approximately 80% of all proteins in milk and are present in 4 major forms: α_{S1} -, α_{S2} -, β -, and κ -CN (Walstra et al., 2006). Before secretion into milk, these caseins can undergo various post-translational modifications (PTM). Phosphorylation is a PTM that can occur in all caseins, whereas glycosylation only occurs in κ -CN (Walstra et al., 2006). On average, approximately 60% of all κ -CN is glycosylated. Up to 6 glycans can be attached to the protein (Holland et al., 2006); glycans attached to κ -CN exist as mono-, di-, tri-, and tetrasaccharides and can consist of *N*-acetylneuraminic acid, galactose, and *N*-acetyl-galactosamine (Saito and Itoh, 1992). The degree of glycosylation of κ -CN is highly variable (Holland et al., 2006).

Received March 7, 2015.

Accepted August 21, 2015.

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Variation in casein composition and PTM profiles results in variation in functional properties of milk. The proportion of κ -CN of total casein is positively related to renneting properties of milk (Wedholm et al., 2006). The glycosylation degree of κ -CN has been shown to affect the chymosin hydrolysis rate of κ -CN in simplified model systems but not in milk (Dziuba and Minkiewicz, 1996). Curd firmness after chymosin hydrolysis of κ -CN in milk was positively correlated with the *N*-acetylneuraminic acid content of κ -CN (Robitaille et al., 1993). Small casein micelles resulting from a high degree of glycosylation could explain this increased curd firmness (Robitaille et al., 1993; Bijl et al., 2014). Several potential functionalities for the newborn calf have been ascribed to glycosylation of κ -CN, such as immune regulating, probiotic, and antimicrobial properties (Brody, 2000). However, the main reason for naturally occurring variation in glycosylation degree of κ -CN in milk is not clear.

Milk casein composition varies between different stages of lactation and is largely constant during mid lactation (Ostersen et al., 1997; de Kruif and Huppertz, 2012). Progressing lactation decreases α _S-CN and κ -CN fractions in milk, whereas it increases the β -CN fraction (Ostersen et al., 1997). Progressing lactation increased glycosylation of κ -CN (Robitaille et al., 1991; Bonfatti et al., 2014), although colostrum κ -CN has the highest degree of glycosylation (Guérin et al., 1974; Fiat et al., 1988). Another factor influencing κ -CN glycosylation is the genetic variant of κ -CN. In general, the B variant of κ -CN is more glycosylated than the A variant (Robitaille et al., 1991; Bijl et al., 2014). The influence of parity on glycosylation of κ -CN is unclear; Robitaille et al. (1991) observed a decrease in glycosylation with increasing parity, whereas Bonfatti et al. (2014) reported the highest levels in second lactation. Plasmin, the main proteolytic enzyme in milk, is another cause of variation in casein composition. Of the caseins, β -CN is most prone to degradation by plasmin in milk (Politis et al., 1989). Plasmin activity increases with increasing parity and stage of lactation (Politis et al., 1989; Bastian et al., 1991).

The aim of this study was to evaluate the effect of shortening or omitting the dry period on casein composition in pre- and postpartum milk. Outcomes can be used for better understanding of the effect of dry period length on processability of milk.

MATERIALS AND METHODS

Experimental Design, Animals, and Sampling

The Institutional Animal Care and Use Committee of Wageningen University and Research centers (Wa-

geningen, the Netherlands) approved the experimental protocol. Milk samples were obtained from an experiment that has been described previously (van Knegsel et al., 2014). In short, Holstein-Friesian dairy cows ($n = 168$) were selected from the Dairy Campus Research dairy herd (WUR Livestock Research, Lelystad, the Netherlands). Cows were blocked for parity (primiparous or multiparous), expected calving date, milk production in the previous lactation, and BCS, and were randomly assigned to 1 of 3 lengths of dry period (0, 30, or 60 d dry) and 1 of 2 early-lactation rations (glucogenic or lipogenic), resulting in a 3×2 factorial design. Cows were housed in freestalls with slatted floors and cubicles. During lactation, cows were milked twice daily (0500 and 1630 h). The drying off protocol for cows with the 30-d and 60-d dry periods consisted of transition to a far-off ration at d 7 before drying-off and milking once daily at d 4 before drying-off. At drying-off, cows were treated with an intramammary antibiotic (Supermastidol, Virbac Animal Health, Barneveld, the Netherlands). Milk yield was recorded daily, and milk samples for fat and protein analysis (ISO, 2000; Qlip, Zutphen, the Netherlands) were collected 4 times per week (Tuesday afternoon, Wednesday morning, Wednesday afternoon, and Thursday morning).

