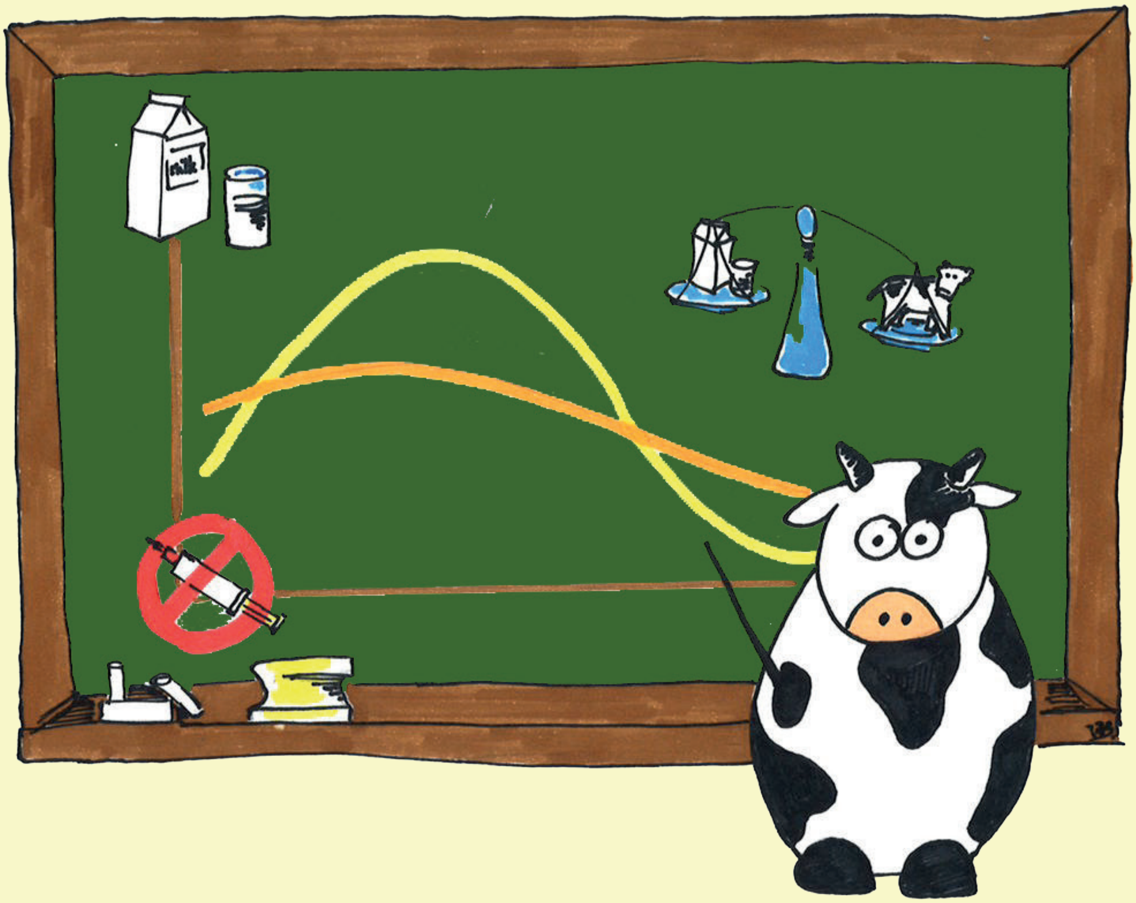

Metabolic status, lactation persistency, and udder health of dairy cows after different dry period lengths

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Thesis

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Table of contents


1066
1139
1292

CHAPTER 1

General introduction

1.1 Challenges in dairy farming

Dairy cows kept in domestic husbandry start producing milk after calving for, traditionally, about 305 days, followed by a 60-d dry period and the subsequent calving (**Voelker**, 1981). In the past decades, annual milk production of dairy cows has increased tremendously (**Figure 1.1**) (**FAO STAT, 2017**). Especially after calving, when energy demands for milk production are increasing rapidly, feed intake is too limited to cover the energy requirements for maintenance and milk production, resulting in a 'negative energy balance' (**Waterman *et al.***, 1972; **Grummer**, 1995; **Van Knegsel *et al.***, 2014a). During a 'negative energy balance' energy reserves from the body are mobilized to cover the needs for maintenance and milk production (**Figure 1.2**). A negative energy balance is associated with a higher incidence of diseases and disorders in early lactation such as ketosis, milk fever, clinical mastitis, displaced abomasum, retained placenta, endometritis, lameness, and decreased fertility (**Collard *et al.***, 2000; **Butler**, 2003; **Ingvartsen *et al.***, 2003). Shortening or omitting the dry period length was opted as a management strategy to partly move milk production in early lactation to the end of the previous lactation (**Grummer and Rastani**, 2004), which improves the energy balance in early lactation (**Rastani *et al.***, 2005). Due to the improvement of the energy balance, it can be expected that a shorter or no dry period is beneficial for cow health and reduces the risk for health and fertility problems in dairy cows.

This introduction focuses on the history of dry period management, and discusses the existing knowledge on effects of dry period length on milk production, metabolic status, lactation curve characteristics, and udder health.

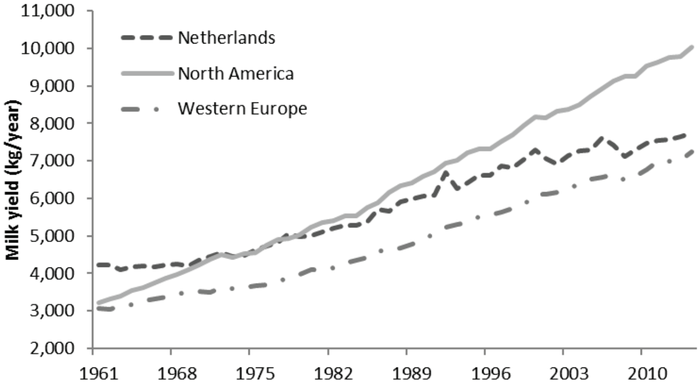

Figure 1.1: Average milk yield per cow per year in Northern America and Europe between 1961 and 2014 (**FAOSTAT,** 2017).

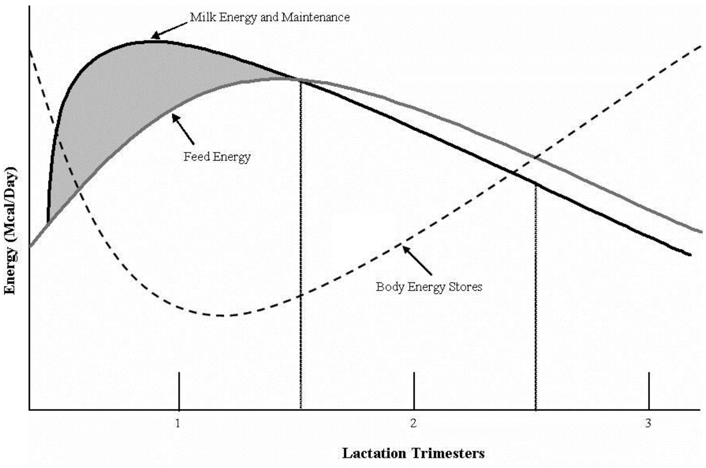

Figure 1.2: Energy requirements for milk yield and body maintenance, energy intake through feed, and body energy stores of dairy cows during lactation (**Hoffman *et al.***, 2000).

1.2 History of the dry period and effects of dry period length on milk yield

The dry period, a non-lactating period preceding calving, is a naturally occurring phenomenon in mammals (**Lee *et al.***, 1991; **Enríquez *et al.***, 2011). In domestic husbandry, a two month dry period was traditionally applied to achieve maximal milk yield in the subsequent lactation (**Arnold and Becker**, 1936). Increasing milk yield in the past decades and the associated negative effect on energy balance in early lactation may demand a change of vision and the decision to shorten or omit the dry period (**Grummer and Rastani**, 2004).

The importance of a dry period was already stressed in the early 1900's when it was stated that a two month dry period was optimal to maximize milk production in the subsequent lactation (**Arnold and Becker**, 1936). It was suggested that the required dry period length depends on individual cow milk production and body condition (**Woodward and Dawson**, 1926). Another study from that period concluded that a dry period length of at least 35 days was necessary to maximize milk yield in subsequent lactation (**Gavin**, 1912). In a review of a total of 10 commonly used textbooks, including the two foregoing authors, Arnold and Becker (1936) concluded that a dry period length between 31 and 60 days in cows resulted in maximum daily milk yield post-partum (**Arnold and Becker**, 1936).

Since the publication of Arnold and Becker (1936) an increasing number of studies have evaluated effects of dry period length on milk production. After World War II maximal milk production was pursued with implementation of a 305 day lactation period and a 2 month dry period, as earlier reviewed (**Bachman and Schairer**, 2003). In the middle of the 1900s, it was hypothesized that a dry period was needed from a nutritional point of view to restore body condition after the previous lactation in order to stimulate milk production in the subsequent lactation (**Swanson**, 1965). However, a dry period actually appeared beneficial for mammary tissue to rejuvenate (**Swanson**, 1965). Part of studies on effects of dry period length on milk yield in the subsequent lactation were retrospective studies. These retrospective studies included substantial numbers of cows, although a shortened or omitted dry period was not the result of assignment to treatment with a specific dry period length, but was rather a result of too early calving for example due to a late abortion twinning or miscalculation of the expected calving date. Subsequent milk yields were lower, possibly due to the fact that cows were not optimally managed for a short dry period (**Bachman and Schairer**, 2003; **Grummer and Rastani**, 2004). Cows with a 1 – 2 month dry period were reported to produce 9% more milk than cows with a 0 – 1 month dry period (**Figure 1.3**) (**Klein and Woodward**, 1943). Other retrospective studies reported that cows with a dry period of 40 days or less produced less milk in the subsequent lactation than cows with a 40 – 70 days dry period, and that cows with an average dry period of 60 days achieved the greatest milk production in the subsequent lactation (**Schaeffer and Henderson**, 1972; **Dias and Allaire**, 1982; **Funk *et al.***, 1987).

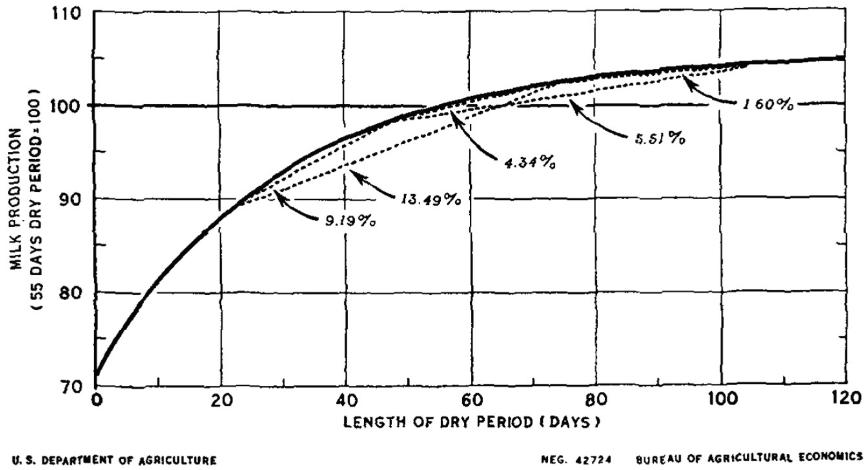

Fig. 1.3: The relationship between dry period length and milk production from Klein and Woodward, (1943). Percentage indicates increase in milk production relative to previous month, and sum of increase of two months combined.

Other studies on effects of different dry period length on milk yield were experimental studies in which cows were managed for specific dry period lengths. These studies allow better evaluation of a dry period strategy although number of cows were somewhat lower than in retrospective studies. One study, using a half udder model, concluded that the lower milk production in the two quarters without a dry period was due to the lack of a dry period and not due to nutritional factors because the plain of nutrition was similar for both udder halves and not limiting (**Smith *et al.***, 1967). Another experimental study reported that cows with a dry period longer than 40 days produced more milk in subsequent lactation than cows with a 10 – 40 days dry period (**Coppock *et al.***, 1974).

More recent experimental studies were able to use a larger number of cows and evaluated effects of a short or omitted dry period on milk yield and milk composition (**Table 1.1**). Compared with a conventional dry period (~60 days), postpartum milk production was on average 18% (range 27 – 9%) lower after an omitted dry period (**Rémond *et al.***, 1992; **Remond *et al.***, 1997; **Annen *et al.***, 2004; **Andersen *et al.***, 2005; **Rastani *et al.***, 2005; **Madsen *et al.***, 2008; **De Feu *et al.***, 2009; **Klusmeyer *et al.***, 2009; **Schlamberger *et al.***, 2010; **Santschi *et al.***, 2011a), and 6% (range 20 – +3%) lower after a shortened dry period (~30 days) (**Coppock *et al.***, 1974; **Sørensen and Enevoldsen**, 1991; **Bachman**, 2002; **Gulay *et al.***, 2003; **Annen *et al.***, 2004; **Rastani *et al.***, 2005; **Pezeshki *et al.***, 2007; **Pezeshki *et al.***, 2008; **Watters *et al.***, 2008; **De Feu *et al.***, 2009; **Klusmeyer *et al.***, 2009; **Soleimani *et al.***, 2010; **Bernier-Dodier *et al.***, 2011; **Santschi *et al.***, 2011a; **Safa *et al.***, 2013; **Khazanehei *et al.***, 2015).

Compared with a conventional dry period, a shortened or omitted dry period resulted in a greater milk protein (**Andersen *et al.***, 2005; **Madsen *et al.***, 2008; **Klusmeyer *et al.***, 2009; **Bernier-Dodier *et al.***, 2011; **Van Knegsel *et al.***, 2014b) and fat concentration (**Van Knegsel *et al.***, 2014b) in the subsequent lactation. The difference in fat- and protein- corrected milk (**FPCM**[1]) yield between dry period lengths was, therefore, smaller than the difference in milk yield (**Rastani *et al.***, 2005; **Santschi *et al.***, 2011b). Compared with a conventional dry period, FPCM yield was on average 15% lower in cows with an omitted dry period which is a 3% smaller difference than milk yield (**Remond *et al.***, 1997; **Rastani *et al.***, 2005; **Madsen *et al.***, 2008; **Van Knegsel *et al.***, 2014b). Compared with a conventional dry period, FPCM yield was on average 6% lower for cows with a shortened dry period, which is similar to the difference in milk yield. For cows with a shortened dry period, some studies reported 3% greater FPCM yield (**Gulay *et al.***, 2003; **Andersen *et al.***, 2005; **Rastani *et al.***, 2005; **Watters *et al.***, 2008; **Klusmeyer *et al.***, 2009; **Bernier-Dodier *et al.***, 2011; **Santschi *et al.***, 2011a; **Van Knegsel *et al.***, 2014b), while others reported lower (**Annen *et al.***, 2004) or not different (**Pezeshki *et al.***, 2007; **Pezeshki *et al.***, 2008), FPCM yield compared with milk yield. The lower postpartum milk and FPCM yield in cows with a short or omitted dry period was partly compensated with prepartum milk yield, compared with cows with a conventional dry period (**Rastani *et al.***, 2005; **Pezeshki *et al.***, 2008; **Klusmeyer *et al.***, 2009; **Santschi *et al.***, 2011a; **Van Knegsel *et al.***, 2014b).

