Dry Period Length of Dairy Cows

Milk Composition and Quality

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Abstract

A dry period of dairy cows is historically seen as a period during which the cow can restore its body condition and regenerate its mammary epithelium in order to be high yielding in the successive lactation. Recent work has indicated that high yielding cows generally experience a severe negative energy balance in early lactation. Dry period reduction is a strategy to improve the energy balance of dairy cows in early lactation. This thesis aimed at evaluating the influence of dry period length on milk composition and milk quality. Milk composition parameters indicate the processing quality of milk for the dairy plant, and may also reflect the physiological condition and energy status of the cow.

In this work, omission of the dry period was related to a reduced β-casein fraction in early lactation milk. Applying a short (4 weeks) instead of a conventional (8 weeks) dry period resulted in increased plasmin activity in milk, but did not affect the β-casein fraction. Increased plasmin activity in relation to a shortened dry period was particularly found in milk of cows of third or higher parity, that generally had relatively high somatic cell count. At low somatic cell counts, dry period reduction or omission only tended to result in an increased plasmin activity due a higher casein concentration in milk. From increased concentrations of a number of low abundant proteins in colostrum of cows with a short dry period it was hypothesized that a short dry period was related to increased proliferation of mammary epithelial cells during the first days in lactation.

Although the casein composition of milk was related to both plasmin activity in milk and the metabolic status of cows, which are both influenced by dry period length, quantitative differences in casein composition of cows with different dry period length were small. It was concluded that shortening or omitting the dry period of cows with good mammary health obtains milk with a higher protein content with little differences in protein composition.

Keywords: dry period length, dairy cows, milk processing quality, casein composition, plasmin activity, milk proteomics, mammary epithelial cells

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List of Publications

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Abbreviations

α-LA	α-lactalbumin
β-LG	β-lactoglobulin
CN	Casein
CZE	Capillary zone electrophoresis
IC	Ion chromatography
ICP-AES	Inductively coupled plasma - atomic emission spectroscopy
IgG	Immunoglobulin G
LC-MS/MS	Liquid chromatography – tandem mass spectrometry
MEC	Mammary epithelial cell
(N)EB	(Negative) energy balance
^{1}H-NMR	Proton - nuclear magnetic resonance
RP-HPLC	Reversed phase – high performance liquid chromatography
SCC	Somatic cell count
SH	Swedish Holstein
SR	Swedish Red
UHT	Ultra-high temperature

1 Introduction

1.1 Historical perspectives on dry period management

During the last century, the dairy chain has developed rapidly, from farm level to the dairy plant (von Keyserlingk et al., 2013). The average milk yield of dairy cows in Western countries has increased over 3-fold since the first half of the last century (Capper et al., 2009, Kristensen et al., 2015). At the same time, industrial milk processing has been optimised, and a constant milk composition is required for obtaining the desired product yield and quality in the dairy plant. The increased demand for long shelf life products such as ultra-high temperature milk, and globalisation of the dairy product market, have also increased the quality requirements of raw milk (ZuivelNL, 2014). Ongoing developments in dairy farming, such as increasing milk production of cows and increasing attention for sustainability and animal welfare, require optimisation of dairy cow management (von Keyserlingk et al., 2013). Optimising the dry period length of dairy cows could be a way to improve cow health, whilst maintaining high quality milk.

A dry period in between lactations has been commonly applied since the early 1900’s (Arnold and Becker, 1936, Woodward et al., 1926). Although the different experiences all described the need of a dry period for high milk production in the successive lactation, there was no consensus about the optimal length of the dry period (Woodward et al., 1926). The first scientific research indicating that a dry period of 6-8 weeks maximizes milk yield in the next lactation was reported by Arnold and Becker (1936). Applying a dry period was also reported to be important for restoring the body condition of the cow (Woodward et al., 1926), and for regeneration of the mammary epithelium (Smith et al., 1966, Swanson, 1965). In the last 2 decades, researchers started re-evaluating the optimal length of the dry period, taking metabolic health of cows into consideration. Although no data are available on the number of farmers that apply a different dry period length to their cows, applying a dry period of 6-8 weeks is still common practice among dairy farmers (Steeneveld et al., 2013).

1.2 Energy balance, milk production and composition

One of the main reasons for shortening the dry period is the improvement of the energy balance of the cow. At the start of lactation, cows switch from a relatively low feed intake and no milk production, to a high milk production. Therefore, in early lactation, cows are generally in a negative energy balance (NEB) (de Vries and Veerkamp, 2000). The severity of the NEB is affected by the milk yield, with an improved energy balance at a lower milk yield (van Knegsel et al., 2005). In the last decades, several controlled trials have been performed in which the performance of dairy cows with different dry period lengths was assessed. The influence of dry period length on milk production traits was assessed in a meta-analysis by van Knegsel et al. (2013). They reported a loss in milk yield in the successive lactation of 5.9 kg/d when no dry period was applied, compared with a conventional dry period of 56 - 63 days. Shortening the dry period to 28 - 35 days also resulted in a statistically significant milk yield loss (-1.4 kg/d). Milk yield loss as a result of dry period reduction was reported to be stronger for primiparous cows than for multiparous cows (Annen et al., 2004, Pezeshki et al., 2007). Cows without a dry period were reported to produce approximately 800 - 1200 kg milk during the last 56 - 60 days of lactation (Schlamberger et al., 2010, Annen et al., 2004, van Knegsel et al., 2014). The total energy corrected milk yield over a whole lactation of continuously milked cows was 600 kg lower compared with cows with a 60 d dry period (Schlamberger et al., 2010). A study that evaluated milk production on practical dairy farms reported a milk yield loss of 1000 kg when the dry period was omitted (Steeneveld et al., 2013), which however was partly compensated by an increased fat and protein yield.

Milk protein content was higher in milk of cows with no (+0.25 percent point) or a short dry period (+0.06 percent point), compared with a conventional dry period (van Knegsel et al., 2013). Schlamberger et al., (2010) reported that cows without a dry period had a higher milk protein content than cows with a 60 d dry period until 20 weeks in lactation. Due to the increased protein content of milk from cows without a dry period, 305 d protein yield was similar for cows with a 0 or a 60 d dry period (Schlamberger et al., 2010). According to a meta-analysis by van Knegsel et al. (2013), milk fat content was not affected by dry period length. Consequently, cows with a 0 d dry period had a lower 305 d fat yield (417 kg) compared with cows with a 60 d dry period (541 kg) (Schlamberger et al., 2010). Steeneveld et al. (2013) reported a somewhat smaller loss in 305 d fat yield of 82 kg. The effect of dry period omission on lactose content in milk is not fully clear. Andersen et al. (2005) reported that cows with a 0 d dry period had a higher lactose content than cows with a 50 d dry period during the first 5 weeks in lactation, whereas van Knegsel et al. (2014) reported that cows without a dry period had a lower lactose content (4.52%) than cows with a 60 d dry period (4.59%) during the first 14 weeks in lactation. Differences in lactose content are relatively small compared with milk protein content, as is commonly the case for healthy cows due to the osmoregulatory function of lactose in milk (Linzell and Peaker, 1971). In the last 8 weeks before parturition, milk protein content of cows without a dry period increased, to an average protein content of 5.1% (van Knegsel et al., 2014). Milk protein content increased up to 7.5% in the last week before calving (Schlamberger et al., 2010), possibly due to increased immunoglobulin transfer into milk during the last weeks before calving (Guy et al., 1994b). Milk fat content of cows without a dry period was 5.2% during the last 8 weeks before parturition (van Knegsel et al., 2014). Little reports are available on detailed compositional analyses of milk from the last weeks of gestation from cows without a dry period, and how this composition relates to the status of the mammary gland during this period.

The reduced milk production of cows without a dry period results in an improved energy balance in early lactation. The NEB of cows with a reduced dry period is less deep, and lasts for a shorter period of time (van Knegsel et al., 2014). Cows with a 60 d dry period were in a NEB during the first 14 weeks of their lactation. Cows with a 30 d dry period were in a NEB for 8 to 12 weeks, and cows with a 0 days dry period were in a NEB for 4 to 5 weeks (van Knegsel et al., 2014, Van Hoeij, Submitted). Besides, the peak NEB can be reduced 1.5 times by applying a dry period of 30 days and 3 times by applying a dry period of 0 days instead of 60 days (van Knegsel et al., 2014).