For the current study, 90 cows within the main experiment were selected based on dry period length and lactation ration, resulting in 6 groups of 15 cows. Milk samples were taken on Friday mornings 6 and 2 wk prepartum and 2, 6, and 12 wk postpartum. Samples were stored at -20°C immediately after collecting. All milk samples of the 90 cows were included in capillary zone electrophoresis (**CZE**) analysis to determine casein composition. The results of the CZE analysis showed that dietary energy source did not influence casein composition of milk. For sample availability reasons, only milk samples from cows with a lipogenic lactation ration (45 cows) were used for quantification of glycosylated and nonglycosylated κ -CN fractions by reversed phase (**RP**)-HPLC.

Analysis of Protein Composition

Milk protein composition was measured by CZE, which is an appropriate method for all caseins, apart from the glycosylated forms of κ -CN (Heck et al., 2008). This method was chosen because of its ability to separate different forms of α -CN and genetic variants of β -CN. Sample preparation, buffer composition, equipment, and run conditions for CZE were as described before (Åkerstedt et al., 2012). D,L-Dithiothreitol was added to the sample buffer at the day of sample preparation. Milk samples of 300 μL were mixed with 700 μL of sample buffer, and subsequently defatted after

centrifugation (10 min, $10,000 \times g$). All chemicals originated from Sigma-Aldrich (Steinheim, Germany). Samples were stored at -20°C before analysis. Fractions of individual proteins were calculated by dividing their peak area by the cumulative peak area of all caseins, α -LA, and β -LG. Because of limited detection of glycosylated isoforms of κ -CN, the total κ -CN fraction was calculated according to the method of Gustavsson et al. (2014). The β -CN fraction was corrected for co-eluting minor forms of κ -CN, as described by Gustavsson et al. (2014).

Analysis of κ -CN Isoforms

It is known that glycosylated and nonglycosylated isoforms of κ -CN can be quantified using RP-HPLC (Visser et al., 1991; Bobe et al., 1998; Bonfatti et al., 2008). Buffer composition and sample preparation for RP-HPLC were according to Bobe et al. (1998). The only exception was solvent A, used for sample dilution, which consisted of 0.1% trifluoroacetic acid (TFA) in water (Bonfatti et al., 2008). Separations were carried out by an Ultimate 3000 LC module equipped with an Aeris Widepore 3.6 μm XB-C18 column, 250×4.6 mm (Phenomenex, Utrecht, the Netherlands). A security guard cartridge system was used as a precolumn (AJ0-8769, Phenomenex). Temperature in the auto-sampler was 4°C , an injection loop of 100 μL was used, and the injection volume was 5 μL . The UV detection wavelength was 214 nm. Two solvents (A and B), consisting of 0.1% TFA in milliQ water (Millipore, Billerica, MA) and 0.1% TFA in acetonitrile, respectively, were used for protein elution. An increasing gradient of solvent B was applied, starting at 33% (vol/vol). Increases of B were 0.2% per min for 5 min, 0.5% per min for 4 min, 0.33% per min for 9 min, 0.25% per min for 2 min, 5% per min for 30 s, 0% per min for 4.5 min, 0.33% per min for 3 min, 0.67% per min for 4.5 min, 0% per min for 5 min, followed by a linear return to the starting conditions in 30 s. A flow rate of 0.25 mL/min was applied for 24 min, followed by an increase to 0.4 mL/min in 3 min. This flow was applied until the end of the run at 41 min. All chemicals originated from Sigma-Aldrich, apart from TFA, trisodium citrate dihydrate (both from Merck, Darmstadt, Germany), and acetonitrile (HPLC-ultra gradient, Biosolve Chemicals, Valkenswaard, the Netherlands). Dionex Chromeleon 7.1.2 software (Thermo Scientific, Waltham, MA) was used for data processing. After every milk sample, a blank sample was run to avoid carry-over effects. Elution times of milk proteins were validated using protein standards (α -CN, α -LA, β -CN, β -LG, κ -CN; purities 70–85%, all from Sigma-Aldrich, St Louis, MO).