[1]FPCM (kg/d) = [(0.337 + 0.116 × fat content (%) + 0.06 × protein content (%)] × milk yield (kg/d) (**CVB**, 2011)

Lower milk production after a shortened or omitted dry period may result from a lower secretory and proliferation capacity of udder cells due to the greater presence of old cells. For cows without a dry period, cell apoptosis and turnover are not stimulated during a period of milk stasis during the dry period (**Capuco *et al.***, 1997). A previous study reported that all udder cells have reached a non-secretory state during involution at around 35 days after drying off (**Capuco *et al.***, 1997). It was previously suggested that a dry period of about 30 days allows sufficient time for involution and cell renewal in order achieve maximal production capacity during the next lactation (**Gulay *et al.***, 2003; **Pinedo *et al.***, 2011).

Recent studies observed that parity affects milk yield after different dry period lengths. Omitting the dry period for cows with postpartum parity 2 resulted in lower milk production in the subsequent lactation compared with cows with greater parity (**Annen *et al.***, 2004; **Van Knegsel *et al.***, 2014b). Cows with parity 2 may need a short or conventional dry period for body and mammary growth (**Annen *et al.***, 2004). Additional to parity, other factors affect milk production such as nutrition (**Friggens *et al.***, 1995), or udder health in the previous lactation (**Green *et al.***, 2008), and can be expected to interact with different dry period lengths (**Van Knegsel *et al.***, 2014b). It would be interesting to evaluate how cows with different dry period lengths should be managed to optimize their performance in milk yield and metabolic status.

Table 1.1: Postpartum milk yield and FPCM yield of cows after a conventional, short or omitted dry period[1] in different studies

	Cows (n=)	DIM	Par[2]	Milk yield (kg/d)			% of conv.	
				Conv.[3]	Short	No	Short	No
Andersen et al., 2005	28	35		40.9		32.1		78
Annen et al., 2004	60	119	2	44.1	38.3	35.1	87	80
Annen et al., 2004	60	119	≥2	47.7	46.6	43.4	98	91
Bachman, 2002	66	168		40.6	38.9		96	
Bernier-Dodier et al., 2011	18	140		38.7	35.2		91	
Coppock et al., 1974	65 herds	305		22.1	20.6		93	
De Feu et al., 2009	40	84	≥ 2	29.4		24.6		84
Gulay et al., 2003	84	70		39.4	37.4		95	
Jolicoeur et al., 2010	12	21		37.7	38.9		103	
Khazanehei et al., 2015	11	112	2	43.9	42.2		96	
Khazanehei et al., 2015	15	112	≥2	42.1	38.4		91	
Klusmeyer et al., 2009	341	294	≥ 2	40.0	39.0	33.5	98	84
Madsen et al., 2008		11	2	18.9		15.6		83
Madsen et al., 2008		17	≥2					
Pezeshki et al., 2007	60	210	2	37.1	33.7		91	
Pezeshki et al., 2007	60	210	≥2	35.4	35.2		99	
Pezeshki et al., 2008	70	210	≥ 2	34.6	32.7		95	
Rastani et al., 2005	65	70		42.4	37.9	33.9	89	80
Remond et al., 1992	26	105		27.6		24.2		88
Remond et al., 1997	26	252		25.1		19.5		78
Santschi et al., 2011	414	305	2	31.5	30.1		96	
Santschi et al., 2011	436	305	≥2	32.7	32.7		100	
Safa et al., 2013	25	60		36.2	28.9		80	
Schlamberger et al., 2010	36	100		44.9		37.8		84
Soleimani et al., 2010	29	60		36.5	34.9		96	
Sorensen and Enevoldson, 1991	366	84		24.5	22.0		90	
Van Knegsel et al., 2014	167	98		33.5	29.7	24.4	89	73
Watters et al., 2008	781	100		39.5	37.7		95	

[1]A short dry period was on average 32-d (range 20 – 40-d) and a conventional dry period was on average 57-d (range 49 – 65-d). [2]Fat- and protein- corrected milk was calculated as: (0.337 + 0.116 × fat content + 0.06 × protein content) × milk yield. [3]Conv. = conventional dry period length. [4]Postpartum parity was 2 for primiparous cows and ≥ 2 for multiparous cows.

Table 1.1: Postpartum milk yield and FPCM yield of cows after a conventional, short or omitted dry period[1] in different studies

				FPCM (kg/d)[2]			% of conv[3].	
	Cows (n=)	DIM	Par[4]	Conv.	Short	No	Short	No
Andersen et al., 2005	28	35		42.3		33.9		80
Annen et al., 2004	60	119	2	41.2	36.7	33.7	89	82
Annen et al., 2004	60	119	≥2	43.7	41.5	40.1	95	92
Bachman, 2002	66	168						
Bernier-Dodier et al., 2011	18	140		36.7	34.5		94	
Coppock et al., 1974	65 herds	305						
De Feu et al., 2009	40	84	≥ 2	29.5		25.6		87
Gulay et al., 2003	84	70		30.8	29.5		96	
Jolicoeur et al., 2010	12	21		35.6	35.2			
Khazanehei et al., 2015	11	112	2	43.5	40.8		94	
Khazanehei et al., 2015	15	112	≥2	40.8	37.3		91	
Klusmeyer et al., 2009	341	294	≥ 2	37.1	36.6	32.2	99	87
Madsen et al., 2008		11	2	18.0		15.7		87
Madsen et al., 2008		17	≥2					
Pezeshki et al., 2007	60	210	2	34.9	31.5		90	
Pezeshki et al., 2007	60	210	≥2	34.3	34.5		101	
Pezeshki et al., 2008	70	210	≥ 2	31.6	29.8		94	
Rastani et al., 2005	65	70		40.5	37.5	33.3	93	82
Remond et al., 1992	26	105		27.9		25.3		91
Remond et al., 1997	26	252		24.6		20.4		83
Santschi et al., 2011	414	305	2	31.1	29.8		96	
Santschi et al., 2011	436	305	≥2	32.4	32.3		100	
Safa et al., 2013	25	60		35.2	28.4		81	
Schlamberger et al., 2010	36	100						
Soleimani et al., 2010	29	60		35.5	33.9		96	
Sorensen and Enevoldson, 1991	366	84						
Van Knegsel et al., 2014	167	98		34.9	31.7	26.8	91	77
Watters et al., 2008	781	100		35.5	34.5		97	

[1]A short dry period was on average 32-d (range 20 – 40-d) and a conventional dry period was on average 57-d (range 49 – 65-d). [2]Fat- and protein- corrected milk was calculated as: (0.337 + 0.116 × fat content + 0.06 × protein content) × milk yield. [3]Conv. = conventional dry period length. [4]Postpartum parity was 2 for primiparous cows and ≥ 2 for multiparous cows.

1.3 Metabolic status, fattening, and lactation persistency

1.3.1 Effects of dry period length on metabolic status in early lactation

In most studies, the improvement of the energy balance (**EB**) after a 0-d dry period was due to a reduction in milk production (**Annen *et al.***, 2004; **De Feu *et al.***, 2009; **Van Knegsel *et al.***, 2014b) and sometimes also due to an increased energy intake (**Rastani *et al.***, 2005), compared with a 30-d or 60-d dry period. Postpartum, cows with a shortened or omitted dry period had a less negative energy balance and consequently lower free-fatty acid (**FFA**) concentration, ketone body formation (lower plasma β-hydroxybutyrate (**BHB**) concentration), and triacylglycerol storage in the liver than cows with a conventional dry period (**Andersen *et al.***, 2005; **Rastani *et al.***, 2005). Furthermore, cows with a shortened or omitted dry period had a greater plasma glucose and insulin concentrations compared with cows with a conventional dry period (**Andersen *et al.***, 2005; **De Feu *et al.***, 2009; **Bernier-Dodier *et al.***, 2010; **Chen *et al.***, 2015). A low plasma FFA and BHB concentration, and greater plasma glucose and insulin concentration reflect a good metabolic status (**Lean *et al.***, 1992). In previous studies, levels of energy content in the ration were kept similar among cows with different dry period lengths (**Andersen *et al.***, 2005; **Rastani *et al.***, 2005; **De Feu *et al.***, 2009; **Van Knegsel *et al.***, 2014b). Cows with an omitted dry period, have a lower milk production and likely a lower energy demand than cows with a shortened or conventional dry period. It is unclear whether feeding cows an energy level according to their requirement for milk yield for cows with an omitted dry period, results in either similar or even lower milk yield or a decreased EB, or both.

1.3.2 Effects of dry period length on metabolic status, fattening, and lactation persistency in mid- and late- lactation

In a previous study, feeding the same energy level (accounting for milk production levels after a conventional dry period) to cows with different dry period lengths was associated with an increased body condition score (**BCS**) at the end of subsequent lactation in cows with a 0-d dry period (**Chen *et al.***, 2016b). The excess energy intake in cows with a 0-d dry period was obviously not used for milk production and thus stored in body reserves. A high BCS was negatively correlated with lactation persistency in late lactation (**Berry *et al.***, 2003; **Roche *et al.***, 2009; **Piccand *et al.***, 2013). In concordance, cows with a 0-d dry period and an increased BCS at the end of lactation dried off spontaneously (**Chen *et al.***, 2016b). Earlier it was hypothesized that improving lactation persistency could partially compensate for milk losses in early lactation and potentially increase total lactation yield in cows with a 0-d dry period compared with cows with a dry period (**Grummer and Rastani**, 2004). Persistency of lactation was previously defined as 'the extent to which peak yield is maintained' (**Wood**, 1967). Studies on effects of dry period length on lactation persistency reported ambiguous results. Shortening or omitting the dry period reduced lactation persistency (**Mantovani *et al.***, 2010), increased lactation persistency in primiparous cows (**Atashi *et al.***, 2013) or did not affect lactation persistency (**Chen *et al.***, 2016a) compared with cows with a conventional dry period length. Differences in lactation persistency among cows with different dry period lengths in different studies could be related with differences in dietary energy availability, dietary energy source, lactogenic hormones, or nutrient partitioning.

Greater energy availability than required for milk production, such as in mid- and late- lactation, results in a more positive energy balance. During positive energy balance, plasma glucose is high, stimulating insulin production in the pancreas (**Figure 1.4**). In the presence of insulin, pituitary derived growth hormone (**GH**) binds GH receptors in the liver and stimulates synthesis of insulin-like growth factor-1 (**IGF-1**) that in turn inhibits pituitary GH release via a negative feedback loop in the somatotropic axis (**Lucy**, 2011). A greater plasma insulin and IGF-1, and lower GH concentration decreased nutrient partitioning towards milk production and increased nutrient partitioning to body reserves (**Hart**, 1983). Lower GH concentration was previously associated with lower lactation persistency (**Butler *et al.***, 2003; **Lucy**, 2008).

At the start of lactation, energy availability does not meet energy demands, plasma glucose concentration is low and as a consequence insulin production from the pancreas is not stimulated (**Lucy**, 2008) (**Figure 1.4**). As a result of a low plasma insulin concentration in early lactation, available glucose is drawn to the insulin-independent mammary gland (**Knight and Peaker**, 1982; **Butler *et al.***, 2003). Furthermore, a low plasma insulin concentration in early lactation results in an uncoupled somatotropic axis, low expression of GH-receptors in the liver and low liver IGF-1 synthesis (**Bradford and Allen**, 2008). Normally, liver derived IGF-1 inhibits the release of pituitary GH, but during the uncoupled phase this inhibition fails and large amounts of GH are being released (**Lucy**, 2011). Growth hormone is a direct regulator of milk production and stimulates gluconeogenesis and breakdown of adipose tissue leading to glucose and FFA which are subsequently used for milk components (**Lucy**, 2008). Growth hormone also stimulates mammary production of IGF-1, which is locally present in mammary tissue as a cell survival factor and anti-apoptotic factor which provides the udder with more functional units in time and consequently improves lactation persistency (**Flint and Knight**, 1997; **Capuco *et al.***, 2003).

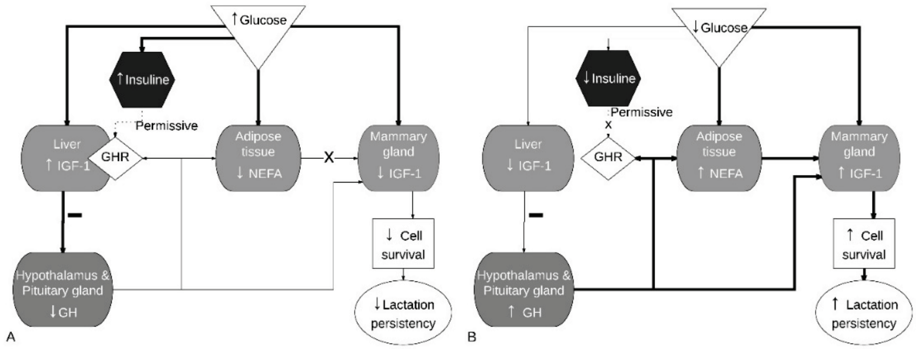

Figure 1.4: Interactions between lactation persistency and metabolites in cows in positive energy balance (A) or negative energy balance (B). The width of lines represents the direction and magnitude of flow of metabolites between tissues. GHR = growth-hormone receptors. IGF-1 = insulin-like growth factor 1. NEFA = non-esterified fatty acids. GH = growth hormone.

The greater energy balance in cows with a shortened or omitted dry period is likely related with a greater plasma insulin concentration, which leads to energy storage in body reserves, and a lower plasma growth hormone concentration, which is the key driver of milk production and lactation persistency. Management factors such as dietary energy level or dietary source may elevate the plasma GH concentration in cows with a 0-d dry period and stimulate lactation persistency and prevent fattening.

1.3.3 Effects of dietary energy level and source on fattening and lactation curve characteristics in mid- and late- lactation

The correlation between the amount of energy fed and amount of milk produced is strong (r^2=0.94 – 0.99, depending on energy density of the ration) (**Friggens *et al.***, 1995). Cows that were relatively underfed produced less milk than cows that were fed a high energy ration in early lactation (**Friggens *et al.***, 1995). Moreover, feeding a dietary energy level below the requirement for milk production in early- or mid-lactation negatively affected persistency (**Chilliard**, 1992). In contrast, greater energy intake in a pasture-based system throughout an extended lactation was associated with more often spontaneously dried off before the intended drying off day, compared with cows fed a control ration (**Grainger *et al.***, 2009). Adjusting dietary energy level to the lower milk yield of cows with a 0-d dry period or feeding a lipogenic ration, compared with a more glucogenic ration, may lower the plasma glucose and insulin concentration, and elevate plasma GH concentration. Lower plasma insulin and greater plasma GH concentration may potentially increase partitioning of energy towards milk yield and lactation persistency, and reduce fattening of cows after a 0-d dry period.

