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Short communication: Influence of shortening the dry period of Swedish dairy cows on plasmin activity in milk

Ruben de Vries,*†[1] Melanie Brandt,‡ Åse Lundh,‡ Kjell Holtenius,* Kasper Hettinga,† and Monika Johansson‡
*Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, PO Box 7024, 750 07 Uppsala, Sweden
†Dairy Science and Technology, Food Quality and Design, Wageningen University, PO Box 8129, 6700 EV, Wageningen, the Netherlands
‡Department of Food Science, Swedish University of Agricultural Sciences, PO Box 7051, 750 07 Uppsala, Sweden

ABSTRACT

The aim of this study was to evaluate the influence of shortening the dry period of Swedish dairy cows on plasmin activity and casein composition in milk. Swedish Holstein and Swedish Red cows, 45 in total, were assigned to a dry period of either 4 or 8 wk. Milk samples were taken 10 and 5 wk prepartum, and 6 and 12 wk postpartum. Plasmin activity and plasminogen activity were measured with a spectrophotometric assay. Casein composition was measured by capillary zone electrophoresis. Prepartum plasminogen activity increased by 22% between 10 and 5 wk prepartum, whereas no change in plasmin activity was observed during the same period. Cows with a 4-wk dry period had 61% higher plasmin activity in postpartum milk than cows with an 8-wk dry period. Cows of third or greater parity tended to have a stronger increase in plasmin activity as a result of applying a short dry period than cows of second parity. Although the α_{S1}- and β-casein fractions declined with increasing plasmin activity, no dry period effects were found. Based on postpartum differences in plasmin activity, it was concluded that particularly multiparous cows require more than 4 wk between lactations for recovery of the mammary epithelium. Changes in casein composition as an effect of plasmin activity are not expected to have a great effect on processing quality of milk, although future work is needed to verify this.
Key words: dairy cow, dry period, milk quality, plasmin activity, casein composition

Short Communication

A conventional dry period of 6 to 8 wk before parturition is known to maximize milk yield of dairy cows in their successive lactation (Kuhn et al., 2005; Watters et al., 2008; van Knegsel et al., 2014). High peak milk production of cows subjected to a conventional dry period may result in a deep negative energy balance in early lactation, which is related to increased risk of metabolic problems (Rastani et al., 2005; van Knegsel et al., 2013) and reduced fertility (Butler, 2003; Gumen et al., 2005). Shortening the dry period is a way to improve the energy balance in early lactation (Rastani et al., 2005; van Knegsel et al., 2014).

When a shorter (28–35 d) instead of a conventional 56- to 64-d dry period was applied, postpartum milk yield was reported to be 1.4 kg/d lower. Protein percentage was 0.06% higher in milk of cows with a short dry period compared with a conventional dry period, whereas milk fat percentage was not affected by shortening the dry period (van Knegsel et al., 2013). The postpartum α_{S1}-casein fraction was 3.8% lower in milk of cows with a 30-d dry period compared with a 60-d dry period, whereas the α_{S2}-CN fraction was 5.5% lower when a 30-d dry period was applied (de Vries et al., 2015). Omitting the dry period resulted in a reduced β-CN fraction, which was suggested to be a result of increased proteolytic activity (de Vries et al., 2015). These recent findings have provided an overview of compositional changes of milk as a result of shortening the dry period of the cow. The cause of compositional changes is, however, not well understood.

Plasmin is the main endogenous protease in bovine milk. It is converted from its inactive zymogen plasminogen by the action of plasminogen activators. Plasmin and plasminogen originate from blood and are transported passively to milk through tight junctions in the mammary epithelium (Kelly and McSweeney, 2003). In the mammary gland, plasmin facilitates tissue remodeling by protein degradation and activation of other enzymes (Politis, 1996). The function of plasmin in milk is not clear yet. Plasmin activity in milk was shown to increase with advancing stage of lactation (Politis et al., 1989a; Bastian et al., 1991), fourth or greater

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[1]Corresponding author: ruben2.devries@wur.nl

parity (Politis et al., 1989a; Bastian et al., 1991), and SCC in milk higher than 300,000 cells/mL (Politis et al., 1989a). Plasmin can hydrolyze α_{S1}-CN, α_{S2}-CN, and β-CN in milk. Reduced casein fractions in milk may lead to defects in processing such as reduced cheese yield and curd firmness (Mara et al., 1998; Srinivasan and Lucey, 2002). However, no relation was found between naturally present plasmin activity and clotting parameters of milk (Bastian et al., 1991). Plasmin is a relatively heat stable enzyme (Prado et al., 2007) that may affect protein stability during storage of UHT milk (Rauh et al., 2014) or milk protein ingredients (Gazi et al., 2014).

The aim of this study was to evaluate the influence of shortening the dry period of dairy cows on plasmin and plasminogen activity, and the consequence of plasmin activity for casein composition.