Statistical Analysis

Prepartum and postpartum data were analyzed separately. For prepartum comparison of the dry period groups, only data of sample week -6 were included in a mixed model (Proc Mixed, SAS 9.3, SAS Institute Inc., Cary, NC). Fixed factors in the model were dry period (0- or 30-d dry), parity (prepartum: 1, 2, or >2), and the interaction dry period $\times$ parity (model 1). Other variables were analyzed by using a mixed model accounting for repeated measures (Proc Mixed, SAS 9.3, SAS Institute Inc.); cow was the repeated subject. For comparison of the prepartum test week, only data from cows in the 0-d dry period group were included. Fixed factors in the model were test week (-2 or -6 wk), parity (prepartum: 1, 2, or >2), and the interaction test week $\times$ parity (model 2). Prepartum parity results are based on the model for comparison of the dry period groups. The postpartum model included the fixed factors dry period (0-, 30-, or 60-d dry), test week (2, 6, or 12 wk), parity (postpartum: 2, 3, or >3), ration (glucogenic or lipogenic), and the interactions dry period $\times$ test week, dry period $\times$ parity, and test week $\times$ parity (model 3). For all variables apart from milk yield and SCC, an autoregressive covariance structure was the best fit and was used to account for within-cow variation. Compound symmetry was the best fit for milk yield and SCC and was used to account for within-cow variation. *P*-values for dry period length were corrected by Bonferroni adjustment. Differences were considered significant when the corrected *P*-value < 0.05 . Correlation coefficients were determined using Proc Corr (SAS Institute Inc.).

RESULTS

Milk Yield and Composition

Cows in the 30-d dry period group had higher milk fat (5.7%) and protein (4.3%) percentages than cows in the 0-d dry period group (5.1% fat and 4.1% protein) at 6 wk prepartum (Table 1). First-parity cows had higher lactose (4.6%) than cows of second (4.4%) or greater (4.3%) parity. A parity $\times$ week interaction was found for lactose percentage prepartum ($P = 0.02$). Milk of cows in third or greater parity at 2 wk prepartum had lower lactose percentage in milk than younger cows or cows in wk 6 prepartum. Cows in third or greater parity had higher SCC (321,000 cells/mL) than first-parity cows (124,000 cells/mL). Between 6 and 2 wk prepartum, milk yield decreased from 16.9 to 9.1 kg/d and lactose percentage decreased from 4.4 to 4.2%. Milk protein (4.1 to 6.1%) and SCC (193,000 to 551,000 cells/mL) increased between 6 and 2 wk prepartum.

Table 1. Milk production, macronutrient composition, and SCC of cows with a dry period of 0, 30, or 60 d (mean $\pm$ SEM; $n = 30$ cows per dry period group)

Item	Dry period length (d)			SEM	<i>P</i> -value ¹		
	0	30	60		Dry period	Week ²	Parity
Prepartum ³							
Milk yield (kg/d)	16.9	14.7		1.5	0.10	<0.01	0.15
Fat (%)	5.1	5.7		0.2	<0.01	0.18	0.58
Protein (%)	4.1	4.3		0.1	0.04	<0.01	0.13
Lactose (%)	4.4	4.4		0.07	0.15	<0.01	<0.01
SCC ⁴ ($\times 10^3$ cells/mL)	193	223		49	0.48	<0.01	<0.01
Postpartum ⁵							
Milk yield (kg/d)	35.5 ^c	39.2 ^b	44.1 ^a	0.4	<0.01	<0.01	<0.01
Fat (%)	4.7	4.6	4.4	0.1	0.07	<0.01	0.47
Protein (%)	3.8 ^a	3.7 ^a	3.4 ^b	0.02	<0.01	<0.01	<0.01
Lactose (%)	4.5	4.6	4.6	0.01	0.04	<0.01	<0.01
SCC ⁴ ($\times 10^3$ cells/mL)	238 ^a	163 ^b	110 ^b	24	<0.01	<0.01	<0.01

^{a-c}Values with different superscripts within a row differ ($P < 0.05$) between different dry periods.