Feeding different dietary energy sources, such as a glucogenic or lipogenic ration, was previously suggested to affect milk production and metabolic status (**Drackley**, 1999). Feeding a lipogenic ration may increase lactation persistency (**Cannas *et al.***, 2002). In mid- and late- lactation, a lipogenic ration did not affect milk yield, lactose or protein yield, but was associated with increased milk fat content, more energy partitioned to milk, decreased plasma glucose concentration, and lower BCS (**Mahjoubi *et al.***, 2009). Lipogenic ingredients do not provide glucose precursors which results in decreased plasma insulin and IGF-1 concentration. A low plasma IGF-1 concentration does not inhibit release of GH from the pituitary gland, which results in a high plasma GH concentration. Additionally, feeding lipogenic ingredients that limit the formation of propionate in the rumen, or feeding ingredients that limit absorption of glucose from the small intestine (e.g., ingredients low in rumen bypass starch) is expected to reduce plasma glucose concentration. A low plasma glucose concentration reduces the plasma insulin concentration and glucose channeling to muscle and adipose tissue which prevents fattening (**NRC**, 2001). In previous studies, feeding a lipogenic ration resulted in a lower plasma glucose (**Chen *et al.***, 2016c), insulin (**Van Knegsel *et al.***, 2007a; **Van Knegsel *et al.***, 2007b; **Chen *et al.***, 2016c), and IGF-1 concentration (**Chen *et al.***, 2016c) in early lactation compared with a more glucogenic ration. Also in mid- and late-lactation, a lipogenic ration reduced the plasma glucose and insulin concentration compared with a more glucogenic ration (**Voelker and Allen**, 2003; **Mahjoubi *et al.***, 2009). It can be hypothesized that feeding a lipogenic ration from peak milk yield onwards would lower the insulin and IGF-1 concentration, and reduce fattening of cows without a dry period. Additionally, it can be hypothesized that feeding a lipogenic ration increases plasma GH concentration after peak yield that would increase milk production and lactation persistency. Greater lactation persistency could possibly compensate for the lower postpartum milk yield of cows with an omitted dry period.

1.3.4 The relationship between metabolic status and behavior

Diseases often occur in early lactation, coinciding with a period of negative energy balance (**Collard *et al.***, 2000; **Butler**, 2003; **Ingvartsen *et al.***, 2003). Plasma metabolites and hormones can be an indicator for metabolic status, but require a more invasive method for collection and determination than taking behavioral observations. Moreover, also changes in lying, walking, and feeding behavior of dairy cows may be an indicator for metabolic status or diseases (**Edwards and Tozer**, 2004). Behavioral indicators for metabolic status would allow earlier observation of a detrimental metabolic status. A previous study reported that cows with a lower plasma FFA concentration had a greater walking activity postpartum, compared with cows with a greater plasma FFA concentration (**Adewuyi *et al.***, 2006). To our knowledge, the relation between behavior and other plasma metabolites and hormones was not described previously. Better insight in the relation of metabolites and hormones with behavior may improve interpretation of behavior with respect to metabolic status in early lactation and could possibly lead to earlier intervention in case of metabolic disease.

1.4 Effects of dry period length on udder health

1.4.1 Relationships between metabolic status and udder health

Metabolic status plays an important role in mastitis incidence (**Ingvartsen and Moyes**, 2013). Cows with subclinical ketosis during the first 2 wks postpartum had a 9.5 times greater odds of developing clinical mastitis during that period than cows without subclinical ketosis (**Suthar *et al.***, 2013). Moreover, post-partum ketotic cows, with a high β-hydroxybutyrate (**BHB**) and high free fatty acid (**FFA**) concentration, and a low plasma glucose concentration, had more severe mastitis, indicated by greater bacterial growth in the quarter inoculated with *Escherichia coli*, than non-ketotic cows in wk 3 – 6 of lactation (**Kremer *et al.***, 1993). Greater FFA concentration stimulates phagocytosis-associated oxidative burst activities of polymorph nuclear leucocytes and may lead to cell and tissue damage (**Scalia *et al.***, 2006). Phagocytosis itself was not affected (**Scalia *et al.***, 2006; **Moyes *et al.***, 2009; **Ingvartsen and Moyes**, 2013). The rapid mobilization of lipids post-partum leads to a greater incidence of mastitis due to a reduced cell viability and increased oxidative stress (**Ingvartsen and Moyes**, 2013). The number of leucocytes in plasma are lower during an episode of ketosis (**Suriyasathaporn *et al.***, 2000). During negative energy balance, leucocytes also have a slower response in producing chemo attractants, a slower endothelial migration and a lower production of superoxide anions (**Suriyasathaporn *et al.***, 2000). Therefore, reducing the effects of negative energy balance through shortening or omitting the dry period may improve udder health during the subsequent lactation.

1.4.2 Somatic cell count and intramammary infections

Contrasting results have been reported on the effects of dry period length on somatic cell count (**Annen *et al.***, 2004). Studies showed that following a 30-d or 0-d dry period, SCC in the subsequent lactation tended to decrease (**Gulay *et al.***, 2003; **Pinedo *et al.***, 2011), was not affected (**Rastani *et al.***, 2005; **Church *et al.***, 2008; **Watters *et al.***, 2008; **Bernier-Dodier *et al.***, 2010), or tended to increase compared with a 60-d dry period (**Kuhn *et al.***, 2006; **Pezeshki *et al.***, 2007; **Steeneveld *et al.***, 2013). Additionally, postpartum SCC was greater during both of two consecutive lactations without a dry period, compared with cows with a dry period (**Chen *et al.***, 2016b).

In previous studies, a 30-d dry period tended to reduce the number of new intramammary infections (**IMI**) postpartum (**Natzke *et al.***, 1975; **Pezeshki *et al.***, 2007; **Pinedo *et al.***, 2011), but other studies indicated no effect of dry period length on clinical mastitis in the subsequent lactation compared with a 60-d dry period (**Church *et al.***, 2008; **Watters *et al.***, 2008; **Shoshani *et al.***, 2014). To our knowledge, bacterial culture on quarter milk samples of cows after different dry period lengths was not performed yet. Expanding determination of udder health using average SSC in milk with parameters such as occurrence of elevations of SCC, cases of clinical mastitis, or bacterial culture of minor and major mastitis pathogens, may provide a more accurate view on udder health of cows with different dry period lengths.

Altogether, findings in literature are contradicting and no conclusions can be drawn on the effect of dry period length on udder health in general or on SCC specifically. In most studies, cows with conventional of shortened dry periods were preventively treated with dry cow antibiotics, whereas cows where the dry period was omitted where not treated (**Gulay *et al.***, 2003; **Rastani *et al.***, 2005; **Kuhn *et al.***, 2006; **Pezeshki *et al.***, 2007; **Church *et al.***, 2008; **Watters *et al.***, 2008; **Bernier-Dodier *et al.***, 2010; **Pinedo *et al.***, 2011; **Santschi *et al.***, 2011b; **Steeneveld *et al.***, 2013; **Shoshani *et al.***, 2014). Thus, dry period length and use of dry cow antibiotics were confounded, and differences in SCC could not be attributed to either dry period length or use of antibiotics.

The value of SCC concentration in milk as a measure for udder health can be debated. Somatic cell count is increased through clinical or subclinical mastitis (**Sharma *et al.***, 2011), but also through increased prepartum and early postpartum cell apoptosis and proliferation (**Capuco *et al.***, 1997; **Fitzgerald *et al.***, 2007), and lower milk yield (**Green *et al.***, 2006).

Differences in postpartum udder health were earlier related with parity, calving month, occurrence of clinical mastitis in the previous lactation, SCC before drying-off, and milk yield during the days before drying-off (**Green *et al.***, 2007; **Pantoja *et al.***, 2009). Additionally, a previous study reported that differences in milk production after different dry period lengths was associated with prepartum cow characteristics parity, and daily milk yield and reduction in daily milk yield in mid- and late- lactation (**Steeneveld *et al.***, 2014). It could be hypothesized that also differences in udder health after different dry period lengths could be associated with prepartum cow characteristics. Recently, preventive use of dry cow antibiotics is not allowed anymore in some European countries, among which the Netherlands (**EC**, 2/2015 299/04; **Dutch M. o E. a.**, 2017). Effects of dry period length without use of dry cow antibiotics on udder health are yet unknown.

1.5 Aim and outline of this thesis

The main aims of this thesis were 1) to evaluate effects of dietary energy level and source during lactation on milk production, metabolic status, fattening, and lactation persistency in cows with different dry period lengths, 2) to evaluate relationships between metabolic status and cow behavior of cows in early lactation, 3) to study dry period lengths with and without use of dry cow antibiotics on udder health of cows with different dry period lengths, and 4) to identify prepartum cow characteristics that determine udder health after different dry period lengths.

To study these aims, two large experiments were conducted. Chapter 2, 3, 4, and 6 describe experiment 1. In this experiment, 123 cows were assigned randomly to a 0-d dry period (2/3 of cows) or a 30-d dry period (1/3 of cows). Cows with a 0-d dry period were fed either a (**LOW**) energy level, which was based on the requirement for their expected milk yield (35.4 kg FPCM/d) [**0-d DP(LOW)**] or a standard (**STD**) energy level [**0-d DP(STD)**] which was based on the energy level for the expected milk yield of cows with a 30-d dry period [**30-d DP(STD)**] (40.4 kg/d). Chapter 2 describes the effects of different (LOW or STD) energy levels after a 0 or 30 days dry period on milk production, feed intake, and plasma metabolites in wk 1 – 7 of lactation. In Chapter 3 we aimed to prevent fattening and improve lactation persistency by lowering plasma insulin and increasing plasma GH concentration through 1) adjusting dietary energy level to expected milk yield of cows with a 0-d dry period, and 2) feeding a lipogenic ration, compared with a more glucogenic ration from wk 8 in lactation onwards. Chapter 3 describes the effects of different dietary energy levels and energy sources after a 0 and 30 days dry period on lactogenic hormones, EB during a complete lactation, and lactation curve characteristics. Chapter 4 focusses on the relations of feeding behavior, lying, and steps with plasma metabolites and hormones in wk 4 of lactation.

Chapter 5 and 6 focus on udder health. Chapter 5 describes experiment 2, in which 167 cows were assigned randomly to a 0-d, 30-d, or 60-d dry period. At dry off, cows with a 30-d or 60-d dry period were treated with dry cow antibiotics. Chapter 5 describes the effects of dry period length, with use of dry cow antibiotics, on udder health and the individual cow characteristics that are associated with udder health after a 0, 30 or 60 days dry period. Recently, preventive use of dry cow antibiotics is not allowed anymore in European countries, among which the Netherlands (**EC**, 2/2015 299/04; **Dutch M. o E. a.**, 2017). Therefore, cows with a 30-d dry period in experiment 1 (Chapter 6), were not treated with dry cow antibiotics. Chapter 6 focusses on udder health across the dry period and in the subsequent lactation after a 0 or 30 days dry period without use of dry cow antibiotics. In chapter 7 results of this thesis are discussed. Specifically what cows are suitable for a 0-d or a 30-d dry period with regard to udder health in the subsequent lactation, and how these cows should be managed to optimize their performance in milk yield, udder health, metabolic status, and general health after a certain dry period length.

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CHAPTER 2

The effect of dry period length and postpartum level of concentrate on milk production, energy balance and plasma metabolites of dairy cows across the dry period and in early lactation

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2.1 Abstract

Shortening or omitting the dry period (**DP**) improves energy balance (**EB**) in early lactation, due to a reduction in milk yield. Lower milk yield results in lower energy demands and requires less energy intake. The aim of this study was to evaluate effects of DP length and concentrate level postpartum on milk yield, feed intake, EB and plasma metabolites between week -4 and 7 relative to calving of cows of second or higher parity. Holstein-Friesian dairy cows (N = 123) were assigned randomly to one of 2 DP lengths (0-d DP, n = 81 or 30-d DP, n = 42). Prepartum, cows with a 0-d DP received a lactation ration based on grass silage and corn silage (6.4 MJ net energy for lactation (NE)/kg dry matter (DM)). Cows with a 30-d DP received a dry cow ration based on grass silage, corn silage and straw (5.4 MJ NE/kg DM). Postpartum, all cows received the same basal lactation ration as provided to lactating cows prepartum. Cows with a 0-d DP were fed a low level of concentrate up to 6.7 kg/d based on requirement for their expected milk yield (0-d DP(LOW), n = 40) or the standard level of concentrate up to 8.5 kg/d (0-d DP(STD), n = 41), which was equal to the concentrate level for cows with a 30-d DP (30-d DP(STD), n = 42), based on requirements for their expected milk yield. Prepartum dry matter intake (**DMI**), concentrate intake, basal ration intake, energy intake, plasma β-hydroxybutyrate (**BHB**) and insulin concentrations were greater, plasma free–fatty-acids (**FFA**) and glucose concentrations were lower, but EB was not different of cows with a 0-d DP than of cows with a 30-d DP. During wk 1-3 postpartum, milk fat yield and plasma BHB concentration were lower, and DMI, and concentrate intake were greater in cows with a 0-d DP than in cows with a 30-d DP. During wk 4-7 postpartum, fat- and protein-corrected milk (**FPCM**), lactose content, and lactose and fat yield were lower in cows with 0-d DP(LOW) or 0-d DP(STD), compared with cows with a 30-d DP(STD). Basal ration intake, EB, BW, plasma glucose, insulin and IGF-1 concentrations were greater, and plasma FFA and BHB concentrations were lower in cows with a 0-d DP(LOW) or a 0-d DP(STD), compared with cows with a 30-d DP(STD). Concentrate and energy intake were lower in cows with a 0-d DP(LOW) than in cows with a 0-d DP(STD) or 30-d DP(STD). Milk yield and concentrations of plasma metabolites did not differ in week wk 4-7, although EB was lower in week 6 and 7 postpartum of cows with a 0-d DP(LOW), than of cows with a 0-d DP(STD). In conclusion, a 0-d DP reduced milk yield, and improved EB and metabolic status of cows in early lactation, compared with a 30-d DP. Reducing postpartum level of concentrate of cows with a 0-d DP did not affect FPCM yield or plasma FFA and BHB concentrations in early lactation, but did reduce EB in week 6 and 7 postpartum.

Key words: continuous milking, metabolic status, parity, dietary energy level

2.2 Introduction

A shortened or omitted dry period (**DP**) improved energy balance (**EB**) in early lactation compared with a conventional DP (**Rastani *et al.***, 2005; **Van Knegsel *et al.***, 2014). Moreover, the incidence of ketosis tended to be lower in cows with a shortened or omitted DP compared with cows with a conventional DP (**Santschi *et al.***, 2011b; **Van Knegsel *et al.***, 2013). Not only EB and health status were affected by DP length, but also plasma metabolite concentrations differed among cows with different DP lengths. Postpartum, cows with a omitted DP had a greater plasma glucose concentration and lower plasma free fatty acids (**FFA**) and β-hydroxybutyrate (**BHB**) concentrations than cows with a conventional DP (**Andersen *et al.***, 2005; **De Feu *et al.***, 2009; **Bernier-Dodier *et al.***, 2011).

