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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*-acetylgalactosamine (Saito and Itoh, 1992). The degree of glycosylation of κ-CN is highly variable (Holland et al., 2006).

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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 (Wageningen, 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