1.3 Metabolic status of the cow in relation to dry period length

The NEB is related to various health problems of the cow, such as weight loss, ketosis, and reduced fertility (Butler, 2003, Grummer et al., 2004). In controlled trials there has been little evidence for the relation between dry period length and energy balance-related disease incidence. This is probably due to the insufficient power of the trials performed, with relatively low numbers of animals in controlled trials compared to the number that would be needed for examining disease incidence. Only ketosis incidence tended to reduce when no dry period was applied (van Knegsel et al., 2013). For fertility traits, no uniform relation with dry period length was found (van Knegsel et al., 2013). The metabolic status of cows was improved by dry period omission. Cows without a dry period had increased glucose concentrations and lower non-esterified fatty acid concentrations in blood than cows with a conventional dry period. This indicates that cows with an improved energy balance have lower mobilization of body fat reserves, which is also reflected in the fatty acid composition of milk. Cows with a conventional dry period had higher concentrations of long chain fatty acids in early lactation milk than cows without a dry period (van Knegsel et al., 2014). High concentrations of long chain fatty acids of cows in severe NEB originate from body fat mobilisation (Jorjong et al., 2014). Cows in severe NEB had lower ATP production in mammary epithelial cells due to reduced activity of the citric acid cycle. Lower mitochondrial activity during NEB can result in less *de novo* synthesis of fatty acids in mammary epithelial cells (Lu et al., 2013). Therefore, cows with a 0 d dry period had higher concentrations of *de novo* synthesised short-chain fatty acids in early lactation milk than cows with a 60 d dry period (van Knegsel et al., 2014).

Milk proteomics and metabolomics were found to reflect the energy balance of cows in early lactation. Compounds such as stomatin and galactose-1-phosphate were found to be higher in milk of cows with a positive energy balance (Lu et al., 2013). Milk proteomics has provided further understanding of mammary health of dairy cows. The milk proteome can give a detailed insight in the response towards infections of the mammary gland (Alonso-Fauste et al., 2012, Danielsen et al., 2010, Smolenski et al., 2014). Proteomics was also used to show the changes occurring during transition from colostrum to milk in the immune protein profile after 2-3 days in lactation (Zhang et al., 2015). The metabolome of milk consists of low molecular weight molecules that are formed by rumen fermentation and in the energy metabolism pathways (Antunes-Fernandes et al., 2016). Another metabolomics study showed that the glycerophosphocholine and choline concentrations in milk could be used as markers for ketosis (Klein et al., 2012). All in all, milk proteomics and metabolomics were proven to be useful tools to improve understanding of the health of dairy cows in general, and mammary gland health in particular.

1.4 Mammary epithelial health status

A conventional dry period allows the mammary gland of the cow to turn into a high-productive state before the successive lactation. During the dry period, accelerated mammary epithelial cell (MEC) renewal takes place compared with a lactating mammary gland in the same stage prior to parturition (Capuco et al., 1997, Collier et al., 2012). Since extensive regeneration of MECs was observed during a 60 d dry period, less viable MECs seem to be present after a reduced dry period (Capuco et al., 1997, Collier et al., 2012). Epithelial cell proliferation and apoptosis in the first 20 days of lactation did not differ between mammary glands of cows with either a conventional or no dry period (Annen et al., 2007). Only at 2 and 4 days postpartum, cows without a dry period had a higher MEC proliferation index than cows with a 60 d dry period (Annen et al., 2008).

Cows without a dry period have a lower colostrum production at first milking (5.1 kg) than cows with a 60 d dry period (7.7 kg) (Mayasari et al., 2015). Continuous milking until parturition results in the absence of an accumulation of milk prepartum. Immunoglobulin G (IgG), an essential immune protein in colostrum for the calf, is secreted into milk starting several weeks before parturition (Guy et al., 1994a). Milking until parturition reduces accumulation of IgG in the last weeks of lactation, resulting in a 2.5 fold lower IgG concentration in colostrum of cows with a 0 d dry period compared with cows with a 30 d or 60 d dry period (Mayasari et al., 2015). Hence, applying no dry period negatively affects both the volume and IgG concentration in colostrum. However, dry period length of the cow did not affect growth of calves and immunoglobulin concentrations in blood plasma of calves after immunisation (Mayasari et al., 2015).

Milk stasis does not only cause accumulation of IgG before parturition, but also residual milk accumulates after drying off. Residual milk in the udder serves as a nutrient source for bacteria, making the dry period a risk period for udder infections (Bradley and Green, 2004). Therefore, it was hypothesized that no accumulation of milk due to dry period omission would reduce the incidence of mammary infections (Collier et al., 2012). However, no relation was found between the incidence of mastitis in early lactation and dry period length (Church et al., 2008, Santschi et al., 2011, Watters et al., 2008) One of the main indicators in milk for mammary health in general, and infection status in particular, is somatic cell count (SCC). Milk that contains pathogens usually has SCC > 600,000 cells/ml (Dohoo and Meek, 1982), whereas subclinical mastitis is indicated by SCC > 250,000 cells/ml (Runciman et al., 2010). SCC was reported to be higher in early lactation milk of cows that had no dry period compared with a dry period of either 30 or 60 days (van Knegsel et al., 2014). In other work, however, no differences in SCC in early lactation milk were found between cows with different dry period lengths (Schlamberger et al., 2010, Watters et al., 2008, Rastani et al., 2005, Andersen et al., 2005).

Studies that indicated no effect of dry period length on mastitis incidence all used intramammary antibiotic treatment at drying off. In the Netherlands, it used to be a common practice to treat the cow with an intramammary antibiotic at drying off. A stricter antibiotics policy nowadays does, however, not allow the use preventive antibiotics at drying off of healthy cows. In Sweden, application of medical products, such as antibiotics, without motivation has been prohibited since 1979 by the Swedish board of Agriculture. Therefore, cow management practices are being explored in Sweden to reduce the risk of microbial infections after drying off (Ekman, 2003). Between 2005 and 2012, 44% of the antibiotics in dairy farming in the Netherlands were used for dry-off therapy of cows (Kuipers et al., 2016). Without considering potential benefits of lower veterinary costs when no dry period is applied, the lower management costs and higher milk protein contents seem to result in sufficient cost reduction and income to compensate for the reduction in milk yield in the Dutch situation. Hence, omitting the dry period may be economically beneficial for Dutch dairy farmers (Heeren et al., 2014). No economic analysis has been reported for Swedish dairy farmers.

1.5 Milk protein composition

In contrast to milk fatty acid composition, milk protein composition has not been studied extensively in relation to dry period length. Bovine milk has a protein content of about 3.4%, of which 20% are whey proteins and 80% are caseins. The whey protein fraction predominantly consists of α-lactalbumin, which plays an essential role in lactose synthesis, and β-lactoglobulin. The casein fraction consists of 4 major proteins, α_{s1}-, α_{s2}-, β- and κ-casein, in a ratio of 11 : 3 : 10 : 4 (Walstra et al., 2006). These caseins form casein micelles due to hydrophobic interactions and calcium phosphate nanoclusters of phosphorylated serine residues and colloidal calcium and inorganic phosphate. The α_{s1}-, α_{s2}- and β-caseins are predominantly located in the core of the casein micelle, whereas κ-casein is located more on the outer part, forming a ‘hairy layer’ at the surface of the micelle, stabilizing the micelle. Caseins undergo posttranslational modifications in the Golgi apparatus of mammary epithelial cells. Phosphorylation is a posttranslational modification that occurs in different degrees for all casein fractions, whereas glycosylation is unique for κ-casein (Walstra et al., 2006). During a lactation with a conventional dry period, the glycosylated κ-casein fraction is increasing towards the end of lactation (Bonfatti et al., 2014).

The casein composition of milk can be affected by proteolytic activity. The main proteolytic enzyme in milk is plasmin, which is the active form of its zymogen plasminogen (Kelly et al., 2006). Plasminogen is transported transcellularly from blood to milk by golgisomes, in the presence of casein micelles (Silanikove, 2016). Plasmin can hydrolyse α_{s1}- α_{s2}- and β-casein fractions (Grufferty and Fox, 1988), with the β-casein fraction being the most susceptible in milk (Politis et al., 1989). Plasminogen can be activated by either tissue-type or urokinase-type activators, the latter being related to somatic cells (Ismail and Nielsen, 2010). Plasmin activity is related to mammary epithelial tissue remodelling (Nørgaard et al., 2008), which increases in the last 20 days prior to calving when the dry period is omitted (Collier et al., 2012). Plasmin activity in milk was increasing in the last two weeks before parturition of cows that did not have a dry period, with maximum activity around parturition (Dupont et al., 1998). The effect of dry period length on plasmin activity in early lactation milk has not been studied yet, neither the consequence this would have for casein composition.