The improvement of EB after an omitted DP was mostly due to a reduction in milk yield in the first 10 weeks (**Rastani *et al.***, 2005), 12 weeks (**De Feu *et al.***, 2009), or 14 weeks (**Van Knegsel *et al.***, 2014) of the subsequent lactation. More specifically, an omitted DP resulted in an 11% (**Rastani *et al.***, 2005), 12% (**Van Knegsel *et al.***, 2014), or 16% (**De Feu *et al.***, 2009) lower milk yield, compared with cows with a shortened DP. Additionally, omitting the DP, compared with a short DP, resulted in a larger reduction in milk yield in the subsequent lactation in cows with parity 2, compared with cows with parity ≥3 (**Annen *et al.***, 2004) (**Van Knegsel *et al.***, 2014). Lower milk yield results in lower energy demands and potentially requires less energy intake. To our knowledge, in all experimental studies that compared cows with different DP lengths, the same energy level was offered to cows in all experimental groups during lactation, and energy level was not adjusted to the lower expected milk yield after a short or omitted DP. Moreover, the lower milk yield of cows with a 0-d DP was related to an increase in BCS in mid and late lactation (**Chen *et al.***, 2016). It is unclear whether feeding less energy to cows with a 0-d DP, according to their requirement for expected milk yield, results in either an even lower milk yield or a decreased EB, or both. Performance of cows with a 0-d DP and a low energy level is relevant compared with both cows with a DP and an energy level for their expected milk yield, and cows with a 0-d DP and the same energy level as cows with a DP. In several earlier studies, a reduction of concentrate level was related with a lower total dry matter intake (**DMI**), energy intake, milk yield, and a lower EB and greater plasma BHB concentration in early lactation, although cows may increase roughage intake to compensate for a lower concentrate intake (**Reist *et al.***, 2003; **Kokkonen *et al.***, 2004; **Dieho *et al.***, 2016). According to **Reist *et al.*** (2003) a lower concentrate level also resulted in lower plasma glucose, insulin, IGF-1 and greater FFA concentration, while **Kokkonen *et al.*** (2004) suggested that FFA and insulin were not affected by a lower concentrate level. Additionally, no parity effect was observed for plasma IGF-1 and BHB concentration, but the plasma insulin concentration was greater and the plasma FFA concentration was lower in multiparous cows than in primiparous cows (**Meikle *et al.***, 2004). Cows in these studies most probably had a conventional DP length of approximately 60 days. It is unclear if there are differences in plasma metabolite concentrations among parities fed a lower concentrate level, following a 0-d DP. A lower energy intake of cows with a 0-d DP is of interest because it might reduce feed costs and is potentially beneficial for net herd returns, and may reduce the risk of over-conditioning of cows with a 0-d DP and is potentially beneficial for cow health and welfare, if EB is not negatively affected.

The aim of this study was to evaluate the effects of DP length and postpartum concentrate level on milk yield and composition, feed intake, EB and plasma metabolites between week -4 and week 7 relative to calving, of cows of second or higher parity.

2.3 Materials and methods

2.3.1 Animals, housing, and experimental design

The Institutional Animal Care and Use Committee of Wageningen University & Research (the Netherlands) approved the experimental protocol in compliance with the Dutch law on Animal Experimentation (protocol number 2014125). The experiment was conducted at the Dairy Campus research herd (Lelystad, the Netherlands) between January 27th 2014 and August 26th 2015. The research herd was composed of 400 lactating Holstein cows. Cows were selected based on 1) being bred with a Holstein sire, 2) expected calving interval <490 days, 3) daily milk yield >16 kg at 90 days before the expected calving date, and 4) no clinical mastitis or high SCC (>250,000 cells/mL) at final two test-days before drying off. In total, 141 cows entered the experiment, including 6 cows that entered twice. Eighteen cows were excluded, due to various reasons including Johne's disease (n = 1), a broken hip (n = 1), severe clinical leg or claw problems (n = 2), severe clinical mastitis (n = 2), acute death (n = 3), incorrect ration composition (n = 5), or an unplanned DP for cows assigned to a 0-d DP (n = 4). The final dataset consisted of 123 cows (58 with postpartum parity 2 and 65 with postpartum parity ≥3). To obtain a balanced distribution of cows across treatments, groups were made of 3 cows with similar expected calving date, milk yield in the previous lactation, and parity (2, ≥3) in the subsequent lactation. Within groups, cows were assigned randomly to a DP length of 0 days (2/3) or 30 days (1/3). Within the group of cows with a 0-d DP, cows were assigned randomly to either a low level of concentrate based on the requirement for their expected milk yield (**LOW**) or a standard (**STD**) level of concentrate based on the requirement for the expected milk yield of cows with a 30-d DP. This resulted in the following 3 treatments: cows with a 30-d DP fed the STD level of concentrate required for their expected milk yield (**30-d DP(STD)**) (n=42), cows with a 0-d DP fed the same STD concentrate level as cows with a 30-d DP (**0-d DP(STD)**) (n=41), and cows with a 0-d DP fed a LOW concentrate level (**0-d DP(LOW)**) (n=40). The LOW level of concentrate was calculated based on the expected milk yield of cows with a 0-d DP. This calculation was based on previous research showing that cows with a 0-d DP (35.4 kg FPCM/d) produced 12% less fat- and protein-corrected milk (**FPCM**), than cows with a 30-d DP (40.4 kg FPCM/d) (**Van Knegsel *et al.***, 2014). The experiment started 4 weeks prepartum and lasted until 7 weeks postpartum. Cows were housed in a freestall with a slatted floor and cubicles. Cows were milked twice daily at ~06:00 hours and ~18:00 hours. Disease incidence and treatment were recorded daily. Treated cows were kept in the experiment. The drying off protocol for cows with a 30-d DP consisted of a transition to the dry cow ration at day 7 before drying off and transition to milking once daily at day 4 before drying-off. At drying-off no dry cow antibiotics were used.

2.3.2 Rations

Prepartum, cows with a 0-d DP received a lactation ration that consisted of grass silage, corn silage, soybean meal, sugar beet pulp, wheat straw, urea, and vitamins and minerals (6.4 MJ net energy (**NE**)/kg DM) (**Table 2.1**). Lactating cows received 1 kg/d of standard concentrate in the milking parlor (**Table 2.2**). Cows with a 30-d DP received a dry cow ration that consisted of grass silage, corn silage, wheat straw, rapeseed meal, urea, and vitamins and minerals (5.4 MJ NE/kg DM). Postpartum, all cows received the same lactation basal ration up to

49 DIM as provided to the prepartum lactating cows including 1 kg/d of standard concentrate in the milking parlor. All cows received the experimental concentrate at a level of 1 kg/d from on average 10 days before the expected calving date (**Table 2.2**). The experimental concentrate increased stepwise by 0.3 kg/d from 4 DIM up to 8.5 kg/d at 28 DIM for cows receiving STD concentrate level (30-d DP(STD) and 0-d DP(STD)), or stepwise by 0.3 kg/d from 4 DIM up to 6.7 kg/d at 22 DIM for cows receiving the LOW concentrate level (0-d DP(LOW)). Rations were optimized aiming at a difference of 12% net energy intake through the basal ration and concentrate. Experimental concentrate was provided individually over 6 periods within 24 hours by a computerized feeder located in the freestall that was available to all cows at all times (Manus VC5, DeLaval, Steenwijk, the Netherlands). Concentrate was supplied separately from the basal ration. Cows had free access to water and the basal ration (dry cow or lactation) throughout the experiment. Rations were mixed once daily at ~10:00 h and fed twice daily at ~10:00 h and ~17:00 h. Dry matter content of basal rations was measured daily. Forage samples were taken weekly and stored at −20°C until analysis. Forage samples were pooled per batch (average 8 weeks; each batch of silage was harvested on the same day) for analysis of dry matter content, net energy (**NE**), intestinal digestible protein (**IDP**), and rumen-degradable protein balance (**RDPB**) (near-infrared spectrometry (NIRS); Eurofins Agro Laboratories, Wageningen, the Netherlands). Net energy was calculated using the Dutch net energy (**NE**) system for lactation (**VEM**), and IDP and RDPB were calculated according to the Dutch protein evaluation system (DVE/OEB-system) (**Tamminga *et al.***, 1994), based on NIRS values. Concentrate composition was available per batch (each batch covering 2 – 3 weeks) and used to calculate NE, IDP and RDPB per batch (median 3 weeks). Values for NE, IDP, and RDPB per batch of concentrate, rapeseed meal, and soybean meal were obtained from the manufacturer (Agrifirm, Apeldoorn, The Netherlands). Values for NE, IDP, and RDPB of wheat straw and urea were obtained from CVB Feed Tables 2011 (**CVB**, 2011). Forage and concentrate samples were pooled per 6 months period for analysis of DM [(**EC**, 152/2009), part III A], crude protein [Kjeldahl, (**EC**, 152/2009), L54/15], crude fat [(**EC**, 152/2009), part III method A], neutral detergent fiber [method Van Soest, (**ISO 13906**, 2008)], acid detergent fiber, and acid detergent lignin [method Van Soest, (**ISO 16472**, 2006)], starch [enzymatic, (**ISO 15914**, 2004)], sugar [glucose, (**EEC**, 1971) L155/ 29-32], and crude ash [(**EC**, 152/2009), L54/50]. Masterlab (Boxmeer, the Netherlands) conducted all analyses according to the listed ISO references and EC regulations.

Table 2.1: Ingredient and nutrient composition of the average dry cow ration and lactation ration

Ingredient (g/kg DM)	Dry cow ration	Lactation ration
Grass silage[1]	473	437
Corn silage[2]	181	338
Sugar beet pulp	-	97
Rapeseed meal	81	-
Soybean meal (High rumen bypass)[3]	-	84
Wheat straw	243	20
Urea[4]	7.2	3.7
Vitamins and minerals[5]	14	20
Nutrient composition (g/kg DM)		
DM (g/kg product)	547	418
Ash	88	82
Crude protein	114	137
Crude fat	32	33
NDF	528	419
ADF	316	237
ADL	35	20
Starch	70	124
Sugar	63	62
IDP[6]	53	79
RDPB[7]	8.1	7.8
NE (MJ/kg DM)[8]	5.4	6.4

[1]Chemical composition of grass silage (g/kg DM): DM 407 g/kg product, crude ash 115, crude protein 132, crude fat 42, NDF 484, ADF 281, ADL 19, sugar 113, IDP 66, RDPB 1.8, NE 6.2 MJ/kg DM. [2]Chemical composition of corn silage (g/kg DM) DM 361 g/kg product, crude ash 51, crude protein 65, crude fat 31, NDF 384, ADF 220, ADL 18, starch 352, sugar 3.7, IDP 48, RDPB 36, NE 6.8 MJ/kg DM. [3]Soybean meal was treated with formaldehyde. [4]Urea was fed in the dry cow ration between April 2015 and August 2015. Urea was added to the dry cow ration when crude protein content was lower than 150 g/kg DM or RDPB was lower than 0. [5]The composition of minerals and vitamins for the dry cow ration was: Ca 20 g/kg, P 30 g/kg, Na 34 g/kg, Cl 59 g/kg, Mg 250 g/kg, I 100 mg/kg, Cu 2200 mg/kg, Mn 3000 mg/kg, S total 25 g/kg, Zn 3300 mg/kg, Se 35 mg/kg, Co 35 mg/kg, Vitamin A 400000 IU, Vitamin D3 125000 IU, Vitamin E 10000 IU. The composition of minerals and vitamins (g/kg product) for the lactation ration was: Ca 155 g/kg, P 0 g/kg, Na 75 , Cl 116 g/kg, Mg 120 g/kg, S total 40 g/kg, I 100 mg/kg, Cu 1600 mg/kg, Mn 1500 mg/kg, Zn 1500 mg/kg, Se 34 mg/kg, Co 45 mg/kg, Vitamin A 500000 IU, Vitamin D3 65000 IU, Vitamin E 5000 IU. [6]IDP = Intestinal digestible protein, calculated according to the Dutch DVE/OEB-system (**Tamminga *et al.*,** 1994). [7]RDPB = rumen-degradable protein balance, calculated according to the Dutch DVE/OEB-system (**Tamminga *et al.*,** 1994). [8]NE = Net energy, calculated according to the Dutch VEM-system (**Van Es**, 1975). NE, IDP and RDPB values for grass silage, corn silage, and sugar beet pulp were based on near-infrared spectrometry (Blgg AgroXpertus, Wageningen, the Netherlands). Values for IDP, RDPB and NE of rapeseed meal and soybean meal were obtained from the manufacturer (Agrifirm, Apeldoorn, The Netherlands). Values for wheat straw and urea were obtained from CVB Feed Tables 2011 (**CVB,** 2011).

Table 2.2: Ingredient and nutrient composition of the standard and experimental concentrate

Ingredient (g/kg)	Standard concentrate	Experimental concentrate
Palm kernel, expeller	148	197
Corn	241	48
Lupine		2.9
Sugar beet pulp (max 12% sugar)	23	
Citrus pulp	157	132
Wheat	46	141
Wheat meal		1
Soybean hulls		186
Soybean meal	32	3.0
Soybean meal (High rumen bypass)	14	60
Rapeseed meal	94	50
Rapeseed meal (High rumen bypass)	87	77
Molasses-cane	29	39
Molasses-beet	12	
Protamylasse[1]	35	
Beet vinasses[2]	51	
Citrocol[3]	11	39
Linseed oil		2.0
Palm oil	2.6	3.9
Calcium carbonate	2.5	2.3
Sodium chloride	4.5	4.8
Magnesium oxide	4.4	4.5
Urea	4.5	3.0
Vitamin E	0.8	1.0
Selenium	1.8	2.0
Methionine source[4]	0.5	0.6
Nutrient composition (g/kg)		
DM (g/kg product)	887	869
Crude protein	173	173
Ash	56	58
Crude fat	42	41
NDF	255	337
ADF	139	205
ADL	41	43
Starch	208	118
Sugar	76	78
IDP[5]	119	123
RDPB[6]	22	16
NE (MJ/kg DM)[7]	7.7	7.4

[1]Protamylasse is potato juice concentrate. Protamylasse, Avebe, Veendam, The Netherlands. Chemical composition of protamylasse (g/kg DM): DM 548 g/kg product, crude ash 290, sugar 57, crude protein 327, IDP 52, RDPB 228, NE 6.2 MJ/kg DM. [2]Chemical composition of beet vinasses (g/kg product): DM 663, crude ash 174, crude protein 214, sugar 37, NDF 2, ADF 1, IDP 34, RDPB 148, NE 4.1 MJ/kg DM. [3]Citrocol (Citrique Belge, Tienen, Belgium) was used as a binding agent in the rations. [4]Methionine as 2-hydroxy-4-methylthiobutanoic acid isopropyl ester. MetaSmart dry, Adisseo, Antony, France. [5]IDP= Intestinal digestible protein, calculated according to the Dutch DVE/OEB-system (**Tamminga *et al.*,** 1994). [6]RDPB= rumen-degradable protein balance, calculated according to the Dutch DVE/OEB-system (**Tamminga *et al*.**, 1994). [7]NE= Net energy, calculated according to the Dutch VEM-system (**Van Es**, 1975). Values for IDP, RDPB and NE were obtained from the manufacturer (Agrifirm, Apeldoorn, The Netherlands).