Forty-five clinically healthy cows with proper udder health of the Swedish Holstein (**SH**, n = 21) or Swedish Red (**SR**, n = 24) breed were included in this study. A dry period of 4 wk (n = 26, of which 13 SH, 13 SR) or 8 wk (n = 19, of which 8 SH, 11 SR) was applied to the cows. The dry period groups consisted of both primiparous and multiparous cows (4 wk dry, primiparous n = 15, multiparous n = 11; 8 wk dry primiparous n = 13, multiparous n = 6). Cows were randomly assigned to either a 4-wk or an 8-wk dry period. Cows that, 1 wk before the experiment, yielded less than 15 kg of milk/d or had signs of reduced udder health were excluded from the study (n = 3). The cows were housed at the Swedish Livestock Research Centre, Lövsta, in an indoor loose house system with slatted floor and cubicles with rubber mats and chopped straw as bedding. Lactating cows were batch-milked twice daily at 0600 and 1600 h in an automatic milking rotary (DeLaval AMR, Tumba, Sweden). Before drying off, the cows were fed silage ad libitum and concentrate according to milk production. The week before drying off, concentrate was withdrawn and during the dry-off procedure the cows were fed 4 kg DM of silage and straw ad libitum and no concentrate. No intramammary antibiotics were used at drying off. During the dry period, cows were fed a blend of silage and straw ad libitum and concentrate was stepwise increased to 3 kg at parturition. After calving, silage was provided ad libitum while the supply of concentrate was increased stepwise to 13.5 kg/d. Water was always available ad libitum.

Milk samples from the morning milking were taken 10 and 5 wk prepartum, and 6 and 12 wk postpartum. Bronopol (0.3%) was added as a preservative to milk samples. Milk samples were stored at −20°C directly after collection. Milk yields were automatically recorded in the robot, whereas milk fat and protein percentage and SCC were analyzed at the Department of Animal Nutrition and Management, SLU Uppsala, using Fourier transform infrared spectroscopy and flow cytometry with fluorescence cell staining (Foss Electric, Hillerød, Denmark). Prepartum plasmin and plasminogen activity were only analyzed in milk of cows with a 4-wk dry period, allowing the comparison of wk 5 and 10 prepartum. No prepartum comparison between dry period groups was made for plasmin and plasminogen activity. The Uppsala Local Ethics Committee approved the experimental protocol (C178/12).

Milk samples were analyzed for both plasmin activity and plasminogen activity by a method modified from Korycha-Dahl et al. (1983). Chemicals were obtained from Sigma-Aldrich (Sigma-Aldrich Inc., Stockholm, Sweden), except where stated differently. The reaction mixture in which plasmin and plasminogen activity were measured consisted of 0.5 mL of milk and 7.5 mL of plasmin buffer (50 m*M* Trizma-HCl pH 7.4, 117 m*M* NaCl, 25 m*M* ε-amino-n-caproic acid, pH 7.4). After 1 h incubation at room temperature, the milk serum containing the plasmin and plasminogen was separated from casein micelles by ultracentrifugation (LKB Ultrospin, Sweden) with an RP55T angle rotor, 12 mL × 12 at 100,000 × *g* for 1 h at 4°C. Plasmin activity was measured in the serum with the pyro-GLU-Phe-Lys-*p*-nitroanilide hydroxychloride chromogenic substrate [2.5 mg/mL; Biophen CS-41(03), Aniara, Westchester, OH] in a Sarstedt 96-well plate (Sarstedt, Helsingborg, Sweden). Plasminogen activity was determined after addition of urokinase (49.5 plough units) for activation of plasminogen into plasmin. Plasmin and total activity (i.e., combined plasmin and plasminogen activity) were measured continuously every third minute for 120 min by a multi-mode microplate reader (FLUOstar Omega, BMG Labtech, Ortenberg, Germany) at 37°C. The change in absorbance (ΔA405/Δt) was used for the measurement of plasmin activity, where the formation of *p*-nitroanilide was calculated from the linear part of the absorbance versus time curve. Plasminogen activity was calculated as the difference between the total activity and plasmin activity. Plasmin and plasminogen activities were expressed in the same units, with 1 unit being defined as the amount of enzyme that produces a ΔA405 of 0.001 per minute at pH 7.4 and 37°C due to *p*-nitroanilide released from CS-41(03) substrate in the defined reaction mixture. The intra-assay coefficient of variation was 5% for both plasmin and plasminogen activity, with a maximum CV of 10% per 96-well plate.

Casein composition of milk samples was determined by capillary zone electrophoresis as described by de Vries et al. (2015).

A mixed model accounting for repeated measures (SAS 9.3, SAS Institute Inc., Cary, NC) was used for comparison between treatments, in which cows were the