1.6 Processing quality of milk

Few studies have been done on the relation between dry period length of cows and milk composition and quality for processing. The current work includes analyses of protein composition, plasmin activity and mineral composition of milk. Two products that are sensitive for changes in milk protein composition and plasmin activity are cheese and ultra-high temperature (UHT) milk.

A high casein content in milk has been related to an increased cheese yield per kg milk (Wedholm et al., 2006, Walstra et al., 2006). Cheese making properties, such as rennet coagulation time and curd firmness also improved with increasing milk casein content (Joudu et al., 2008, Walstra et al., 2006). Milk with a large κ-casein fraction generally showed good coagulation during renneting (Wedholm et al., 2006). A high degree of glycosylation of κ-casein has been related to higher curd firmness after renneting of milk (Robitaille et al., 1993), possibly as a result of small casein micelles (Bijl et al., 2014). Very late lactation milk, which was collected >46 weeks in lactation, also showed good coagulation properties (Wedholm et al., 2006). The authors did not explicitly report the dry period length that was applied, so the weeks until calving were not indicated.

The physical stability and sensory properties of ultra-high temperature (UHT) milk can be affected by proteolytic action of plasmin (Rauh et al., 2014b, Rauh et al., 2014a). Although most plasmin is inactivated during UHT treatment, residual plasmin activity can hydrolyse caseins into peptides during prolonged storage at room temperature (Rauh et al., 2014b). Casein hydrolysis can cause age gelation of UHT milk. Besides, the peptides that are formed during casein hydrolysis may have bitter off flavours in UHT milk (Rauh et al., 2014a). Hence control of plasmin activity is an important quality attribute of milk for UHT processing.

2 Objectives

The aim of this work is to evaluate the effect of shortening or omitting the dry period on milk composition and quality. The biological background of dry period-induced differences in milk composition is addressed, and differences in milk composition are used for predicting the processing quality of milk. Most dry period research thus far has focused on major production traits of cows, whereas this more detailed study provides an indication for the applicability of a reduced dry period from a milk quality perspective.

In paper 1, casein composition in early and late lactation milk is analysed of cows with either a 0, 30 or 60 days dry period. The possible biological origin of differences between casein compositions is discussed, which is further evaluated in papers 2, 3 and 5.

In paper 2, plasmin and plasminogen activity and casein composition of milk from cows with a dry period of either 4 or 8 weeks is evaluated. The influence of the factors parity and somatic cell count on plasmin activity of cows with a shortened dry period are addressed. In addition, the relation between plasmin activity and casein composition is evaluated.

In paper 3, outcomes of more detailed compositional analyses are presented to predict processing quality of milk from cows with a dry period of either 0 or 30 days. Plasmin activity and mineral composition are related to casein composition of casein micelles and milk serum.

In paper 4, the proteomes of colostrum serum (first secretion after calving) and transition milk serum (fifth milking after calving) of Swedish dairy cows with a dry period of either 4 or 8 weeks are compared.

In paper 5, variation in glucose concentration of blood in cows is related to milk protein composition and lactose production in early lactation. The milk metabolome is presented to indicate the involvement of metabolic pathways that use glucose as a substrate in milk component synthesis in the mammary gland.

3 Materials and Methods

3.1 Animal experiments

This research is based on three controlled animal experiments. All three experiments were used for comparing the effect of different, pre-determined, dry period lengths on cow health and milk composition. The current work focusses on milk composition. All experiments have been approved by the authorized ethical committees. An overview of the three experiments and the conducted analyses is provided in Table 1.

3.2 Milk composition analyses

Milk samples were stored at -20°C after collection. Milk macronutrient composition was determined routinely with infra-red analysis. Milk serum for among others proteome, mineral composition and metabolome analyses was obtained by ultracentrifugation.

Table 1. *Overview of the three animal experiments which the current thesis is based on, the papers they have contributed to, and the milk analyses that were done with the samples*.

	Why Dry?	**Dry period study Uppsala**	**Customised dry period length**
Location	Dairy campus Lelystad (NL)	SLU university farm Lövsta (SE)	Dairy campus Lelystad (NL)
Paper	I	II + IV	III + V
Dry period length (d)	0, 30, 60	28, 56	0, 30
Sampling moments (wk relative to calving)	-6, -2, 2, 6, 10	-10, -5, 6, 12, 1st and 5th sample postpartum	5-8, 20-28
Analyses in the current work	Major milk proteins (CZE, RP-HPLC)	Plasmin activity Major milk proteins (CZE) Proteome (LC-MS/MS)	Major milk proteins (RP-HPLC) Mineral composition (IC, ICP-AES) Plasmin activity Metabolomics (^{1}H-NMR)

3.2.1 Major milk protein analyses

Two methods were used to determine the major milk protein composition in milk: capillary zone electrophoresis (CZE, paper I and II) and reversed phase – high performance liquid chromatography (RP-HPLC, paper I and III). With CZE, caseins with different degrees of phosphorylation can be distinguished, whereas RP-HPLC is a suitable method for quantifying glycosylated κ-casein. Sample preparation, buffer composition, equipment and run conditions for CZE were described before (Åkerstedt et al., 2012). Buffer composition and sample preparation for RP-HPLC were also described before (Bobe et al., 1998, 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, 250x4.6 mm. An increasing gradient of acetonitrile with 0.1% trifluoroacetic acid in water was used for separation of caseins and the major serum proteins α-LA and β-LG. Protein fractions are expressed as percentage of the sum of all proteins in the electropherogram (CZE) or chromatogram (RP-HPLC).

3.2.2 Colostrum and transition milk proteome

The proteome of individual colostrum and milk serum from 12 Swedish Holstein (SH) and 12 Swedish Red (SR) cows (paper IV) was analysed by liquid chromatography- tandem mass spectrometry (LC-MS/MS). Samples were prepared by filter aided sample preparation (FASP) and dimethyl labelling (Lu et al., 2011). By dimethyl labelling, protein concentrations could be compared with a reference sample, consisting of a mixture of all samples. Samples of SH and SR were analysed separately.

3.2.3 Plasmin activity

Plasmin and plasminogen activity measurements were based on the methods of Korycka-dahl et al. (1983) (Paper II) and Rollema et al. (1983) (Paper III). Both methods are spectrophotometric assays of the cleavage of Biophen CS-41(03) chromogenic substrate by plasmin, or by plasminogen after activation by urokinase. Cleavage resulted in an increasing light intensity over time, which was quantified spectrophotometrically. Analyses were done in duplicate. Activities of plasmin and plasminogen are expressed as absorbance change per time unit, indicating the amount of substrate converted by plasmin.

3.2.4 Milk metabolome

The metabolite profile of milk samples (Paper V) was determined by ^{1}H-NMR. The procedure is described in detail by Lu et al. (2013). In short, milk serum (175 μL) was mixed with phosphate buffer (175 μL; 0.3 M, pH 6.0, and 1 mM of 3-trimethylsilyl-2,2,3,3-tetradeuteropropionate (TSP; Sigma-Aldrich, Germany)). Nuclear magnetic resonance spectrometer Avance III with a 600 MHz/54 mm UltraShielded Plus magnet equipped with a CryoPlatform cryogenic cooling system, a BCU-05 cooling unit, an ATM automatic tuning and matching unit (Bruker, Rheinstetten, Germany) was used to obtain spectra (one-dimensional nuclear Overhauser enhancement spectroscopy (1-D-NOESY)) for all milk serum samples. The peak area of each assignment is relative to the calibration standard TSP, resulting in a relative peak area in arbitrary units that was used for statistical analyses.

3.2.5 Mineral composition

Concentrations of calcium, magnesium and phosphorus in milk and milk serum were measured by inductive coupled plasma - atomic emission spectroscopy (ICP-AES; ISO 15151, 2010). Citrate concentrations were measured by anion exchange chromatography using an IonPac AS19 column (4 x 250 mm, Dionex, Thermo Scientific, Sunnyvale CA) (Gaucheron et al., 1996). Sample preparations and analyses were done in duplicate. Micellar mineral fractions were obtained by subtracting the serum fractions from the total fractions in milk.

3.3 Statistical analyses

Postpartum differences between dry period groups were analyzed statistically using a mixed model, accounting for repeated measures (Proc Mixed, SAS 9.3, SAS Institute Inc., Cary, NC). Fixed factors in the model were dry period, lactation week and parity. Cows were the random factors in the model. An autoregressive covariance structure (AR1) was used to account for within-cow variation. P-values were corrected by Bonferroni adjustment. Differences were considered significant if $P < 0.05$.