2.3.3 Measurements

Milk yield and milk composition. Milk yield was recorded daily from 4 weeks prepartum until 7 weeks postpartum. Milk samples for fat, protein and lactose analysis (ISO 9622, Qlip, Zutphen, The Netherlands) were collected four times per week (Tuesday afternoon, Wednesday morning, Wednesday afternoon, and Thursday morning) and were analyzed as a pooled sample per cow per week. FPCM was calculated as:

FPCM = (0.337 + 0.116 × fat content + 0.06 × protein content) × milk yield (**CVB**, 2011).

Feed intake, body weight, body condition score and energy balance. Basal ration was provided and daily intake was measured individually using roughage intake control (RIC) troughs (Insentec, Marknesse, The Netherlands). The stocking density was 2 cows per trough. The actual quantity of concentrate dispensed (kg/d) was recorded. Body weight (BW) was recorded daily before each milking. Dry cows were weighed once per week. Body condition was scored every 4 weeks by the same person using a 1 to 5 scale (**Ferguson *et al.***, 1994).

Energy balance was calculated per week according to the Dutch VEM system for lactation (**Van Es**, 1975), as the difference between intake of VEM and the requirements of VEM for maintenance, milk yield, and pregnancy (1,000 VEM = 6.9 MJ of NE). According to the VEM system, the daily requirement for maintenance is 42.4 VEM/kg$^{0.75}$ BW, the requirement for milk yield is 442 VEM/kg FPCM, and the daily requirement for pregnancy is 2700 VEM in the last 4 weeks prepartum. Energy intake and EB are expressed in kJ/ kg$^{0.75}$ ·day (**Van Es**, 1975).

Blood collection and analysis. Blood was collected weekly from 3 weeks prepartum until 7 weeks postpartum. Blood samples were collected after the morning milking and between 3 and 1 hours before the morning feeding. Blood (10 mL) was collected from the coccygeal vein or artery into evacuated EDTA tubes (Vacuette, Greiner BioOne, Kremsmunster, Austria). Blood samples were kept on ice before centrifugation for plasma isolation (3,000 × *g* for 15 min, 4°C). Plasma samples were stored at −20°C. Concentrations of FFA and BHB were measured enzymatically using kit no. 994–75409 from Wako Chemicals (Neuss, Germany) and kit no. RB1007 from Randox Laboratories (Ibach, Switzerland,) respectively (**Graber *et al.***, 2012). The plasma glucose concentration was measured using kit no. 61269 from BioMerieux (Marcy l'Etoile, France) (**Graber *et al.***, 2012). The plasma insulin concentration was measured using kit no. PI-12K from EMD Millipore Corporation (Billerica, MA, USA). The plasma IGF-1 concentration was measured using kit no. A15729 from Beckman Coulter (Fullerton, CA, USA).

2.3.4 Statistical analyses

Preliminary analysis showed that repeated use of the 6 cows in this experiment did not affect the results of this study. Statistical analyses were performed using a repeated measures analyses in a mixed linear model [PROC MIXED, SAS version 9.3] (**SAS Institute Inc.**, 2011) with cow as the subject, considered as a random effect, for repeated weekly observations on individual cows. A first-order autoregressive covariance matrix was the best fit according to Akaike's corrected information criterion and was used to account for within-cow variation.

Data were analyzed separately for 3 periods of the experiment: prepartum, week 1 to 3 postpartum, and week 4 to 7 postpartum. Prepartum and during week 1 to 3 postpartum, feed intake, EB, and plasma metabolites were analyzed for fixed effects of treatment (0-d DP or 30-d DP), postpartum parity (2, ≥3), week relative to calving (week -4 to -1, or week 1 to 3), and their two-way interaction effects. For week 4 to 7 postpartum, milk production, milk composition, feed intake, EB, and plasma metabolites were analyzed for fixed effects of treatment (0-d DP(LOW), 0-d DP(STD) or 30-d DP(STD)), postpartum parity (2, ≥3), week relative to calving (week 4 to 7), and their two-way interaction effects. Body condition score was analyzed using the same model but with month relative to calving rather than week relative to calving. Cows were blocked for calving date, milk yield in the previous lactation, and parity (2, ≥3) in the subsequent lactation. Block was included in all analyses as a random effect. All pairwise differences of LSmeans for transition treatments and parities were assessed per week. The natural logarithm was calculated for plasma BHB and FFA concentration to approximate normality. Results are presented as least square means with their SEM, unless otherwise stated.

2.4 Results

Between week 1 and 7 postpartum, 5 cows were treated for milk fever (3x 0-d DP(LOW), 2x 30-d DP(STD)), 3 cows were treated for left displaced abomasum (1x 0-d DP(LOW), 2x 30-d DP(STD)), 2 cows were treated for ketosis (1x 0-d DP(LOW), 1x 0-d DP(STD)), 19 cows were treated for clinical mastitis (6x 0-d DP(LOW), 7x 0-d DP(STD), 6x 30-d DP(STD)), 12 cows were treated for retained placenta (3x 0DPLOW, 5x 0DPSTD, 4x 30DPSTD), 15 cows were treated for chronic endometritis (2x 0DPLOW, 7x 0DPSTD, 6x 30DPSTD), and 5 cows were treated for pyometra (2x 0DPLOW, 1x 0DPSTD, 2x 30DPSTD.

2.4.1 Milk yield

Prepartum, cows with a 0-d DP produced 11.3 ± 0.6 kg/d of milk or 13.9 ± 0.7 kg/d of FPCM (**Table 2.3**). Total milk yield was 323 ± 17 kg for cows with a 0-d DP in the 4 weeks prepartum. Milk yield, FPCM yield, lactose content, and lactose, fat, and protein yield were greater and protein content was lower for cows with postpartum parity 2, than for cows with parity ≥ 3 ($P < 0.01$). There was an interaction between parity and week relative to calving for milk yield, FPCM yield, lactose, fat and protein content, and lactose, fat and protein yield. Milk and FPCM yield and lactose content were greatest for cows with parity 2 at 4 weeks prepartum (17.2 ± 0.8 kg/d; 20.4 ± 0.9 kg/d; 4.73 ± 0.06%), and lowest for cows with parity ≥ 3 at 1 week prepartum (5.7 ± 0.9 kg/d; 7.4 ± 1.2 kg/d; 3.56 ± 0.13%) ($P < 0.01$).

During week 1 to 3 postpartum, milk fat yield was lower for cows with a 0-d DP, compared with cows with a 30-d DP ($P < 0.01$; **Table 2.4a**). During week 1 to 3, the effect of DP length on milk yield (**Figure 2.1a**), milk protein content (**Figure 2.1b**), and lactose yield (**Figure 2.1c**) depended on parity. Milk lactose content was lower for cows with parity ≥3, compared with cows with parity 2 ($P < 0.05$). Milk and lactose yield were greater and milk protein content was lower for cows with parity ≥3 after a 0-d DP compared with parity 2 ($P < 0.05$), while the effect of parity was not present for cows after a 30-d DP ($P \geq 0.05$).

During week 4 to 7 postpartum, FPCM yield, milk lactose content, and milk lactose and fat yield were lower for cows with a 0-d DP(LOW) or 0-d DP(STD), than for cows with a 30-d DP(STD) ($P < 0.05$; **Table 2.4b**). During week 4 to 7, the effect of DP length on milk yield (**Figure 2.1a**), milk protein content (**Figure**

2.1b), and milk fat content (**Figure 2.1d**) depended on parity. Milk lactose content was lower for cows with parity ≥3, compared with cows with parity 2 ($P < 0.05$). Milk and lactose yield was greater and milk protein content was lower for cows with parity ≥3 after a 0-d DP(LOW) or 0-d DP(STD) compared with parity 2 ($P < 0.05$), while the effect of parity was not present for cows after a 30-d DP(STD) ($P \geq 0.05$). Milk fat content was lower for cows with parity ≥3, compared with parity 2, after a 0-d DP(LOW) or 0-d DP(STD) ($P < 0.05$), while the effect of parity was not present for cows after a 30-d DP ($P \geq 0.05$). During week 4 to 7, there was an interaction between treatment and week relative to calving for milk yield and FPCM yield (**Figure 2.2a**), and milk fat yield (**Figure 2.2b**) ($P < 0.05$).

Table 2.3: Prepartum[1] milk yield and milk composition of cows with a 0 days dry period (LSM ± SEM)

	Dry period length		Parity			*P*-value[2]		
	0	SEM	2	≥3	SEM	P	W	P × W
Cows (n)	81		40	41				
Milk yield (kg/d)	11.3	0.6	13.5	9.1	0.9	<0.01	<0.01	<0.01
FPCM[3] (kg/d)	13.9	0.7	16.6	11.2	1.0	<0.01	<0.01	<0.01
Lactose (%)	4.37	0.05	4.67	4.08	0.08	<0.01	<0.01	<0.01
Fat (%)	5.20	0.08	5.36	5.05	0.11	0.06	0.03	<0.01
Protein (%)	5.61	0.13	5.25	5.97	0.18	<0.01	<0.01	<0.01
Lactose (kg/d)	0.52	0.03	0.64	0.40	0.04	<0.01	<0.01	0.01
Fat (kg/d)	0.59	0.03	0.71	0.46	0.04	<0.01	<0.01	0.01
Protein (kg/d)	0.55	0.03	0.63	0.47	0.04	<0.01	<0.01	0.02

[1]Prepartum = week -4 to -1 before calving. [2]P = Parity; W= Week relative to calving. [3]FPCM = Fat- and protein- corrected milk.

Table 2.4a: Postpartum[1] milk yield and milk composition during week 1 to 3 of cows after a 0 or 30 days dry period. Cows with a 0-d DP were provided either a low (0-d DP(LOW)) or the standard (0-d DP(STD)) level of concentrate that cows with a 30-d DP received (30-d DP(STD)) (LSM ± SEM)

WK 1-3	Dry period length			Parity			*P*-value[2]					
	0-d DP	30-d DP	SEM	2	≥3	SEM	DP	P	W	DP × P[4]	DP × W	P × W
Cows (n)	81	42		58	65							
Milk yield (kg/d)	23.2	26.5	1.4	24.9	24.8	1.4	<0.01	0.93	<0.01	<0.01	0.24	0.56
FPCM[3] (kg/d)	27.6	31.4	1.5	29.4	29.7	1.5	<0.01	0.83	<0.01	0.03	0.98	0.48
Lactose (%)	4.39	4.44	0.04	4.48	4.36	0.04	0.31	0.02	<0.01	0.48	0.24	0.28
Fat (%)	5.08	5.14	0.09	5.07	5.14	0.09	0.65	0.60	<0.01	0.14	0.19	0.17
Protein (%)	4.42	4.14	0.09	4.40	4.17	0.09	0.02	0.02	<0.01	<0.01	0.25	0.02
Lactose (kg/d)	1.02	1.18	0.07	1.13	1.08	0.07	<0.01	0.49	<0.01	<0.01	0.12	0.12
Fat (kg/d)	1.20	1.40	0.08	1.28	1.32	0.07	<0.01	0.54	<0.01	0.08	0.60	0.33
Protein (kg/d)	1.00	1.06	0.04	1.04	1.02	0.04	0.08	0.77	<0.01	0.09	0.14	0.12

[1]Postpartum = week 1 to 3 after calving. [2]DP = Dry period length; P = Parity; W = Week relative to calving. [3]FPCM = Fat- and protein-corrected milk. [4]Interactions of treatment with parity (T × P) ($P < 0.05$) are shown in Figure 2.1.

Tabel 2.4b: Postpartum[1] milk yield and milk composition during week 4 to 7 of cows after a 0 or 30 days dry period. Cows with a 0-d DP were provided either a low (0-d DP(LOW)) or the standard (0-d DP(STD)) level of concentrate that cows with a 30-d DP received (30-d DP(STD)) (LSM ± SEM)

WK 4-7	Treatment[2]				Parity			*P*-value[3]					
	0-d DP(LOW)[1]	0-d DP(STD)	30-d DP(STD)	SEM	2	≥3	SEM	T	P	W	T × P[5]	T × W	P × W
Cows (n)	40	41	42		58	65							
Milk yield (kg/d)	30.4^{a}	30.7^{a}	36.4^{b}	1.6	31.6	33.4	1.5	<0.01	0.23	<0.01	0.03	<0.01	0.33
FPCM[4] (kg/d)	33.9^{a}	33.5^{a}	38.4^{b}	1.5	34.6	35.9	1.4	<0.01	0.35	0.02	0.18	<0.01	0.56
Lactose (%)	4.59^{a}	4.57^{a}	4.65^{b}	0.02	4.63	4.57	0.02	0.01	0.01	0.09	0.68	0.59	0.20
Fat (%)	4.66^{a}	4.46^{ab}	4.36^{b}	0.09	4.60	4.39	0.07	0.01	0.02	<0.01	0.02	0.51	0.14
Protein (%)	3.78^{a}	3.77^{a}	3.40^{b}	0.08	3.73	3.57	0.07	<0.01	0.03	<0.01	<0.01	0.94	0.99
Lactose (kg/d)	1.39^{a}	1.39^{a}	1.69^{b}	0.07	1.46	1.52	0.07	<0.01	0.41	<0.01	0.06	0.06	0.46
Fat (kg/d)	1.46^{a}	1.41^{a}	1.63^{b}	0.06	1.47	1.53	0.06	<0.01	0.32	0.55	0.41	<0.01	0.26
Protein (kg/d)	1.14	1.15	1.23	0.04	1.16	1.18	0.04	0.06	0.68	<0.01	0.60	0.14	0.61

[a-b]Values with different superscript differ ($P < 0.05$). [1]Postpartum = week 4 to 7 after calving. [2]Treatment: Cows with a 0-d DP were fed either a low (0-d DP(LOW)) level of concentrate, based on their requirement for their expected milk yield, or a standard (0-d DP(STD)) level of concentrate that cows with a 30-d DP (30-d DP(STD)) received based on the requirement for their expected milk yield. [3]T = Treatment; P = Parity; W = Week relative to calving. [4]FPCM = Fat- and protein- corrected milk. [5]Interactions of treatment with parity (T × P) ($P < 0.05$) are shown in Figure 2.1.