¹Also included in the model: lactation ration and the interactions dry period $\times$ parity, dry period $\times$ test week, and parity $\times$ test week.

²Prepartum week effects were based on 2- and 6-wk prepartum samples of 0-d dry cows.

³Prepartum means, SEM, and dry period and parity effects were based on 6-wk prepartum samples of 0- and 30-d dry cows.

⁴*P*-values are based on the natural logarithm of SCC.

⁵Postpartum test weeks were 2, 6, and 12 wk after calving.

Postpartum (2, 6, 12 wk), milk yield was lower for cows with a 30-d dry period (4.9 kg/d) and cows with a 0-d dry period (8.6 kg/d) compared with cows with a 60-d dry period ($P < 0.01$; Table 1). Second-parity cows had lower milk yield (35.9 kg/d) than cows of third (41.8 kg/d) or greater parity (41.4 kg/d). The interaction dry period $\times$ test week indicated that milk yield increased with increasing dry period length in wk 2 and 6 postpartum, but milk yield did not differ between cows with a 0-d or 30-d dry period at 12 wk postpartum ($P = 0.02$). Cows with a 0-d or 30-d dry period had higher milk protein percentages than cows with a 60-d dry period. Also, cows with 0-d dry period had a greater milk protein percentage compared with cows with 30-d dry period ($P = 0.03$). Second-parity cows with a dry period of 0 d had a higher milk protein percentage (4.2%) than older cows or cows with a longer dry period. Ration did not affect milk yield or macronutrient composition, except for fat percentage. Cows fed the lipogenic ration had a greater fat percentage than cows fed the glucogenic ration (lipogenic: 4.7%, glucogenic: 4.4%; $P = 0.03$). Cows of greater than third parity had lower lactose percentage (4.5%) than cows of second (4.7%) or third (4.7%) parity at 6 wk postpartum ($P = 0.01$). In wk 2 and 12 postpartum, no difference in lactose percentage was found between parities. Cows that had a 0-d dry period had higher SCC than cows that had a dry period of 30 or 60 d. At 2 wk in lactation, SCC was higher than at 6 wk in lactation. Second- and third-parity cows had lower SCC than fourth- or greater-parity cows.

Casein Composition

At 6 wk prepartum, cows in the 0-d dry period group had a higher α_{S2} -CN fraction (7.7%) in milk than cows in the 30-d dry period group (6.9%; $P = 0.02$; Table 2). Cows in the 0-d dry period group had a lower β -CN fraction (32.2%) than cows in the 30-d dry period group (34.5%; $P = 0.01$). First-parity cows had a higher α_{S1} -CN fraction (30.8%) than second-parity cows (29.3%; $P = 0.02$) 6 wk prepartum and had a lower α_{S2} -CN fraction (6.4%) than cows of second (7.8%) or greater (7.8%) parity ($P < 0.01$). First-parity cows had a higher β -CN fraction (35.1%) than third or greater parity cows (31.6%; $P = 0.01$) and a lower total κ -CN fraction (11.0%) than cows of third or greater parity (12.2%; $P = 0.02$).