Proteomic results (paper IV) were analyzed by comparing dimethyl ratios of individual cows with a two-samples T-test (Perseus 1.3.0.4, Max Planck Institute of Biochemistry, 2012), using a false discovery rate of 0.05. Blood glucose and insulin concentrations, energy balance, milk yield and milk component concentrations among dry period lengths and parities in paper V were analyzed with a general linear model (Proc GLM) in SAS.

4 Results

This chapter provides a summary of the main findings. Experiments in the different research papers partially overlap. The research paper that corresponds to the results is indicated (I-V).

4.1 Milk macronutrient and casein composition (Paper I)

Between 6 and 2 wk prepartum, milk yield of cows without a dry period decreased from 16.9 to 9.1 kg/d and lactose content decreased from 4.4% to 4.2%. Milk protein (4.1% to 6.1%) and SCC (193,000 cells/mL to 551,000 cells/mL) increased between 6 and 2 wk prepartum. First parity cows had higher lactose (4.6%) than second (4.4%) or greater (4.3%) parity cows. A parity x week interaction was found for lactose content prepartum ($P = 0.02$). Milk of cows in third or greater parity at 2 weeks prepartum had lower lactose content in milk than younger cows or cows in week 6 prepartum. Cows in third or greater parity had higher SCC (321,000 cells/mL) than first parity cows (124,000 cells/mL).

Postpartum (2, 6 and 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 (Table 2). Second parity cows had lower milk yield (35.9 kg/d) than third (41.8 kg/d) or greater parity (41.4 kg/d) cows. Cows with a 0 d or 30 d dry period had higher milk protein content than cows with a 60 d dry period. Also, cows with a 0 d dry period had greater milk protein content compared with cows with 30 d dry period ($P = 0.03$). Cows that had a 0 d dry period had higher SCC than cows that had a dry period of 30 d 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.

Table 2. *Milk production, macronutrient composition and SCC of cows with a dry period of 0, 30 or 60 d (Mean ± SEM). All dry period groups consist of 30 cows.*

	Dry period (days)				P-values[1]		
	0	30	60	SEM	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] ($x10^3$ 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] ($x10^3$ cells/mL)	238[a]	163[b]	110[b]	24	<0.01	<0.01	<0.01

[a-c] Values with different superscript within a row differ ($P < 0.05$) between different dry periods.
[1] Also included in the model: lactation ration and the interactions dry period x parity, dry period x test week and parity x test week.
[2] Prepartum week effects are based on 2 and 6 wk prepartum samples of 0 d dry cows.
[3] Prepartum means, SEM, and dry period and parity effects are based on 6 wk prepartum samples of 0d and 30d dry cows.
[4] P-values are based on the natural logarithm of SCC.
[5] Postpartum test weeks are 2, 6 and 12 wk after calving.

Between 6 and 2 wk prepartum, the glycosylated κ-CN faction increased from 7.8% to 12.0% and the non-glycosylated κ-CN fraction decreased from 6.2% to 3.7% (Figure 1). Differences were stronger for cows that have the B genetic variant of κ-CN (6.2% increase) than for cows that are homozygous AA for κ-CN (2.5% increase).

Cows with a 0 d dry period had the lowest β-CN fraction. The β-CN fraction was inversely related with parity: 32.9%, 32.4% and 31.5% (parities 2, 3 and >3 respectively). The interaction dry period x test week influenced the β-

CN fraction ($P < 0.01$): milk samples 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 x test week combination. 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, $P < 0.01$).

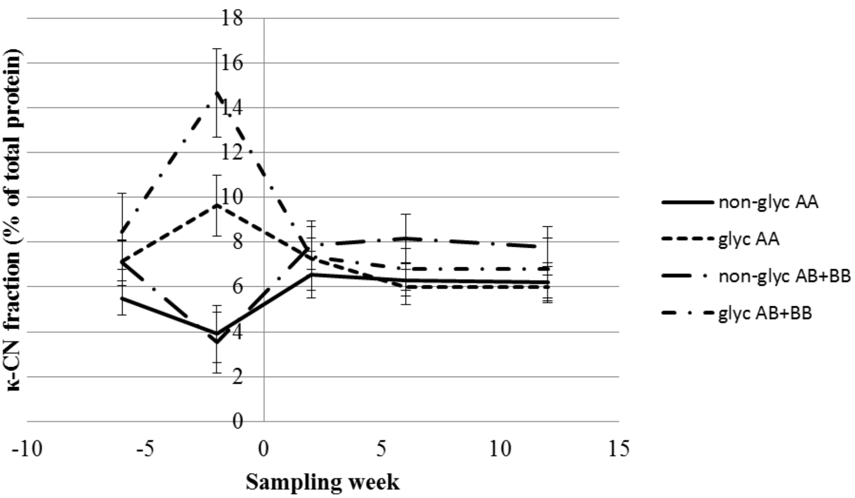

Figure 1. Changes in glycosylated (glyc) and non-glycosylated (non-glyc) κ-CN fractions in the peripartum period of continuously milked cows with κ-CN genotype AA (8 cows) or AB (+BB) (7 cows), measured with RP-HPLC. Fractions are represented as percentage of the sum of total casein, α-LA and β-LG.

4.2 Casein composition and plasmin activity (Paper II)

In paper II, plasmin and plasminogen activities were studied in milk samples of cows with either a 4 wk or an 8 wk dry period. Plasmin activity in milk samples 6 or 12 wk postpartum was higher of cows with a 4 wk dry period (5.0 units/mL) than of cows with an 8 wk dry period (3.1 units/mL) ($P < 0.01$). Cows of third or greater parity had higher plasmin activity (5.8 units/mL) than cows of second parity (3.5 units/mL, $P = 0.02$). Dry period length and parity tended to interact with each other ($P = 0.06$), and plasmin activity was particularly high in milk of cows of third or greater parity that had a 4 wk dry period (7.4 units/mL, Figure 2A). Plasminogen activity was not affected by dry period length, or by an interaction between dry period length and parity (Figure 2B). In contrast to plasmin activity, none of the casein fractions was affected by dry period length in paper II. Plasmin activity correlated negatively with the β-CN fraction (R = -0.46, P < 0.01) and the α_{s1}-CN fraction (R = -0.45, P < 0.01) when including all postpartum milk samples. These results indicate that shortening the dry period to 4 wk can affect plasmin activity in milk, although the effect on casein composition is limited.

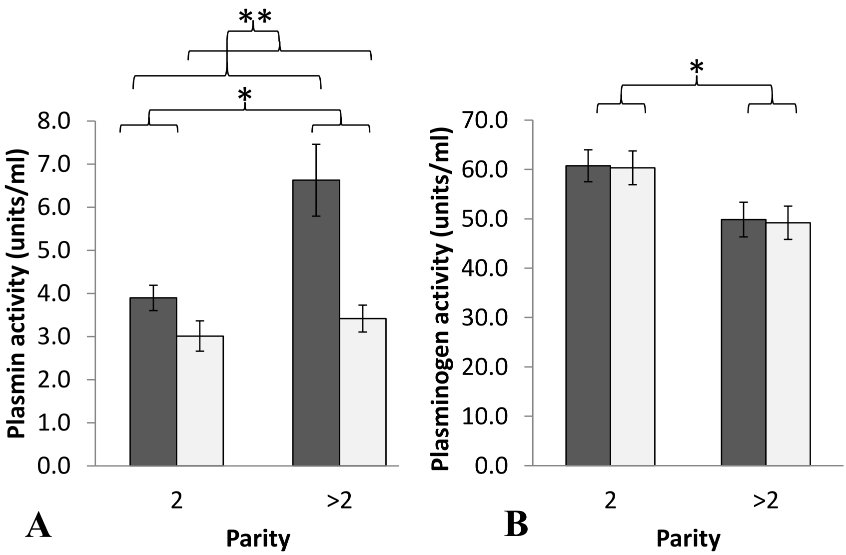

Figure 2. Plasmin activity (**A**) and plasminogen activity (**B**) in milk samples (6 and 12 wk postpartum) of cows with a dry period of either 4 wk (■) or 8 wk (□), and of second, or greater than second parity. * P < 0.05, ** P < 0.01.