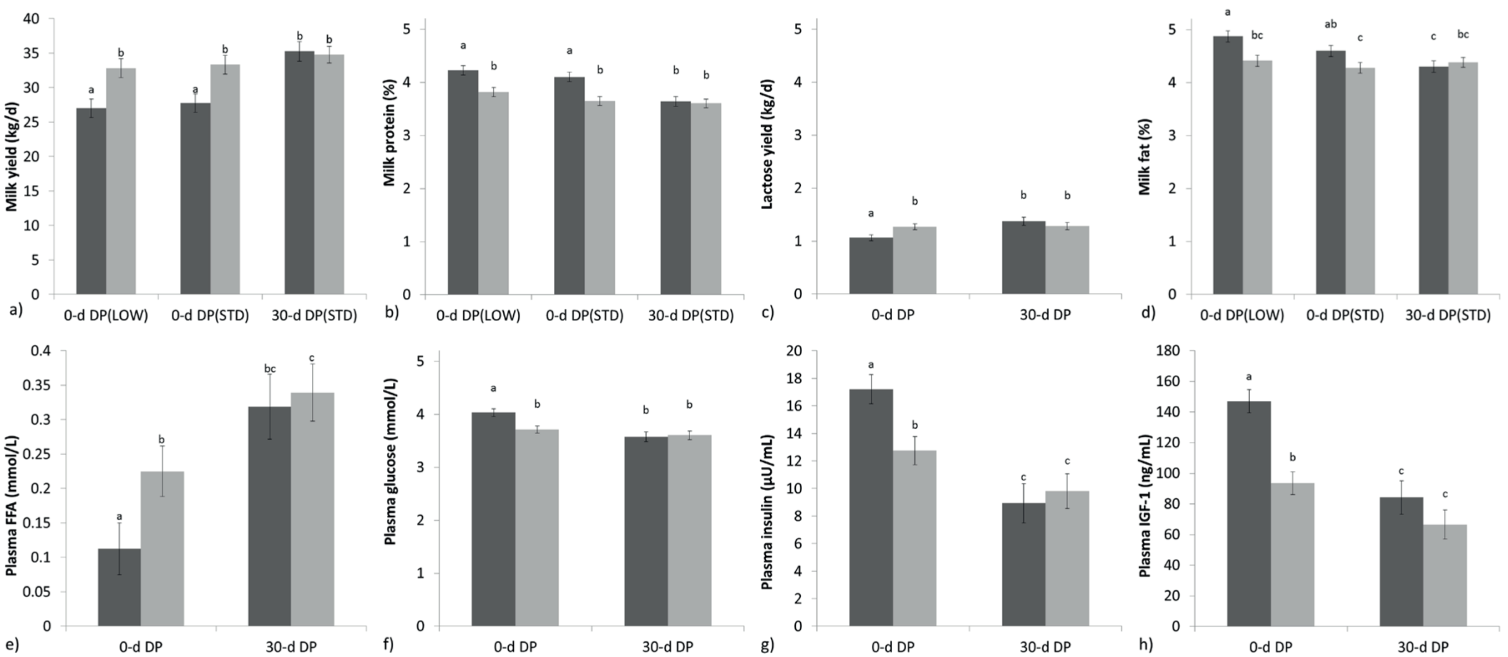

Figure 2.1: Interaction between postpartum treatment (0-d DP or 30-d DP during week 1 to 3, or 0-d dry period (DP) and a low level of concentrate based on the requirement for their expected milk yield, [0-d DP(LOW)], or a 0-d DP and standard level of concentrate, [0-d DP(STD)], or a 30-d DP and a standard level of concentrate based on the requirement for their expected milk yield, [30-d DP(STD)] between week 4 to 7) and postpartum parity (2 in dark grey or ≥ 3 in light grey) for milk yield during wk 1-3 and 4-7 postpartum (a), milk protein content during wk 1-3 and 4-7 postpartum (b), lactose yield during wk 1-3 postpartum (c), milk fat content during wk 4-7 postpartum (d), plasma FFA concentration during wk 1-3 postpartum (e), plasma glucose concentration during wk 1-3 postpartum (f), plasma insulin concentration during wk 1-3 postpartum (g), and plasma IGF-1 concentration during wk 1-3 postpartum (h). Values with different letters differ ($P < 0.05$). Results were presented as LSM ± SEM.

2

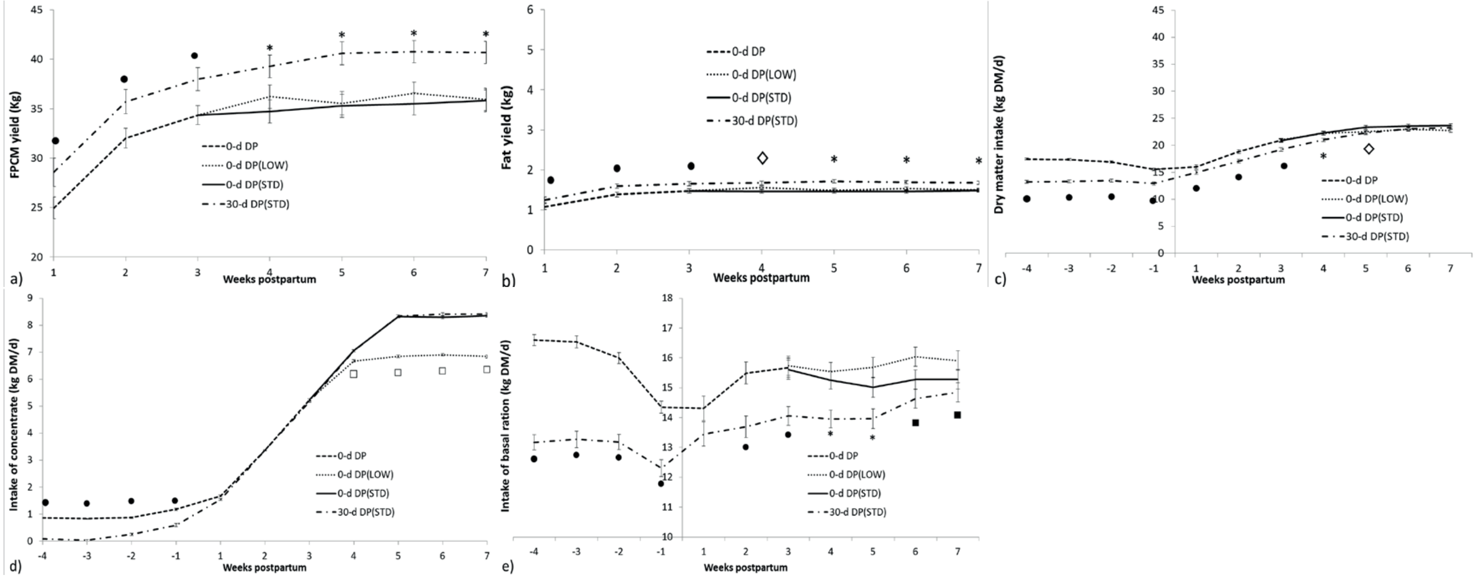

Figure 2.2: The effect of treatment (0-d dry period (DP) and a low level of concentrate, based on the requirement for their expected milk yield, 0-d DP(LOW); a 0-d DP and standard level of concentrate, 0-d DP(STD); and a 30-d DP and a standard level of concentrate, based on the requirement for their expected milk yield, 30-d DP(STD)) on fat- and protein- corrected milk (FPCM) yield (a), fat yield (b), dry matter intake (c), concentrate intake (d), and intake of basal ration (e). 0-d DP represents the average of both 0-d DP(LOW) and 0-d DP(STD) prepartum. ● 0-d DP differs from 30-d DP, * 30-d DP(STD) differs from 0-d DP(LOW) and 0-d DP(STD), ◊ 30-d DP(STD) differs from 0-d DP(STD), but not from 0-d DP(LOW), ■ 0-d DP(LOW) differs from 30-d PP(STD), but not from 0-d DP(STD), □ 0-d DP(LOW) differs from 0-d DP(STD), but not from 30-d DP(STD). Data were analyzed separately for 3 periods of the experiment: prepartum, week 1 to 3 postpartum, and week 4 to 7 postpartum. Results were presented as LSM ± SEM.

2.4.2 Dry matter intake, energy balance, metabolites, and disease

Prepartum, DMI, concentrate intake, basal ration intake, and energy intake expressed per kg metabolic body weight, were lower for cows with a 30-d DP, than for cows with a 0-d DP (*P* < 0.01; **Table 2.5**). Plasma FFA and glucose concentrations were lower of cows with a 0-d DP, than of cows with a 30-d DP (*P* < 0.01). Plasma BHB and insulin concentrations were greater of cows with a 0-d DP, than of cows with a 30-d DP (*P* < 0.05). Dry matter intake, basal ration intake, and BW were lower for cows with parity 2, than for cows with parity ≥ 3 (*P* < 0.05). Energy intake, expressed per kg metabolic body weight, was greater for cows with parity 2, than of cows with parity ≥ 3 (*P* < 0.01). The plasma insulin concentration was lower of cows with parity 2, than of cows with parity ≥ 3 (*P* < 0.05). Energy balance was not different between DP lengths, or between parities (*P* ≥ 0.05). The interaction of DP length with week relative to calving was present for DMI (**Figure 2.2e**), concentrate intake (**Figure 2.2f**), basal ration intake (**Figure 2.2g**), and energy intake (**Figure 2.3a**), BW, BCS, and the plasma BHB concentration (**Figure 2.3c**; *P* < 0.05).

During week 1 to 3 postpartum, DMI, concentrate intake, basal ration intake, and energy intake were greater, of cows with a 0-d DP, compared with cows with a 30-d DP (*P* < 0.01; **Table 2.6a**). The plasma BHB concentration was lower of cows with a 0-d DP, compared with cows with a 30-d DP (*P* < 0.01). Energy intake was greater, but BW was lower of cows with parity 2, compared with cows with parity ≥ 3 (*P* < 0.01). During week 1 to 3 postpartum, the effect of DP length on EB, the plasma FFA (**Figure 2.1e**), glucose (**Figure 2.1f**), insulin (**Figure 2.1g**), and IGF-1 (**Figure 2.1h**) concentration depended on parity. The plasma FFA concentration was greater of cows with parity ≥3 after a 0-d DP compared with parity 2 (*P* < 0.05), while the effect of parity was not present for cows after a 30-d DP (*P* ≥ 0.05). The plasma glucose, insulin, and IGF-1 concentration was lower of cows with parity ≥3 after a 0-d DP compared with parity 2 (*P* < 0.05), while the effect of parity was not present for cows after a 30-d DP (*P* ≥ 0.05). Energy intake was greater, but BW was lower for cows with parity 2, compared with cows with parity ≥ 3 (*P* < 0.01). During week 1 to 3 postpartum, there was an interaction of treatment with week relative to calving for DMI (**Figure 2.2c**), concentrate intake (**Figure 2.2d**), basal ration intake (**Figure 2.2e**), BW, and the plasma BHB (**Figure 2.3a**) and IGF-1 concentration (**Figure 2.3b**) (*P* < 0.05). The interaction of parity with week relative to calving revealed that BW decreased with week relative to calving in cows with parity ≥ 3, and increased with week relative to calving in cows with parity 2 between week 1 and 3 ($P < 0.01$).

During week 4 to 7 postpartum, concentrate and energy intake were lower of cows with a 0-d DP(LOW), than of cows with a 0-d DP(STD) or a 30-d DP(STD) (**Table 2.6b**). Basal ration intake, EB, BW, and the plasma glucose, insulin and IGF-1 concentration were greater, and the plasma FFA concentration was lower of cows with a 0-d DP(LOW) or a 0-d DP(STD), compared with cows with a 30-d DP(STD) ($P < 0.05$). Energy intake, and the plasma glucose and IGF-1 concentration were greater, but DMI, basal ration intake, BW, and the plasma BHB concentration were lower of cows with parity 2, than of cows with parity ≥ 3 ($P < 0.05$). During week 4 to 7, there was an interaction between treatment with week relative to calving for DMI (**Figure 2.2c**), concentrate intake (**Figure 2.2d**), energy intake (**Figure 2.3c**), and EB (**Figure 2.3d**) ($P < 0.05$).

2.5 Discussion

Reducing the level of concentrate for cows with a 0-d DP did not affect EB in early lactation in week 4 and 5 postpartum, compared with cows with a standard concentrate level and a 0-d DP. After the concentrate contrast was complete (from week 5 postpartum onwards), EB of cows with a 0-d DP(LOW) was lower in week 6 and 7 postpartum than cows with a 0-d DP(STD). In the current study, the lower concentrate intake of cows with a 0-d DP(LOW) did not affect average DMI or NE intake in week 4 to 7 postpartum. When the concentrate contrast was complete, cows with 0-d DP(LOW) consumed 19% less concentrate (6.8 ± 0.0 kg/d DM vs. 8.4 ± 0.0 kg/d DM), which resulted in a 5% lower energy intake (1151 ± 14 kJ/ $kg^{0.75}$·d vs. 1216 ± 14 kJ/ $kg^{0.75}$·d), compared with cows with a 0-d DP(STD). In earlier studies, a lower concentrate level resulted in lower DMI and lower NE intake (**Reist *et al.***, 2003; **Kokkonen *et al.***, 2004; **Zbinden *et al.***, 2016). The differences in concentrate level between treatment groups in these studies were as large as 20% of individual requirements. These studies showed that reduction of concentrate level resulted in a small reduction in energy-corrected milk (**Reist *et al.***, 2003; **Kokkonen *et al.***, 2004). In the current study, when the concentrate contrast was complete, milk yield, FPCM yield and yield of milk components were not different between cows with a 0-d DP(LOW) or 0-d DP(STD).

Table 2.5: Prepartum[1] dry matter intake, energy balance and plasma metabolites of cows with a 0 or 30 days dry period (LSM ± SEM)

	Dry period length			Parity			*P*-value[2]					
	0	30	SEM	2	≥3	SEM	DP	P	W	DP × P	DP × W	P × W
Cows (n)	81	42		58	65							
DMI (kg DM/d)	16.8	13.2	0.2	14.7	15.4	0.2	<0.01	0.02	<0.01	0.85	<0.01	<0.01
Concentrate (kg DM/d)	0.9	0.2	0.02	0.6	0.6	0.02	<0.01	0.35	<0.01	0.32	<0.01	0.01
Basal ration[3] (kg DM/d)	15.9	13.0	0.2	14.1	14.8	0.2	<0.01	0.02	<0.01	0.98	<0.01	<0.01
EI[4] ($kJ/kg^{0.75}\cdot d$)	786	529	9	676	639	9	<0.01	<0.01	<0.01	0.39	0.02	0.09
EB[5] ($kJ/kg^{0.75}\cdot d$)	208	234	15	212	230	15	0.23	0.41	<0.01	0.10	0.06	0.13
BW (kg)	717	702	8	660	759	8	0.19	<0.01	<0.01	0.34	<0.01	0.30
BCS[6]	3.1	3.2	0.07	3.1	3.3	0.07	0.31	0.13	0.99	0.41	0.02	0.26
FFA[7,8] (mmol/L)	0.08 (0.07-0.10)	0.13 (0.11-0.16)		0.11 (0.09 – 0.13)	0.10 (0.09 – 0.12)		<0.01	0.67	<0.01	0.65	0.90	0.07
BHB[7,8] (mmol/L)	0.60 (0.58 – 0.64)	0.47 (0.44 – 0.50)		0.53 (0.50 – 0.57)	0.53 (0.50 – 0.56)		<0.01	0.85	0.23	0.19	0.01	0.06
Glucose[7] (mmol/L)	4.06	4.30	0.06	4.25	4.12	0.06	<0.01	0.15	0.71	0.64	0.23	0.90
Insulin[7] (μU/mL)	18.3	15.3	0.9	15.4	18.1	0.1	0.03	0.04	0.03	0.39	0.79	0.04
IGF-1[7] (ng/mL)	184.5	191.6	7.2	196.9	189.2	7.3	0.49	0.09	<0.01	0.64	0.10	0.88

[1]Prepartum = week -4 to -1 before calving. [2]DP = Dry period length; P = Parity; W = Week relative to calving. [3]The basal ration was a dry cow ration for cows with a 30-d DP and a lactation ration for cows with a 0-d DP. [4]EI = Energy intake. [5]EB = Energy balance. [6]BCS = Body condition score; calculated without a repeated measures effect, but with month relative to calving (1 and incidentally 2) as a fixed effect. [7]Concentration in plasma was measured weekly between week -3 and -1 precalving. [8]FFA and BHB were transformed to the natural logarithm for statistical analysis, but are shown in actual concentrations. The confidence limits (CL) are shown instead of SEM.