Postpartum fractions of α_{S1} -, α_{S2} -, and β -CN in milk were different for cows with different dry period lengths. Cows with a 60-d dry period had 0.8 percentage point higher α_{S1} -CN fraction than cows with a 0-d dry period, and 1.2 percentage point higher α_{S1} -CN fraction than cows with a 30-d dry period. Cows with a 30-d dry period had the highest β -CN fraction ($P < 0.01$). Cows with a 0-d dry period had the lowest β -CN fraction. The β -CN fraction was inversely related to parity: 32.9, 32.4, and 31.5% for parities 2, 3, and >3 , respectively ($P = 0.04$). The interaction dry period $\times$ test week influenced the β -CN fraction ($P < 0.01$): milk samples at 2 wk postpartum of cows with a 0-d dry period had a lower β -CN fraction (29.0%) than samples from any other postpartum dry period $\times$ test week combination.

Table 2. Milk casein fractions (% of the sum of total casein, α -LA, and β -LG) from cows with a dry period of 0, 30, or 60 d (30 cows per group), measured with capillary zone electrophoresis (mean $\pm$ SEM)

Item	Dry period length (d)			SEM	<i>P</i> -value ¹		
	0	30	60		Dry period	Week ²	Parity
Prepartum ³							
α_{S1} -CN	29.8	30.2		0.5	0.58	0.81	0.02
α_{S2} -CN	7.7	6.9		0.3	0.02	0.62	<0.01
β -CN	32.2	34.5		0.9	0.01	0.78	0.01
κ -CN	11.4	11.3		0.4	0.93	0.03	0.02
Postpartum ⁴							
α_{S1} -CN	30.8 ^{ab}	30.4 ^b	31.6 ^a	0.1	0.02	0.24	0.63
α_{S2} -CN	8.1 ^a	7.7 ^a	7.3 ^b	0.1	<0.01	<0.01	0.27
β -CN	30.7 ^b	33.8 ^a	32.4 ^a	0.2	<0.01	<0.01	0.04
κ -CN	12.1	11.9	11.8	0.1	0.57	0.04	<0.01

^{a,b}Values with different superscripts within a row differ ($P < 0.05$) between different dry periods.
¹Also included in the model: lactation ration and the interactions dry period $\times$ parity, dry period $\times$ test week, and parity $\times$ test week.
²Prepartum week effects were based on 2- and 6-wk prepartum samples of 0-d dry cows.
³Prepartum means, SEM, and dry period and parity effects were based on 6-wk prepartum samples of 0- and 30-d dry cows.
⁴Postpartum test weeks were 2, 6, and 12 wk after calving.

At 12 wk in lactation, the β -CN fraction of cows with a 0-d dry period (31.3%) did not differ from the β -CN fraction of 30-d dry cows (32.5%). The β -CN fraction was negatively correlated with SCC ($r = 0.37$, $P < 0.01$). The α_{S2} -CN fraction was greater at 2 wk after calving (7.9%) than in later postpartum samples ($P < 0.01$). Casein composition was not influenced by feeding either a glucogenic or a lipogenic ration.

Glycosylation of κ -CN

Separation of milk proteins with RP-HPLC obtained 5 different κ -CN fractions for milk of cows heterozygous for κ -CN (Figure 1). The nonglycosylated, mo-

nophosphorylated A, B, and E genetic variants could be distinguished, as well as 3 regions of isoforms with different PTM patterns (Bobe et al., 1998; Bonfatti et al., 2008). The glycosylated κ -CN fraction is described as the sum of the 3 glycosylated κ -CN regions (peaks 1, 2, and 4; Figure 1). Of cows with a 0-d dry period that were used for detailed κ -CN analysis, 8 had κ -CN genotype AA, 6 had AB, and 1 had BB.

Between 6 and 2 wk prepartum, the glycosylated κ -CN fraction increased from 7.8 to 12.0% and the nonglycosylated κ -CN fraction decreased from 6.2 to 3.7% (Figure 2). Differences were stronger for cows that had the B genetic variant of κ -CN (6.2 percentage point increase) than for cows that were homozygous AA for

Figure 1. Example of a reversed phase-HPLC chromatogram of raw bovine milk, focused on the κ -CN fractions. Peaks 1, 2, and 4 = glycosylated κ -CN isoforms, peak 3 = nonglycosylated κ -CN A, peak 5 = nonglycosylated κ -CN B.