4.3 Casein micelle composition (Paper III)

Paper III provides a more integrative view on the findings in papers I and II by analyzing casein and mineral composition of casein micelles, and the effect of plasmin activity on casein micelle composition in milk of cows with a 0 d or 30 d dry period. Cows with a 0 d dry period tended to have higher plasmin activity in milk (12.4 units/mL) than cows with a 30 d dry period (9.1 units/mL) (P = 0.07). Cows with a 0 d dry period had a lower β-casein fraction in milk (32.6%) than cows with a 30 d dry period (33.7%) (P = 0.02). The distribution of β-casein between casein micelles and milk serum was not influenced by dry period length. Consequently, the β-casein fraction that was present in casein micelles was also lower in milk of cows with a 0 d dry period (31.3%) compared with a 30 d dry period (32.7%). ectionThe sum of potential casein breakdown products measured with RP-HPLC did not differ between cows with either a 0 or a 30 d dry period (P = 0.11). The casein number, which was calculated as the sum of the micellar α_{s1}-, α_{s2}-, β- and κ-casein fraction, tended to be higher in milk of cows with a 30 d dry period (86.7%) compared with cows with a 0 d dry period (85.0%) ($P = 0.07$). Cows with a 0 d dry period had higher magnesium concentration in casein micelles (2.0 mmol/kg defatted milk) than cows with a 30 d dry period (1.6 mmol/kg defatted milk, $P = 0.02$). Cows with a 0 d dry period had higher micellar phosphorus concentration (21.1 mmol/kg defatted milk) than cows with a 30 d dry period (18.5 mmol/kg defatted milk, $P = 0.04$). Dry period length did not affect the ratios between micellar casein and micellar phosphorus, calcium and magnesium. Cows with a 0 d dry period had a lower calcium concentration in milk serum (9.1 mmol/kg defatted milk) than cows with a 30 d dry period (10.8 mmol/kg defatted milk) ($P < 0.01$). The calcium concentration in milk serum correlated negatively with milk protein content (R = -0.52, $P < 0.01$), and positively with the citrate concentration in milk (R = 0.51, $P < 0.01$).

4.4 Colostrum proteome (Paper IV)

In paper IV, the proteome of colostrum and transition milk (5th milking after calving) of cows with either a 4 wk or an 8 wk dry period were compared. In total, 222 proteins were quantified based on their dimethyl ratios in the current sample set. Concentrations of 18 proteins were higher (FDR ≤ 0.05) in colostrum of cows with a 4 wk dry period compared with an 8 wk dry period. Upregulated proteins in SR colostrum of 4 wk dry cows were 3 – 58 times more abundant compared with colostrum of 8 wk dry cows. Differences in protein concentrations are visualized in Figure 3. Hierarchical clustering of colostrum proteomes of SH cows showed numerical differences in the same proteins between cows with either a 4 wk or an 8 wk dry period. Colostrum of cows with a 4 wk dry period did not have any proteins with lower abundance compared with an 8 wk dry period. In transition milk samples, 18 proteins were upregulated (FDR = 0.05) in milk of cows that had a 4 wk dry period compared with an 8 wk dry period. Of these 18 proteins, 10 were upregulated in their colostrum samples as well. Like in colostrum, concentrations of individual proteins in transition milk of SH did not differ between cows with either a 4 wk or an 8 wk dry period.

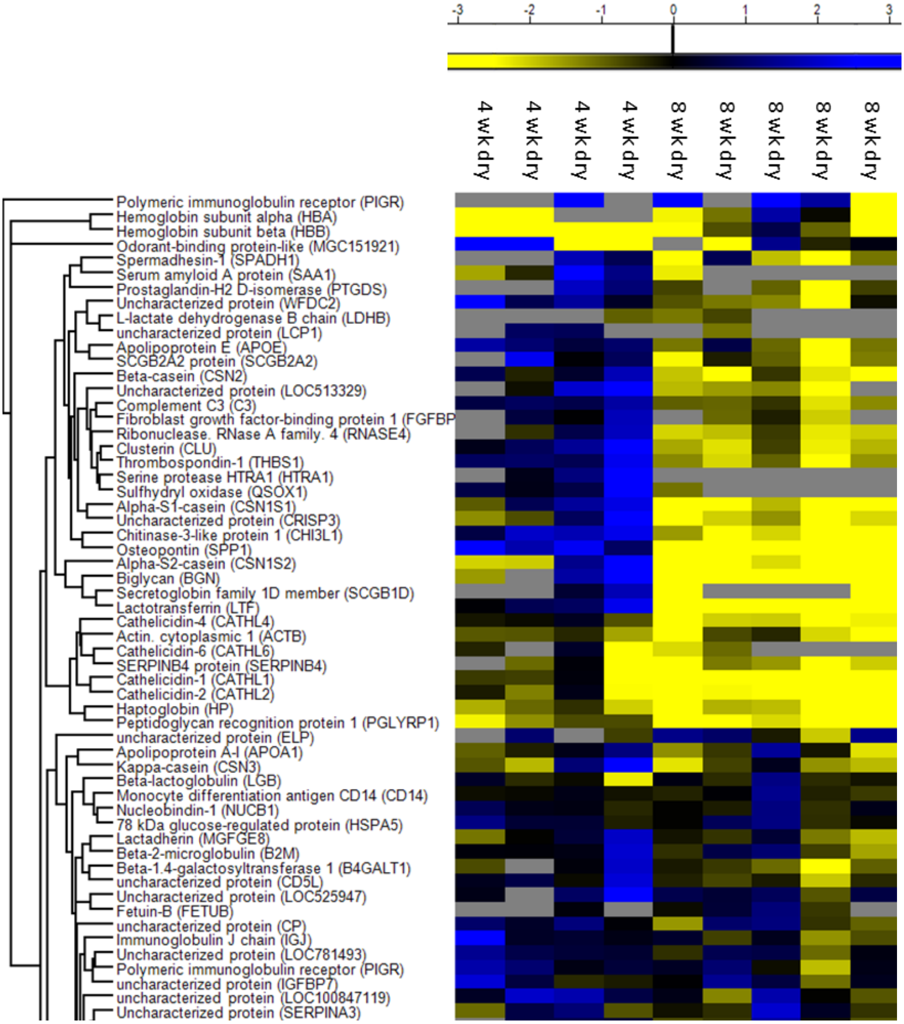

Figure 3. Hierarchically clustered dimethyl ratios of Swedish Red colostrum proteins (rows) shown as a heat map. Columns represent individual samples of cows with a 4 wk or an 8 wk dry period, ordered manually.

4.5 Blood glucose, milk metabolome, and milk composition (Paper V)

In paper V, the relation between the glucose concentration in blood, and milk composition was studied in early lactation milk. The metabolome of milk was studies, as metabolites are intermediates in cellular processes such as lactose and milk protein synthesis. The glucose concentration in blood correlated positively with the EB of the cows (R = 0.65, *P* = 0.02). Both blood glucose and the major milk protein fractions α-lactalbumin, β-lactoglobulin, α_{s1}-casein, β-casein, and κ-casein correlated with metabolite concentrations in milk. None of the metabolites correlated with the α_{s2}-casein fraction in milk. Nine metabolites (sugar A, lactate, ethanol, methylmalonate, alanine, acetate, N-acetylsugar A, malonate and hippurate) correlated with blood glucose, as well as with fractions of α-lactalbumin, β-lactoglobulin, α_{s1}-casein and β-casein. All correlations between β-lactoglobulin or κ-casein fractions and concentrations of metabolites that correlated with blood glucose were positive. Correlations between α-lactalbumin, α_{s1}-casein or β-casein fractions and metabolites that correlated with blood glucose were all negative.

5 General discussion

During the last 2 decades, increasing attention has been paid to find the optimal dry period of dairy cows. Cows with a traditional dry period of approximately 60 days generally experience in a severe negative energy balance after calving. It was suggested that improving the energy balance by shortening or omitting the dry period could improve the metabolic status and fertility of cows. Cow health and milk production were the main issues that have been studied in relation to shortening or omitting the dry period, whereas only limited studies had indicated the impact on milk composition. Milk composition in relation to dry period length can increase knowledge about the status of the mammary epithelium and the synthesis of milk components. Next to that, high quality milk is essential for the production of high quality milk products for human consumption. Therefore, in this thesis the effect of shortening or omitting the dry period on milk composition was evaluated.

This thesis about the relation between dry period length of dairy cows and milk composition and quality comprises two main research questions: *how is milk composition influenced by applying different dry period lengths to cows with regard to the processing quality of milk?* And, *how does milk composition of cows with different dry period lengths relate to the lactation physiology of the cow?* Paper 1, 2 and 3 provide data that indicate the effect of dry period length on processing quality of milk. Paper 4 and 5 focus on the relation between dry period length and mammary gland physiology. However compositional data in paper 1, 2 and 3 are also used as an indication of processes going on in the mammary gland. In this chapter, firstly, new insights from the different papers about mammary gland physiology are discussed (section 5.1). Secondly, implications of applying different dry period lengths for milk processing quality are evaluated (section 5.2). Thirdly, the contribution of this work to the opportunities and threats for different actors in the dairy chain of applying different dry period lengths in practice are discussed (section 5.3).