Table 2.6a: Postpartum[1] dry matter intake, energy balance and plasma metabolites during week 1 to 3 of cows after a 0 or 30 days dry period. Cows with a 0-d DP were provided either a low (0-d DP(LOW)) or standard (0-d DP(STD)) level of concentrate that cows with a 30-d DP received (30-d DP(STD)) (LSM ± SEM)

WK 1-3	Dry period length			Parity			P-values[2]					
	0-d DP	30-d DP	SEM	2	≥3	SEM	DP	P	W	DP × P[3]	DP × W	P × W
Cows (n)	81	42		58	65							
DMI (kg DM/d)	18.6	17.1	0.3	17.8	17.8	0.3	<0.01	0.96	<0.01	0.49	0.01	0.71
Concentrate (kg DM/d)	3.4	3.3	0.01	3.4	3.4	0.01	<0.01	0.99	<0.01	0.24	0.02	0.37
Basal ration (kg DM/d)	15.1	13.7	0.3	14.4	14.4	0.3	<0.01	0.98	<0.01	0.43	<0.01	0.61
EI[4] ($kJ/kg^{0.75}$·d)	942	881	14	956	866	14	<0.01	<0.01	<0.01	0.89	0.10	0.82
EB[5] ($kJ/kg^{0.75}$·d)	-62	-235	34	-120	-176	36	<0.01	0.15	<0.01	<0.01	0.11	0.41
BW (kg)	661	648	7	613	696	7	0.21	<0.01	<0.01	0.11	<0.01	<0.01
BCS[6]	2.80	2.76	0.09	2.84	2.72	0.09	0.79	0.36		0.38		
FFA[7] (mmol/L)	0.11 (0.08 – 0.15)	0.23 (0.17 – 0.32)		0.16 (0.12 – 0.20)	0.17 (0.12 – 0.25)		<0.01	0.60	<0.01	<0.01	0.35	0.40
BHB[7] (mmol/L)	0.66 (0.61 – 0.70)	0.77 (0.70 – 0.84)		0.67 (0.61 – 0.73)	0.75 (0.69 – 0.81)		<0.01	0.07	<0.01	0.10	<0.01	0.51
Glucose (mmol/L)	3.69	3.41	0.08	3.68	3.43	0.08	<0.01	<0.01	<0.01	<0.01	0.17	0.47
Insulin (μU/mL)	14.95	9.38	0.83	13.01	11.31	0.82	<0.01	0.14	<0.01	<0.01	0.11	0.07
IGF-1(ng/mL)	120.3	75.4	6.4	115.7	80.04	6.5	<0.01	<0.01	<0.01	0.03	0.03	0.06

[1]Postpartum = week 1 to 3 after calving. [2]DP = Dry period length; P = Parity; W = Week relative to calving. [3]Interactions of treatment with parity (T × P) ($P < 0.05$) are shown in Figure 2.1. [4]EI = Energy intake. [5]EB = Energy balance. [6]BCS = body condition score; analyzed with a repeated measures effect of month relative to calving with cow as subject, instead of weeks relative to calving. [7]FFA and BHB were transformed to the natural logarithm for statistical analysis, but are shown in actual concentrations. The confidence limits (CL) are shown instead of SEM

Table 2.6b: Postpartum[1] dry matter intake, energy balance and plasma metabolites during week 4 to 7 of cows after a 0 or 30 days dry period. Cows with a 0-d DP were provided either a low (0-d DP(LOW)) or standard (0-d DP(STD)) level of concentrate that cows with a 30-d DP received (30-d DP(STD)) (LSM ± SEM)

WK 4-7	Treatment[2]				Parity			P-values[3]					
	0-d DP(LOW)	0-d DP(STD)	30-d DP(STD)	SEM	2	≥3	SEM	T	P	W	T × P[4]	T × W	P × W
Cows (n)	40	41	42		58	65							
DMI (kg DM/d)	22.6	23.2	22.4	0.3	22.4	23.1	0.3	0.10	0.02	<0.01	0.98	<0.01	0.10
Concentrate (kg DM/d)	6.7[a]	8.1[b]	8.1[b]	0.02	7.6	7.6	0.02	<0.01	0.47	<0.01	0.23	<0.01	0.84
Basal ration (kg DM/d)	15.8[a]	15.1[a]	14.3[b]	0.3	14.7	15.5	0.3	<0.01	0.03	<0.01	0.96	0.10	0.09
EI[5] ($kJ/kg^{0.75}$·d)	1145[a]	1200[b]	1189[b]	14	1203	1153	11	0.01	<0.01	<0.01	0.52	<0.01	0.10
EB[6] ($kJ/kg^{0.75}$·d)	-2[a]	55[b]	-110[c]	30	4	-42	27	<0.01	0.13	<0.01	0.08	0.04	0.15
BW (kg)	673[a]	664[a]	643[b]	8	630	691	7	0.03	<0.01	<0.01	0.20	0.54	0.06
BCS[7]	2.90	2.80	2.80	0.08	2.90	2.76	0.07	0.58	0.15	0.59	0.22	0.33	0.83
FFA[8] (mmol/L)	0.07[a] (0.05 – 0.10)	0.06[a] (0.05 – 0.08)	0.13[b] (0.10 – 0.17)		0.08 (0.06 – 0.09)	0.09 (0.06 – 0.12)		<0.01	0.26	<0.01	0.49	0.64	0.40
BHB[8] (mmol/L)	0.72 (0.61 – 0.84)	0.69 (0.59 – 0.81)	0.80 (0.68 – 0.94)		0.67 (0.60 – 0.76)	0.80 (0.67 – 0.96)		0.13	0.02	0.03	0.88	0.95	0.17
Glucose (mmol/L)	3.90[a]	3.93[a]	3.67[b]	0.05	3.93	3.74	0.05	<0.01	<0.01	<0.01	0.17	0.37	0.83
Insulin (μU/mL)	18.80[a]	19.44[a]	14.37[b]	1.33	16.88	18.20	1.32	<0.01	0.29	0.48	0.16	0.66	0.14
IGF-1(ng/mL)	130.8[a]	136.0[a]	107.9[b]	6.8	138.6	111.2	6.2	<0.01	<0.01	0.36	0.29	0.74	0.57

[a,b,c] Values with different superscript differ ($P < 0.05$). [1]Postpartum = week 4 to 7 after calving. [2]Treatment: Cows with a 0-d DP were fed either a low (0-d DP(LOW)) level of concentrate, based on their requirement for their expected milk yield, or a standard (0-d DP(STD)) level of concentrate that cows with a 30-d DP (30-d DP(STD)) received based on the requirement for their expected milk yield. [3]T = Treatment; P = Parity; W = Week relative to calving. [4]Interactions of treatment with parity (T × P) ($P < 0.05$) are shown in Figure 2.1. [5]EI = Energy intake. [6]EB = Energy balance. [7]BCS = body condition score; analyzed with a repeated measures effect of month relative to calving with cow as subject, instead of weeks relative to calving. [8]FFA and BHB were transformed to the natural logarithm for statistical analysis, but are shown in actual concentrations. The confidence limits (CL) are shown instead of SEM.

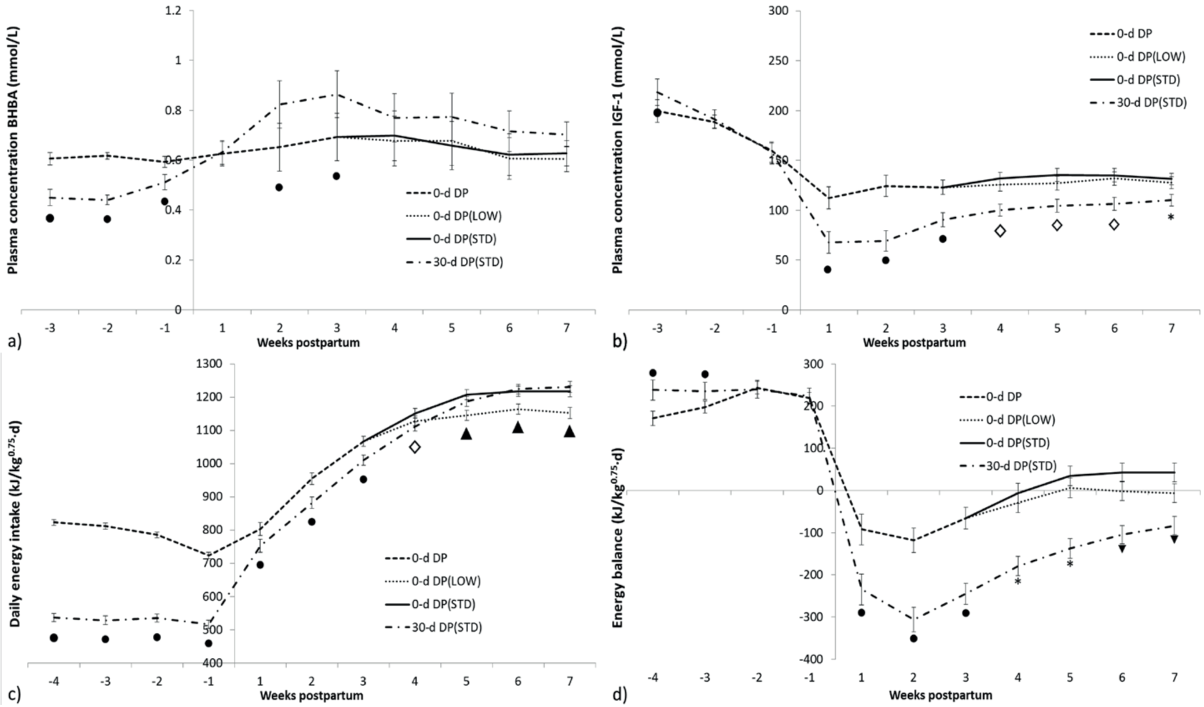

Figure 2.3: The effect of treatment (0-d dry period (DP) and a low level of concentrate, based on the requirement for their expected milk yield, 0-d DP(LOW); a 0-d DP and standard level of concentrate, 0-d DP(STD); and a 30-d DP and a standard level of concentrate, based on the requirement for their expected milk yield, 30-d DP(STD)) on plasma β-hydroxybutyrate (a) and IGF-1 concentration (b), energy intake (c), and energy balance (d). 0-d DP represents the average of both 0-d DP(LOW) and 0-d DP(STD) prepartum. ● 0-d DP differs from 30-d DP, * 30-d DP(STD) differs from 0-d DP(LOW) and 0-d DP(STD), ◇ 30-d DP(STD) differs from 0-d DP(STD), but not from 0-d DP(LOW), ▲ 0-d DP(LOW) differs from 0-d DP(STD) and 30-d DP(STD), ▼ 0-d DP(LOW), 0-d DP(STD) and 30-d DP(STD) differ from each other. Data were analyzed separately for 3 periods of the experiment: prepartum, week 1 to 3 postpartum, and week 4 to 7 postpartum. Results were presented as LSM ± SEM.

The level of concentrate for cows with a 0-d DP can, thus, be lowered without an effect on average milk yield in week 1 – 7 postpartum, although EB was lower in week 6 and 7 of cows with a lower level of concentrate for cows with a LOW level of concentrate.

The absence of an effect of concentrate level [0-d DP(LOW) vs. 0-d DP(STD)] on total DMI intake in week 4 to 7 postpartum is caused by a numerically greater consumption of the basal ration of cows with a 0-d DP(LOW), compared with cows with a 0-d DP(STD). Cows with a 0-d DP(LOW) tended to consume 4% more basal ration, when the concentrate contrast was complete, which partially compensated for the lower concentrate intake. This partial compensation of lower concentrate intake (6.8 ± 0.0 kg/d vs. 8.4 ± 0.0 kg/d) with a greater basal ration intake (15.8 ± 0.3 kg/d vs. 15.1 ± 0.3 kg/d) resulted in a similar DMI (22.6 ± 0.3 kg/d vs. 23.2 ± 0.3 kg/d) between cows with a 0-d DP(LOW) and 0-d DP(STD). Similarly, Dieho et al. (2016) reported that a daily difference in concentrate supply of 6.8 kg DM/d (day 16 relative to calving) vs. 3.3 kg DM/d (day 30 relative to calving) did not affect total DM intake since animals receiving lower concentrate supply increased their intake of the basal ration. Compensation of lower concentrate with basal ration can only occur to a certain extent due to difference in satiety value of concentrate versus basal ration (**Faverdin *et al.***, 1991). The satiety value of a feed indicates the extent to which a feed limits intake, and is calculated based on feed chemical composition (DM, crude protein, crude fiber, ash, sugar, and starch) and in vitro feed digestibility (**Zom *et al.***, 2012). In the current study the satiety value of the basal ration was 0.92/kg DM and the satiety value of the concentrate was 0.35/kg DM (**Zom *et al.***, 2012). Based on these satiety values, the reduced level of concentrate (1.6 kg DM/d) can be expected to result in an increase of 0.6 kg DM/d of basal lactation ration. In the current study cows with a 0-d DP(LOW) consumed 0.6 kg DM/d more basal lactation ration than cows with a 0-d DP(STD). So, the estimated satiety value of the basal ration is similar to the actual satiety value of the basal ration. The partial compensation of concentrate intake with basal ration intake, in cows with a 0-d DP(LOW), results in sufficient energy intake to provide enough energy for milk yield. The lower concentrate intake, nevertheless, tended to decrease EB and increase milk fat content when the concentrate contrast was complete for cows with a 0-d DP(LOW), compared with a 0-d DP(STD). Feeding a reduced level of concentrate after a 0-d DP may be beneficial for roughage intake and rumination, and may be relevant to prevent subacute ruminal acidosis in early lactation (**González *et al.***, 2012). Moreover, reduction of the level of concentrate may also reduce feed costs.