κ -CN (2.5 percentage point increase). In postpartum milk, no differences in nonglycosylated κ -CN were found between the different dry period lengths (Figure 3A). Postpartum milk of cows with a 0-d dry period contained a higher glycosylated κ -CN fraction (6.7%) than milk of cows with a 60-d dry period (5.2%, $P < 0.01$; Figure 3B). Differences were still present ($P < 0.05$) at 6 and 12 wk postpartum. The glycosylated κ -CN fraction was not correlated with milk yield and SCC when all postpartum sampling moments were combined. At 2 wk postpartum, the glycosylated κ -CN fraction correlated negatively ($r = -0.69$, $P < 0.01$) with milk yield (Figure 4).

DISCUSSION

The current results show that dry period length influenced postpartum casein composition of milk. Cows with a 30-d dry period had a lower α_{S1} -CN fraction in milk than cows with a 60-d dry period. Applying a 0-d dry period did not affect the α_{S1} -CN fraction. Both cows with a 0-d and a 30-d dry period had increased α_{S2} -CN fractions in milk compared with cows with a 60-d dry period. Differences in α_{S1} -CN and α_{S2} -CN between cows with a 60-d dry period and cows with a 0- or 30-d dry period cannot be compared with prepartum casein composition because no prepartum samples

were available before 6 wk prepartum. Postpartum differences may thus be a result of dry period length. The nature of the differences in α -CN fractions cannot be explained by the current results. Cows with a 0-d dry period had a 5.2% lower postpartum β -CN fraction than cows with a 60-d dry period, and 9.2% lower than cows with a 30-d dry period. In 6 wk prepartum samples, a 6.7% lower β -CN fraction was found in milk of cows in the 0-d dry period group compared with milk of cows in the 30-d dry period group. This unexpected prepartum difference in β -CN fractions may partly explain the postpartum difference. Overall, we cannot exclude the possibility that postpartum differences in casein composition between dry period groups originate from prepartum differences.

In the current study, the α_{S2} -CN fraction was found to be maximal at 2 wk postpartum, whereas the α_{S1} -CN fraction was not influenced by postpartum sampling week. Previously, α_S -CN was reported to decrease as lactation progressed, without distinguishing between α_{S1} - and α_{S2} -CN (Ostersen et al., 1997). An increase in β -CN fraction as a result of lactation week in early lactation has been observed before (Ostersen et al., 1997).

Besides the separate effects of dry period length and lactation stage on postpartum β -CN fractions, we found an interaction between dry period and lactation stage. At 2 wk in lactation, the β -CN fraction of cows with a

Figure 2. Changes in glycosylated (glyc) and nonglycosylated (non-glyc) κ -CN fractions in the peripartum period of continuously milked cows with κ -CN genotype AA (8 cows) or AB (+BB) (7 cows), measured with reversed phase-HPLC. Fractions are represented as percentage of the sum of total casein, α -LA, and β -LG. Data points represent means $\pm$ SE.

Figure 3. Postpartum fractions of nonglycosylated (A) and glycosylated (B) κ -CN in milk of cows with a 0-, 30-, or 60-d dry period (15 cows per dry period group), measured with reversed phase-HPLC. Fractions are represented as percentage of the sum of total casein, α -LA, and β -LG. * $P < 0.05$; ** $P < 0.01$. Data points represent means $\pm$ SE.

0-d dry period was lower than the β -CN fraction of cows with a 30-d or 60-d dry period. At 12 wk in lactation, the β -CN fraction was no longer related to dry period length. The β -CN fraction was corrected for co-eluting

minor forms of κ -CN using a fixed value. This correction factor may be underestimated for cows with a 0-d dry period due to an increased glycosylated κ -CN fraction. Therefore, the β -CN fraction of cows with a 0-d