5.1 New insights in mammary gland physiology

Cows with a short or with no dry period have a lower milk yield than cows with a conventional dry period. Dry period length also influences milk composition on macronutrient level, but also within the fat fraction (van Knegsel et al., 2014) and the protein fraction (this thesis). The physiological background of changes in the fat fraction in early lactation milk of cows with different dry period lengths is well understood (Jorjong et al., 2014). However the mechanism underlying differences in milk protein composition in relation to dry period length have not been reported so far. In this work, shortening or omitting the dry period was related to a reduction in milk yield and β-casein fraction in milk, and an increase in milk protein content and plasmin activity in milk. In this section, the relation between these changes and the physiology of the mammary epithelium is discussed.

5.1.1 Regeneration of epithelial cells

The dry period is commonly known as a period during which regeneration of the mammary epithelium of the cow takes place (Collier et al., 2012, Capuco et al., 1997). When the dry period is shortened or omitted, lactogenesis and regeneration of the mammary epithelium may overlap. Cows that did not have a dry period had less prepartum proliferation of mammary epithelial cells (MECs), resulting in a lower number of active MECs in their successive lactation compared with cows that had a conventional dry period (Collier et al., 2012). This lower number of active mammary epithelial cells in early lactation of cows that did not have a dry period results in a lower milk yield postpartum (Annen et al., 2007, Annen et al., 2008). In the current work, dry period omission was indeed related to a lower postpartum milk yield, in comparison to a 60 d dry period (Paper 1). Regeneration of MECs has not been described for cows with a 30 d dry period. It can be assumed that numbers of active MECs will be lower for these cows than of cows with a 60 d dry period since the proliferation index is relatively high during the entire dry period (Collier et al., 2012). Indeed, cows with a 28-30 d dry period had a lower milk yield than cows with a 56-60 d dry period (Papers 1 and 2), in accordance to previous findings (van Knegsel et al., 2013).

5.1.2 Enzymatic activity in the mammary epithelium

Plasmin activity in milk is a result of transcellular transport of plasmin, or its precursor plasminogen, from blood to milk (Silanikove, 2016). Plasminogen is transported in golgisomes to milk, in the presence of casein micelles. Plasminogen is activated by either tissue-type activators, that are mainly associated with casein micelles, or urokinase-type activators that originate from somatic cells (Ismail and Nielsen, 2010). Hence, the plasmin activity in milk is dependent on the amount of plasmin and plasminogen that is transported from blood to milk, and the amount of plasminogen that has been activated. Consequently, an increase in plasmin activity can indicate a number of processes in the mammary epithelium. Firstly, it can reflect an increased casein content in milk, since plasminogen and casein are transported together. Secondly, increased plasmin activity can reflect an increase in somatic cell count (SCC) in milk. Thirdly, plasmin activity may reflect physiological processes in the mammary epithelium such as proliferation of mammary epithelial cells (Politis, 1996). In the current work, shortening or omitting the dry period was related to an increase in, or a tendency towards, an increased plasmin activity. In this section, 2 questions are evaluated. Firstly, does shortening or omitting the dry period by itself induce an increase in plasmin activity in the successive lactation? Secondly, are differences in plasmin activity related to the status of the mammary epithelium of cows with different dry period lengths?

The current work indicated that multiparous cows with a 4 wk dry period had increased plasmin activity in milk compared with cows with an 8 wk dry period, but no increased plasminogen activity (Paper 2). These cows with high plasmin activity in milk also had high SCC. Dry period reduction from 30 to 0 d resulted in only a tendency towards higher plasmin activity, which was proportional to plasminogen activity (Paper 3). All cows in the latter study had low SCC. Dry period omission may induce an increase in SCC (van Knegsel et al., 2014), although SCC was not affected by shortening the dry period to 30 d (van Knegsel et al., 2014, Bernier-Dodier et al., 2011). If SCC exceeds 300,000 cells/mL milk, it is related to increased activation of plasminogen (Politis et al., 1989), which was observed in the current work as well. All in all, shortening or omitting the dry period may result in an increased plasmin activity, proportional to the casein content in milk. However, increased SCC, which can be induced by dry period omission, may result in an increased plasminogen activation on top of increased plasmin activity. In the current work, plasmin activity increased with decreasing milk yield between early and mid-lactation. Milk yield declined proportionally between early and mid-lactation for cows with different dry period length (Schlamberger et al., 2010, van Knegsel et al., 2014). Therefore, the suggestion that plasmin activity is higher in milk of cows without a dry period due to the inverse relation with milk yield can be made for both early and mid-lactation.

High plasmin activity in milk of cows that had high SCC (Paper 2) indicated a role related to mammary health. Plasminogen activators from SCC have been related to proliferation of MEC. In primiparous cows, dry period omission did not affect MEC proliferation in early lactation (Annen et al., 2007). The effect of shortening the dry period on MEC proliferation in multiparous cows has not been reported. It is known that regeneration of MEC is slower for multiparous cows compared with primiparous cows (Miller et al., 2006). Several studies reported that the milk yield of multiparous cows is not affected by shortening the dry period (Klusmeyer et al., 2009, Pezeshki et al., 2007, Annen et al., 2004), in contrast to other work that show a drop in milk yield when the dry period is shortened, regardless of parity (van Knegsel et al., 2014). It can be hypothesized that multiparous cows require a high number and activity of MEC, whereas the formation of MEC during a dry period occurs in a lower rate compared with primiparous cows. Subsequently, multiparous cows may have increased postpartum MEC proliferation after a shortened dry period, which is reflected by increased activation of plasminogen into plasmin. Future work is needed to test this hypothesis, with focus on the actual MEC proliferation and the mechanism of plasminogen activation.

All in all, controlling SCC in milk of cows with a short or no dry period is important with regard to controlling plasmin activity. Further work is needed to assess the interactions between dry period length, parity and herd effects as risk factors for high SCC. When SCC can be kept low, shortening or omitting the dry period does not alter the ratio between plasmin activity and casein content in milk. Besides plasmin activity, also free fatty acid (FFA) concentrations were measured in the milk samples that were described in paper 2. However, no difference was found in FFA concentrations between early lactation milk samples of cows with a dry period of either 4 or 8 weeks. Spontaneous lipolysis due to physical damage of the milk fat globule membrane is the major cause for the presence of FFAs in milk (Deeth, 2006). The absence of a relation between lipolysis and plasmin activity of cows with a shortened dry period indicates that plasmin activity is unlikely to be related to physical damage of the epithelial cell membrane. With regard to dry period length, most physical damage to the MEC membrane may take place in the last weeks of lactation of cows without a dry period, due to a high cell turnover (Capuco et al., 1997, Annen et al., 2007). Therefore it is recommended to analyse FFA concentrations in the last weeks of lactation of cows without a dry period, which has not yet been included in the current work.

5.1.3 Milk synthesis in relation to energy status of the cow

The lower number of active MECs of cows without a dry period may be responsible for the lower milk production in early lactation (Collier et al., 2012). Due to the lower milk production, the metabolic status of the cow is improved (van Knegsel et al., 2014), which is reflected in a higher glucose concentration in blood of cows with a short or no dry period (van Knegsel et al., 2013). In paper 5, it was suggested that a higher glucose concentration in blood was not only related to an increased protein content in milk, but also to milk protein composition. In paper 1, only the β-casein fraction in early lactation milk was affected by applying a dry period of 0 d instead of 30 d, as was the case in paper 3. However, in paper 5, also other major milk protein fractions such as α_{s1}-casein and β-lactoglobulin were related to the blood glucose concentration of the cow. It is important to consider that dry period omission itself does not affect milk protein fractions, but it can reduce the number of active MECs, improve the energy balance and increase the blood glucose concentration in early lactation. The current work indicates that variability in glucose concentration in blood, and a reduced number of MECs, have an indirect effect on milk protein composition. The β-casein fraction in early lactation milk was related to both the blood glucose concentration (Paper 5) and plasmin activity (Paper 3). The effect of plasmin on β-casein was particularly found in mid-lactation, whereas no correlation between β-casein and plasmin was found in early lactation. The difference in the β-casein fraction in early lactation milk of cows with different dry period lengths was thus predominantly related to a difference in glucose concentration in blood, since the differences in metabolic status between individual cows are relatively large. In mid-lactation, when cows generally are in a positive energy balance, plasmin activity seems to be the major factor affecting the β-casein fraction in milk. In paper 3, it was addressed that cows with a 0 d dry period tended to have a lower casein number than cows with a 30 d dry period. Part of this tendency can be explained by the positive correlation between glucose metabolism and the β-lactoglobulin fraction in milk (Paper 5). However, a more large scale experiment using a conventional method to determine the casein number would be needed to determine whether dry period omission indeed has an effect on casein number.

5.2 Implications for milk processing

The current work showed that dry period length influenced macronutrient composition, casein composition, plasmin activity and mineral composition of milk. Besides, cows without a dry period have increased milk protein content and glycosylated κ-casein fraction in the last weeks of lactation. The cheese making process is sensitive for differences in casein composition and contents, and differences in mineral contents. Plasmin activity may affect the quality of dairy products during long term storage. Two dairy products that undergo long term storage are ultra-high temperature (UHT) milk, and cheese, in which proteolytic activity plays a major role for texture and flavour formation during ripening. Therefore, UHT milk and cheese are considered as important products with regard to the compositional differences in milk of cows with different dry period length. The current work has focused on late lactation and early lactation milk composition. In practice, bulk milk in the dairy plant contains milk from cows in various lactation stages. In this section, an estimation of the impact of different compositional parameters that are affected by dry period length is done on the processing characteristics of bulk milk.

In modern large-scale dairy processing, milk from large numbers of cows in different lactation stages and from different farms are mixed. This way, the composition of bulk milk is very constant, which is important for sensitive processes such as cheese making. For instance, the proportion of individual proteins is known to influence the cheese yield per amount of milk, and the amount of cheese solids per 100 g milk protein (Wedholm et al., 2006). In the current work, milk composition of individual cows, and of specific moments during lactation, was analysed. In order to be able to estimate the effects of differences in milk composition on cheese and UHT milk processing, a number of assumptions has to be made. Firstly, differences in milk composition are recognized on tanker level in the dairy plant. Milk from an individual tanker can be used in a specific process, based on the composition of the milk. Secondly, it is assumed that all milk comes from farms that do not apply a dry period to all cows. Thirdly, it is assumed that farms use complete randomized calving during the year, so that lactation stage effects can be ignored on bulk level.

5.2.1 Macronutrient contents and yields

The milk yield of cows reduces with progressing lactation, and milk protein content increases (Ostersen et al., 1997). In the current work, milk protein content of cows without a dry period increased up to 6.1% at two weeks before calving (Paper 1). Milk protein content of cows with a 4 wk dry period was 4.1% at 5 weeks before calving, which only tended ($P = 0.06$) to be higher than at 10 weeks before calving (Paper 2). Hence, the main concentration effects seem to take place after 5 weeks before calving. The cheese yield per kg milk is strongly dependent on the casein content in milk (Wedholm et al., 2006). The milk protein content of cows without a dry period was higher compared with cows with a conventional dry period during the first 20 weeks of lactation (Schlamberger et al., 2010), but no differences were found later in lactation. However, a field study indicated that during the first 305 days in milk (DIM), cows without a dry period had on average a 6.6% higher protein content than cows with a conventional dry period. The total cheese yield per cow depends on the total casein yield over the entire lactation. In contrast to the protein content, the average protein yield until 305 DIM of cows with a 0 d dry period was on average lower (33 kg, 9.4%) compared with cows with a conventional dry period (Steeneveld et al., 2013). This, however, does not include the protein yield of the last 60 days of the lactation of cows without a dry period. Assuming a protein content of 4.2% in 16.9 kg milk/d between 60 and 30 days prepartum, and 6.1% in 9.1 kg milk/d between 30 and 0 days prepartum (Paper 1), cows with a 0 d dry period yield an additional 38 kg protein during the last 60 days of their lactation. Hence, the protein loss during the first 305 d in lactation of cows without a dry period is compensated by the additional protein yield in the last 60 d before calving. In bulk milk from farms on which no dry period is applied, a high protein content will obtain a higher cheese yield per kg milk due to the increased protein content. In contrast to cheese yield, UHT milk yield is proportional to the milk yield, and not to protein yield. As the protein content in UHT milk is not standardised, it will be higher when no dry period is applied to cows.

In the current work, cows with different dry period lengths had similar fat content in milk, as was found in a systematic review by van Knegsel et al., (2013). Both cheese and UHT milk have a standardized fat content. Therefore, milk fat content does not seem to play a major role in the processability of milk in relation to dry period length. However, a 82 kg lower fat yield in the first 305 d of lactation of cows without a dry period (Steeneveld et al., 2013) is not fully compensated by the additional 40 kg fat in the last 60 d before calving (van Knegsel et al., 2014). A loss in fat yield results in an economic loss for dairy farmers, as milk fat is one of the constituents of milk that determines the milk price for the farmer. All in all, of the macronutrients, the lower fat yield seems to be the major economic concern for farmers when no dry period is applied. On a milk tanker level, milk of cows without a dry period may obtain a higher cheese yield. Since UHT milk, like other liquid dairy products, is not standardised to protein content, it may be disadvantageous to use milk of cows without a dry period for this application.

5.2.2 Plasmin activity and β-casein

Cows without a dry period had higher postpartum plasmin activity, as it changed proportionally with the milk protein content. Additionally, dry period reduction could increase SCC, which may could be related to increased activation of plasminogen into plasmin. In dairy products, plasmin activity may alter product quality due to proteolysis of caseins during long term storage. Besides, plasmin may affect casein fractions prior to processing. Reduction of casein fractions may result in altered processing properties of milk. Plasmin activity increased proportionally with the casein content in milk when cows did not have a dry period. Therefore it is not expected that dry period omission affects plasmin-induced breakdown of casein in a concentrated casein product such as cheese.

The current work indicated that dry period omission resulted in a reduced β-casein fraction in casein micelles, which in early lactation was related to the blood glucose concentration of the cow and not to plasmin activity. At a proportional change of casein content and plasmin activity, plasmin activity is not expected to affect casein composition. Wedholm et al. (2006) reported no significant influence of the β-casein fraction within the total casein fraction on cheese yield per kg milk. In the current work, however, the reduced β-casein fraction of cows without a dry period was found in total protein instead of total casein, and a lower casein number cannot be excluded. The casein number is one of the major compositional factors in milk affecting cheese yield (Wedholm et al., 2006). However, the difference in protein content on bulk milk level between cows without a dry period or cows with a conventional dry period are higher (6.6%, Steeneveld et al., 2013) than the difference in casein number (-2.6% in early lactation, not significant). It can be concluded that based on casein content, milk of cows without a dry period is suitable for cheese making. However, reductions in casein number are strongly reflected in a reduction of cheese yield per amount of protein (Wedholm et al., 2006). Therefore, it is recommended to do a large scale study about the casein number in bulk milk of cows without a dry period.

Plasmin is thought to be one of the main factors causing instability of UHT milk during long term storage. The potential role of plasmin activity in UHT milk depends on the temperature profile of the UHT treatment. Although plasmin has a certain heat stability, no activity was found after preheating at 95°C for 180 s followed by UHT treatment (direct steam infusion, 150°C, >0.2 s). After preheating at 72°C for 180 s followed by UHT treatment, however, residual plasmin activity was 31% of the plasmin activity in raw milk (Rauh et al., 2014b). Hence, the effect of the temperature profile seems to have a larger effect on plasmin activity in UHT milk than differences in plasmin activity in raw milk that can be induced by dry period length.

5.2.3 Glycosylated κ-casein

The current work showed an increase in the glycosylated κ-casein fraction in late lactation milk of cows without a dry period. At 10 days postpartum, cows without a dry period also had a greater glycosylated κ-casein fraction in milk than cows with a conventional dry period. This effect however was not found later in lactation. It was hypothesised that increased glycosylated κ-casein would result in a reduced casein micelle size, as is the case in middle lactation (Bijl et al., 2014). In house follow-up experiments indicated that casein micelle size and renneting properties remained constant until 4 weeks pre-calving. At 2 weeks pre-calving, however, casein micelle size and renneting properties appeared to vary widely between individual cows. The underlying mechanism of this variation should be subject of further studies. Considering a total lactation yield of 10,633 kg (Schlamberger et al., 2010), and an average yield of 9.1 kg/d during the last 4 weeks of lactation, the contribution of these last 4 weeks is 2.4% of the total yield. Assuming 6.0% of glycosylated κ-casein in the remaining 97.6% of the bulk milk of cows without a dry period, the glycosylated κ-casein fraction in total protein may increase from 6.0 to 6.15% on tank level when none of the cows receives a dry period. In cheese processing, curd firmness increases proportionally with the N-acetyl neuraminic acid content of κ-casein, a measure for the glycosylation of the κ-casein fraction (Robitaille et al., 1993). Therefore, even though the contribution of the glycosylated κ-casein fraction in late lactation is relatively small on bulk level, it may enhance the cheese making properties of milk from cows without a dry period. However, the increase in protein content of at least 6.6% in bulk milk (Steeneveld et al., 2013) is likely to have a much larger contribution than the estimated 2.5% increase in only the glycosylated κ-casein fraction.