dry period may be slightly overestimated. The reduced β -CN fraction of cows with a 0-d dry period at 2 wk postpartum may be the effect of proteolytic breakdown by plasmin. Previous work has shown that plasmin activity increases with increasing lactation stage (Politis et al., 1989; Bastian et al., 1991), so a reduction in plasmin activity in milk can be expected between late lactation and the successive early lactation. These studies did not emphasize the dry period length of the cows. For cows with a 0-d dry period, plasmin activity was found to be similar pre- and postpartum (Dupont et al., 1998). This may indicate increased plasmin activity in early-lactation milk of cows with a 0-d dry period compared with cows with a 60-d dry period. In the study of Dupont et al. (1998) no comparison between postpartum plasmin activities in milk of cows with different dry period lengths could be made, as the study only included cows with a 0-d dry period. In the current study, the β -CN fraction was decreased for cows in fourth or higher parity. A negative correlation was found between β -CN fraction and SCC. Plasmin activity is known to increase with increasing parity and elevated SCC (Politis et al., 1989; Bastian et al., 1991). Increasing plasmin activity with increasing SCC may be the result of increased activation of plasminogen due to decreased epithelial barrier integrity, as is the case for mastitic cows (Politis et al., 1989). The current results indicate that the reduced β -CN fraction in 2 wk

postpartum milk samples of cows with a 0-d dry period may result from increased plasmin activity.

In the current study, the glycosylated κ -CN fraction increased approximately 1.5-fold between 6 and 2 wk prepartum. The increase in glycosylation was stronger when the B variant of κ -CN was present than in milk of cows homozygous for κ -CN A. Only in this late stage of lactation was a genotype effect on κ -CN glycosylation observed, whereas the B variant of κ -CN is known to be associated with higher levels of glycosylation in mid lactation as well (Robitaille et al., 1991; Bijl et al., 2014). Increasing glycosylated and decreasing nonglycosylated κ -CN fractions have been observed before with progressing lactation (Bonfatti et al., 2014). The *N*-acetylneuraminic acid content of κ -CN, an indication of the amount of glycosylated κ -CN, has also been shown to increase when lactation progresses (Robitaille et al., 1991). From the study of Bonfatti et al. (2014), an approximately 1.5-fold increase in glycosylated κ -CN can be calculated between early (5–30 d) and late (350 d) lactation. The strong increase in glycosylation of κ -CN in the last weeks before calving may be related to the onset of colostrum production, because colostrum κ -CN is known to be highly glycosylated (Guérin et al., 1974; Fiat et al., 1988). Secretion of colostrum components such as IgG₁ has also been shown to increase in the last 21 d before calving (Guy et al., 1994). The 1.5-fold increase in glycosylated κ -CN between 6 and 2

Figure 4. Glycosylated κ -CN fraction as a percentage of total protein related to milk yield at 2 wk in lactation, measured with reversed phase-HPLC. A fraction is represented as percentage of the sum of total casein, α -LA, and β -LG. Different markers indicate different dry period lengths (Δ = 0-d dry, $\blacksquare$ = 30-d dry, $\times$ = 60-d dry; 15 cows per dry period group).

wk prepartum that was observed in the current study indicated that the number of days until calving, not the number of days in lactation, was most important in determining the level of κ -CN glycosylation.

The current study showed that the glycosylated κ -CN fraction was higher in postpartum milk samples of cows with a 0-d dry period compared with cows with a 30-d or 60-d dry period. Glycosylation was higher at 2 wk in lactation than in later postpartum samples. At this early time in lactation, the glycosylated κ -CN fraction of milk correlated negatively with milk yield. In previous work, a higher *N*-acetylneuraminic acid content was found in the casein fraction of mastitic milk (Robitaille et al., 1991). This is in agreement with increased activation of the O-glycan biosynthesis pathway found in bovine mammary cells with *Escherichia coli* mastitis (Yang et al., 2014). In other cell types, inhibition of O-glycosylation was shown to result in increasing apoptosis (Chatham et al., 2008). In all 3 aforementioned studies, protein glycosylation was related to dysfunctional cells. Applying a 0-d dry period to dairy cows was shown to result in reduced repair or replacement of damaged mammary epithelial cells prepartum (Capuco et al., 1997), possibly resulting in increased numbers of dysfunctional cells postpartum. Reduced milk yield and increased κ -CN glycosylation may be markers for the presence of an increased proportion of unrepaired mammary epithelial cells. In the current study, the glycosylated κ -CN fraction was not correlated with SCC. Because no mastitic cows were included in our study, our findings do not contradict the work of Robitaille et al. (1991) and Yang et al. (2014). Both high κ -CN glycosylation and β -CN degradation in milk were related to a 0-d dry period. In contrast to a high glycosylated κ -CN fraction, a low β -CN fraction was related to high-parity cows and elevated SCC. Therefore, increased κ -CN glycosylation and β -CN degradation in postpartum milk of cows with a 0-d dry period seem to have different origins. Increased κ -CN glycosylation in milk of cows with a 0-d dry period at 2 wk in lactation seems to be related to mammary epithelial cells that are not functioning optimally.