5.2.4 Calcium concentration

Milk of cows without a dry period had a lower calcium concentration in early and mid-lactation milk serum compared with cows with a 30 d dry period, which was related to a higher milk protein content and a lower citrate concentration in milk. The calcium concentration in milk serum correlates positively with the concentration of free calcium ions (calcium activity) in mid-lactation milk (Bijl et al., 2013). Calcium activity contributes to the coagulation of casein micelles during renneting of milk. Calcium activity was not analysed in the current work. During cheese making, an excess of calcium chloride is added to milk prior to the renneting process. This added calcium is expected to level out differences in calcium activity of milk from cows with different dry period.

5.2.5 Wrapping up: processing of milk

On bulk level, dry period omission had a major effect on protein content of milk. Other compositional differences, such as the increased glycosylated κ-casein fraction, had a lower impact on bulk level since the effect is specific for one lactation stage. The increased protein content seems to make bulk milk from cows without a dry period suitable for cheese production. The increase in protein content of bulk milk from cows without a dry period has much more impact on the casein content in milk than the relatively small potential reduction in casein number. Indications for a reduced casein number in milk of cows without a dry period should be subject for further investigation however, as it may affect the cheese yield per 100 g protein. The small increase in glycosylated κ-casein fraction in bulk milk may have a minor positive contribution to the curd formation of milk from cows without a dry period. Plasmin activity was not expected to affect the cheese making properties of milk from cows without a dry period, since plasmin activity is proportional to the casein fraction of milk. Milk from cows without a dry period seems economically less suitable for UHT milk processing. Firstly, dry period omission results in a lower milk yield with an increased milk protein content, which results in a lower amount of product with a higher milk protein content. Secondly, increased plasmin activity may affect stability of UHT milk when no sufficient temperature profile is applied.

5.3 Dry period management in practice

The aim of shortening or omitting the dry period of dairy cows is to improve the health of dairy cows, without loss of milk quality and profitability. The current work contributes to finding an optimal dry period length by providing a detailed overview of the milk composition of cows with a dry period of either 0, 30 or 60 d. One of the questions that has recently been put forward is whether there is an optimal dry period length for all cows, or whether an individual approach is more suitable (van Hoeij et al., 2016).

The current work showed higher plasminogen activation in early lactation milk of multiparous cows with a short dry period. Although not all studies are in line with each other, multiple studies showed that primiparous cows showed a decrease in milk yield as a result of shortening the dry period from 56 to 35 d, whereas milk yield of multiparous cows was not affected by shortening the dry period (Pezeshki et al., 2007, Klusmeyer et al., 2009, Annen et al., 2004). Primiparous and multiparous cows did not only show a difference in milk yield response towards shortening the dry period, but parity also influenced the metabolic status after shortening the dry period. Primiparous cows with a 35 d dry period had lower non-esterified fatty acid (NEFA) concentrations in blood than primiparous cows with a 56 d dry period, which reflects lower mobilisation of body reserves (Pezeshki et al., 2007). This indicates a positive effect of dry period reduction on the metabolic status of primiparous cows, whereas no effect of dry period length on NEFA concentrations was found for multiparous cows. Metabolic problems during early lactation are related to cows with a high body condition, since these cows tend to mobilise more of their own body reserves and consequently end up in a lower negative energy balance (Rukkwamsuk et al., 1999). Hence, for milk production and optimising the metabolic status of cows, a customised dry period seems to be beneficial, taking factors such as parity and body condition into consideration.

From a milk composition perspective, the choice for a customised dry period length for dairy cows is supported by plasmin activity data (Paper 2) and the proteome of colostrum (Paper 4). It turned out that parity and the breed (Swedish Red or Swedish Holstein) of cows may affect the composition of milk and colostrum in relation to dry period length. Besides, herd effects may play a role, since the parity effect on plasmin activity, and the breed effect on the colostrum proteome were both found in the same herd with cows that had a relatively high SCC. This is supported by the finding that in another herd, there was no interaction between dry period length and parity on the β-casein fraction in milk. The current results do not favour a short dry period for multiparous cows, because of the risk of increased plasmin activity in milk after calving. Possibly a herd effect on SCC, which can contribute to activation of plasminogen, interacts with the parity effect on plasmin activity. The outcome that multiparous cows may have higher plasmin activity in milk after a short dry period is contrasting with other milk production characteristics such as the limited drop in milk yield, which are favourable for applying a reduced dry period to multiparous cows (Pezeshki et al., 2007). Proteome results in paper 4 indicated that Swedish Red cows had higher concentrations of cell proliferation related proteins as a result of shortening the dry period than Swedish Holsteins. It is suggested that this breed effect was related to a different cell proliferation rate of a dual purpose breed (Swedish Red), compared with a milk breed (Swedish Holstein). Shortening the dry period did not result in concentration reduction of any of the low abundant proteins in colostrum, and therefore there seems to be no disadvantage for the cow or its calf. Milk production characteristics did not differ between breeds in response to dry period reduction (Paper 2), so also for the farmer, Swedish Holsteins and Swedish Reds seem equally suitable for applying a shortened dry period. In conclusion, based on milk composition analyses, multiparous cows from herds with a high average SCC may be less suitable for dry period reduction from a milk quality perspective. The current work showed no other arguments for applying a customised dry period based on milk composition.

5.3.1 Late lactation milk: problem or opportunity?

Milk obtained in the last 4 weeks before calving of cows without a dry period was shown to have a small effect on bulk level, for example by increasing the protein content and the glycosylated κ-casein fraction (Paper 1). In addition, IgG production is increased prepartum (Guy et al., 1994b), which in cows with a dry period results in accumulation of IgG in the mammary gland that will eventually end up in colostrum (Mayasari et al., 2015). Cows with no dry period may secrete some of this IgG prepartum in late lactation milk, which is likely to be the cause of the reduced IgG concentration in their colostrum (Mayasari et al., 2015). Both IgG and glycosylated κ-casein are functional components of milk. The glycosylated macropeptide of κ-casein was reported to have both prebiotic and antimicrobial properties (O'Riordan et al., 2014). The additional IgG that is secreted in prepartum milk may be used by farmers for additional feeding of their calves since the IgG concentration in colostrum of cows without a dry period is lower than in colostrum of cows that did receive a dry period (Mayasari et al., 2015). The increased IgG concentration in prepartum milk may however also be used in products for human consumption for specific functional dairy products. In the milk processing discussion (section 5.2), only milk processing at large scale was considered. For small scale, on-farm cheese production, a more flexible milk collection may create opportunities for late lactation milk of cows without a dry period. The different protein content and composition of this milk may result in distinct processing properties. Before utilising late lactation milk of cows without a dry period, an in depth analysis of the variability of milk components and the economic feasibility of their application should be done.

6 Conclusions and recommendations

6.1 Conclusions

- Shortening or omitting the dry period of cows with good mammary health obtains milk with a higher protein content with little differences in protein composition. The tendency towards a lower casein number in milk of cows without a dry period was the major factor that may influence processing of milk, particularly with regard to cheese yield.
- The casein composition of milk is related to both plasmin activity in milk and the metabolic status of cows. The blood glucose concentration is particularly important in early lactation, whereas the contribution of plasmin activity is greater in mid-lactation when all cows are in positive energy balance.
- Low abundant protein concentrations in colostrum, and plasminogen activation in early lactation milk indicate that a dry period of 4 weeks is not sufficient for all cows to regenerate their mammary epithelium.
- Apart from controlling plasmin activity in milk, milk composition analyses did not obtain further arguments for applying a customised dry period to cows.

6.2 Recommendations

- The current work showed a tendency towards a lower casein number in milk of cows without a dry period. This reductions in casein number may cause a lower cheese yield. Therefore it is recommended to study the effect of dry period omission on casein number in a large scale study, using a method better suited to study this parameter.
- A study on the influence of a 0 d dry period on the colostrum proteome can obtain further insight in the role of mammary involution prepartum and mammary epithelial cell proliferation postpartum.
- Further investigation of compositional changes in the last days before calving for both its functional properties and the insights in preparation for a new lactation.
- The suggested effect of dry period omission on cheese processing should be evaluated in practice. Such a study can be done by using tank milk, to evaluate dilution effects of changes in milk composition that were reported in the current work on bulk level.

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