The cows that were used in the current study were a subgroup of the population described by van Knegsel et al. (2014). Similar changes in milk yields and macronutrient composition were found when the dry period was shortened or omitted in the current study as in the previous study. Differences in milk protein percentages when the dry period was shortened from 60 d to 0 or 30 d were greater in the current study (0 d: 0.4% higher, 30 d: 0.3% higher) than in a previously reported meta-analysis (0 d: 0.25% higher, 30 d: 0.06% higher; van Knegsel et al., 2013). The difference between the current study and the previous meta-analysis may be due to

the early stage of lactation in which the samples were taken in the current study. Including samples from a later stage of lactation may reduce differences between groups of cows with different dry period lengths.

Differences in milk composition resulting from different dry period lengths may affect processing properties of the milk. Total protein percentage increased in both early and late lactation when a 0-d or 30-d dry period was applied. It is known that high protein percentage is related to high cheese yield (Wedholm et al., 2006). In previous work, a variation in α_{S1} -CN and β -CN fractions greater than that found in the current study did not influence milk coagulation properties or cheese yield (Wedholm et al., 2006). Therefore, the observed α_{S1} -CN and β -CN reductions in the current study because of dry period shortening may not influence the processability of milk. It is known that plasmin activity negatively affects cheese milk quality and UHT milk stability (Kelly and McSweeney, 2003). The suggested increase in plasmin activity in relation to reduction of dry period length should be studied in more detail in future work. We found increased κ -CN glycosylation in both pre- and postpartum milk of cows with a 0-d dry period. If the increase in κ -CN glycosylation were a separate effect, it could result in increased curd firmness (Robitaille et al., 1993). Besides protein percentage and κ -CN glycosylation, increases in calcium concentration (White and Davies, 1958; Ostensen et al., 1997) and milk pH (White and Davies, 1958) in late lactation may be expected. All of these milk compositional properties are known to affect cheese-making properties (Walstra et al., 2006), and may thus influence cheese-making properties of milk from cows with a 0-d dry period. Protein percentage, composition, and proteolytic activity are all factors that may influence milk processing. Future work is required to investigate the influence of these factors on rennetability, gel strength, and storage stability of early-lactation milk as they relate to dry period length.

CONCLUSIONS

Shortening the dry period of dairy cows from 60 to 30 d resulted in lesser α_{S1} -CN and greater α_{S2} -CN fractions in milk protein. Applying a 0-d dry period resulted in an increase of glycosylated κ -CN in both late and early lactation. A lower β -CN fraction in early-lactation milk of cows with a 0-d dry period is likely to be a result of increased plasmin activity. Changes in α_{S1} -CN and α_{S2} -CN fractions as a result of shortening the dry period from 60 to 30 d are unlikely to influence the processability of milk. The contribution of changes in protein percentages, the glycosylated κ -CN fraction, and possible increase in plasmin activity of milk from

cows with a 0-d dry period to milk processing properties should be subject of future work in this field.

ACKNOWLEDGMENTS

The authors thank the Swedish Farmer's Foundation for Agricultural Research (SLF, Stockholm, Sweden) for funding the project. Thanks to Anders Andrén (SLU, Uppsala, Sweden) for his efforts in initiating the project as well as fruitful discussions over the course of the project. The authors also thank Ruiqi Zhang and the staff of the Dairy Campus (Wageningen UR, Lelystad, the Netherlands) for technical assistance.

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