Postpartum, EB of cows with a 30-d DP was more negative than of cows with a 0-d DP, which is in line with previous studies (**Rastani *et al.***, 2005; **Van Knegsel *et al.***, 2014). The more negative EB of cows with a 30-d DP was earlier mainly due to greater yield of milk and milk components of these cows, compared with cows with a 0-d DP. This greater milk yield of cows with a 30-d DP is possibly related to greater renewal of mammary cells during the DP (**Capuco *et al.***, 1997). Additionally, in the current study, postpartum DMI in week 1 - 7 was 5% lower of cows with a 30-d DP (20.1 ± 0.3 kg/d) compared with cows with a 0-d DP (21.1 ± 0.3 kg/d), which is in line with earlier work (**Rastani *et al.***, 2005). In the current study, during week 5 to 7 postpartum, DMI and energy intake of cows with a 30-d DP(STD) did not differ anymore from cows with a 0-d DP(STD). Cows with a 30-d DP may have a less regular feeding pattern in the first week postpartum than cows with a 0-d DP as a result of more stressors such as greater milk production, the transition from a dry cow ration to a lactation ration, and the transition to another social group (**Huzzey *et al.***, 2005). The more negative EB of cows with a 30-d DP was reflected in a greater plasma FFA and BHB concentration, and a lower plasma glucose, insulin and IGF-1 concentration, compared with cows with a 0-d DP. A metabolic state with a low plasma FFA concentration is related with a greater plasma insulin concentration that allows greater hepatic IGF-1 production (**Lucy**, 2004). By omitting the DP in the current study, the plasma insulin concentration was greater than after a short DP, which likely resulted in greater IGF-1 concentration and an improved EB and is consistent with other studies (**De Feu *et al.***, 2009; **Chen *et al.***, 2015).

Prepartum, the plasma BHB concentration was greater of cows with a 0-d DP, than of cows with a 30-d DP, in line with Chen et al. (2015), which is possibly explained by the greater prepartum DMI and different ration composition of cows with a 0-d DP, than of cows with a 30-d DP. The greater DMI of cows with a 0-d DP may have increased the production of VFA in the rumen, including ruminal butyrate (**Bergman**, 1990). Greater ruminal butyrate formation contributes to a greater plasma BHB concentration (**Bergman**, 1990; **Huhtanen *et al.***, 1993; **Miettinen and Huhtanen**, 1996). Prepartum, the plasma FFA concentration was lower of cows with a 0-d DP, than of cows with a 30-d DP, which is in line with earlier studies (**Andersen *et al.***, 2005; **De Feu *et al.***, 2009), but not all (**Rastani *et al.***, 2005; **Schlamberger *et al.***, 2010).

Prepartum, FFA are partly removed from the body via milk of cows with a 0-d DP, whereas FFA have to be fully metabolized in the body of cows with a 30-d DP.

Prepartum, cows with parity 2 had lower DMI, lower basal ration intake, lower BW and lower BCS, but greater energy intake, compared with cows with parity ≥ 3. Cows with parity 2 have a lower feed intake capacity (unit of feed per unit of time) than cows with parity ≥ 3, when expressed per unit metabolic BW (**CVB**, 2011). In the current study cows with parity 2, nevertheless, had a greater DMI when DMI was expressed per kg metabolic BW, compared with cows with parity ≥ 3 (0.17 vs. 0.16 kg DM/ $kg^{0.75}$·d; $P < 0.01$). The greater DMI per kg metabolic BW resulted in a greater energy intake per kilogram metabolic BW of cows with parity 2, than of cows with parity ≥ 3. Cows with parity 2 and a 0-d DP had the lowest milk yield in subsequent lactation, compared with cows with parity 2 and a 30-d DP, or cows with parity ≥3. Earlier studies also showed that omitting the DP of cows with parity 2 resulted in greater milk yield reduction in the subsequent lactation, compared with cows with parity ≥ 3 (**Annen *et al.***, 2004; **Santschi *et al.***, 2011a; **Steeneveld *et al.***, 2014). Cows with parity 2 may need a short or conventional DP for body and mammary growth (**Annen *et al.***, 2004).

Postpartum, not only energy intake per kg metabolic body weight and EB were greater of cows with parity 2, but also plasma glucose and IGF-1 concentrations were greater, compared with cows with parity ≥ 3. Cows with parity 2 also had a lower plasma FFA and BHB concentrations, compared with cows with parity ≥ 3, which allows coupling of the somatotropic axis. In the somatotropic axis, insulin acts opposite to GH. Insulin allows GH to bind to the GH receptor in the liver which stimulates IGF-1 synthesis in the liver. Liver IGF-1 inhibits GH releasing hormone from the hypothalamus, and thus inhibits GH release from the pituitary gland. Cows with parity 2, nevertheless, already have a greater plasma GH concentration which is related to their priority for growth (**Kertz *et al.***, 1997; **Lucy**, 2011). The greater plasma insulin concentration in the presence of a greater plasma GH concentration of cows with parity 2 resulted in more coupling of the somatotropic axis and a greater IGF-1 concentration (**Lucy**, 2011). The lower plasma IGF-1 concentration of cows with parity ≥ 3 was, most likely, the result of a more negative EB due to greater milk production, compared with cows with parity 2. Cows adapt to a more negative EB by a greater degree of uncoupling their somatotropic axis related with the low plasma insulin concentration (**Gross and Bruckmaier**, 2015; **Zbinden *et al.***, 2016). Uncoupling of the somatotropic axis resulted in uninhibited release of GH (**Lucy**, 2004). The released GH can, nevertheless, not bind its receptor in the liver, which results in lower IGF-1 synthesis in the liver of cows with parity ≥ 3. In earlier studies, greater milk production was negatively related with the plasma concentration of IGF-1 (**Grala *et al.***, 2011; **Gross and Bruckmaier**, 2015). In contrast, the plasma IGF-1 concentration may be greater as a result of a greater plasma insulin concentration. Insulin may reduce persistency of lactation through its inhibition of GH through IGF-1 (**Lucy**, 2008; **Roche *et al.***, 2009). A greater plasma insulin concentration is related with over-conditioning (**Roche *et al.***, 2009) which is a risk in cows with a 0-d DP in late lactation (**Chen *et al.***, 2016). The current study, however, only focused on the first 7 weeks in lactation during which no effect of treatment on BCS was observed. Studies are ongoing to evaluate the effect of dietary energy source and dietary energy level on body condition and persistency of lactation of cows with a 0-d or 30-d DP.

2.6 Conclusions

A 0-d DP reduced milk production and improved the EB and metabolic status of cows in early lactation, compared with a 30-d DP. Reducing the level of concentrate for cows with a 0-d DP increased basal ration intake and milk fat content, reduced energy intake, and EB in week 6 and 7, but did not affect FPCM yield or plasma FFA and BHB concentrations in early lactation, compared with cows with a 0-d DP and a standard concentrate level. Although feeding a reduced level of concentrate of cows after a 0-d DP increased milk fat content and reduced energy balance in early lactation, it may prevent fattening in mid and late lactation, increase roughage intake, stimulate rumination, and reduce feed costs.

2.7 Acknowledgements

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CHAPTER 3

Consequences of dietary energy source and energy level on energy balance, lactogenic hormones and lactation curve characteristics of cows after a short or omitted dry period

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3.1 Abstract

Omitting the dry period (**DP**) generally reduces milk production in the subsequent lactation. The aim of this study was to evaluate the effect of dietary energy source [glucogenic (**G**) or lipogenic (**L**)], and energy level [standard (**STD**) or low (**LOW**)] on milk production, energy balance (**EB**), lactogenic hormones insulin, insulin-like growth factor 1 (**IGF-1**), and GH, and lactation curve characteristics between wk 1 and 44 postpartum in cows after a 0-d or 30-d DP. Cows (N = 110) were assigned randomly to 3 transition treatments, viz. a 30-d DP with a standard (**STD**) energy level required for expected milk yield [**30-d DP(STD)**], a 0-d DP with the same STD energy level as cows with a 30-d DP [**0-d DP(STD)**], and a 0-d DP with a LOW energy level [**0-d DP(LOW)**]. In wk 1 – 7, cows were fed the same basal ration, but level of concentrate increased to 6.7 kg/d for cows fed a LOW energy level and to 8.5 kg/d for cows fed a STD energy level in wk 4. From wk 8 postpartum onwards cows received a G ration, mainly consisting of corn silage and grass silage, or an L ration, mainly consisting of grass silage and sugar beet pulp, with the same energy level contrast (LOW or STD) as in early lactation. Cows fed the G ration had a greater milk, lactose and protein yield, lower milk fat percentage, greater dry matter and energy intake, and plasma IGF-1 concentration, compared with cows fed the L ration. Dietary energy source did not affect EB or lactation curve characteristics. In cows with a 0-d DP, a reduced energy level decreased energy intake, EB, and weekly body weight gain, but did not affect milk production and lactation curve characteristics. A 30-d DP resulted in a greater total predicted lactation yield, initial milk yield after calving, peak milk yield, energy intake, energy output in milk, days to conception (only when compared with 0-d DP(LOW)), plasma GH concentration (only when compared with 0-d DP(STD)), and decreased weekly body weight gain compared with a 0-d DP. A 30-d DP decreased both the increasing and the declining slope parameters of the lactation curve and the relative rate of decline in milk yield indicating a greater lactation persistency, compared with a 0-d DP, and decreased plasma insulin and IGF-1 concentration, and EB. In conclusion, feeding a G ration after wk 7 in milk improved energy intake and milk production, but did not affect EB, compared with an L ration. For cows without a DP, a reduced dietary energy level did not affect milk production and lactation curve characteristics, but did decrease EB and weekly body weight gain. A 30-d DP increased milk yield and lactation persistency, but decreased milk fat and protein content, EB, and plasma insulin and IGF-1, compared with a 0-d DP.

Key words: continuous milking, persistency, body condition, metabolic status

3

3.2 Introduction

A conventional dry period (**DP**) length of 6 to 8 wks has been applied for several decades and maximizes milk production in the subsequent lactation (**Grummer and Rastani**, 2004). High milk yield in early lactation is related with a negative energy balance (**NEB**) with associated metabolic disorders, such as ketosis, fatty liver, and impaired fertility (**Ingvartsen *et al.***, 2003). Shortening or omitting the DP was suggested as a management measure to improve the EB and metabolic status of dairy cows in early lactation (**Annen *et al.***, 2004; **Rastani *et al.***, 2005; **Schlamberger *et al.***, 2010).

Improvement of the energy balance (**EB**) after a 0-d DP is mainly a result of a reduction in milk production, compared with a 30-d or 60-d DP (**Annen *et al.***, 2004; **De Feu *et al.***, 2009), and sometimes also due to an improvement of energy intake (**Rastani *et al.***, 2005). Postpartum milk yield losses as a result of omitting the dry period are partially compensated by additional milk yield in the precalving period (**Rastani *et al.***, 2005; **Van Knegsel *et al.***, 2014). It was hypothesized that a greater persistency of milk yield in the subsequent lactation could also partially compensate for milk losses in early lactation and potentially increase total lactation yield in cows after a short or omitted DP (**Grummer and Rastani**, 2004). Later, experimental studies reported ambiguous effects of dry period length on lactation persistency. Shortening or omitting the DP reduced lactation persistency (**Mantovani *et al***., 2010; persistency expressed as absolute decline in milk yield), increased lactation persistency in primiparous cows (**Atashi *et al.***, 2013; persistency calculated from lactation curve characteristics) or did not affect lactation persistency (multiparous cows, **Atashi *et al.*,** 2013; **Chen *et al***., 2016a; persistency expressed as absolute decline in milk yield) compared with cows with a conventional DP. In the latter study, **BCS** of cows with a 0-d DP increased in late lactation, compared with cows with a 30-d or 60-d DP (**Chen *et al.***, 2016b).

Lactation persistency can be improved by administration of exogenous growth hormone (**GH**) (**Van Amburgh *et al.***, 1997; **Bauman**, 1999), and was found to be slightly related with the endogenous plasma GH concentration (**Sorensen and Knight**, 2002). Growth hormone increases nutrient partitioning towards milk (**Hart**, 1983; **Lucy**, 2004). Growth hormone release from the pituitary gland is inhibited by insulin-like growth factor 1 (**IGF-1**) (**Berelowitz *et al.***, 1981; **Tannenbaum *et al.***, 1983). Hepatic production of IGF-1 is positively related with plasma glucose and insulin concentration (**Butler *et al.***, 2003). Specific dietary measures, such as energy level (**Kokkonen *et al.***, 2004; **Lucy *et al.***, 2009; **Grala *et al.***, 2011) and energy source (**Gong *et al.***, 2002; **Van Knegsel *et al.***, 2007a; **Van Knegsel *et al.***, 2007b) affect lactogenic plasma hormone concentrations such as insulin, IGF-1 and GH, and partitioning of energy towards milk (**Mashek *et al.***, 2002). Several studies evaluated lactation curve characteristics during bST treatments and illustrated how the increasing slope of the lactation curve, peak yield, and lactation persistency were related with plasma IGF-1 and GH concentrations (**Van Amburgh *et al.***, 1997; **Bilby *et al.***, 2006). Differences in the plasma insulin, IGF-1, and GH concentration, due to differences in DP length, or dietary energy level or dietary energy source, can be hypothesized to affect the lactation curve characteristics.

Feeding a lipogenic ration may increase lactation persistency (**Cannas *et al.***, 2002). Feeding a lipogenic ration resulted in a lower plasma glucose (**Chen *et al.***, 2016b), insulin (**Van Knegsel *et al.***, 2007a; **Van Knegsel *et al.***, 2007b; **Chen *et al.***, 2016b), and IGF-1 concentration (**Chen *et al.***, 2016b) in early lactation, compared with a more glucogenic ration. Other studies, however did not find differences in plasma glucose, IGF-1, GH (**Garnsworthy *et al.***, 2008; **Garnsworthy *et al.***, 2009; **Chen *et al.***, 2016a) or insulin concentration (**Chen *et al.***, 2016a) after feeding a more lipogenic, compared with a glucogenic ration. Differences among studies could be related to the number of cows in the study, the contrast in lipogenic and glucogenic nutrient availability between different rations within a study, or the stage of lactation when rations were fed. In mid- and late- lactation, a lipogenic ration reduced the plasma glucose and insulin concentration compared with a more glucogenic ration (**Voelker and Allen**, 2003; **Mahjoubi *et al.***, 2009). In light of the correlation between insulin and GH (**Hart *et al.***, 1979), we hypothesized that a lower plasma insulin concentration after peak milk yield stimulates the plasma GH concentration and lactation persistency.

Feeding an energy level aimed at zero EB, to prevent fattening in late lactation, may be another measure to stimulate lactation persistency of cows with a short or no DP, compared with feeding an excess energy level such as in Chen et al. (2016a). It can be hypothesized that limiting intake of NE_L aiming at zero EB, adjusted for the lower milk yield of cows with a 0-d DP, lowers the plasma glucose and insulin concentration, elevates plasma GH concentration, increases partitioning of energy towards milk yield and lactation persistency, and prevents fattening of cows after a 0-d DP. So far, effects of DP length on milk yield and EB in the subsequent lactation were always evaluated with a lactation ration containing the same energy level for all cows.

The aim of this study was to evaluate the effect of dietary energy source and of dietary energy level on milk production, EB, lactogenic hormones and lactation curve characteristics in cows after a 0-d or 30-d DP.

3.3 Materials and methods

3.3.1 Animals and housing

The Institutional Animal Care and Use Committee of Wageningen University & Research (the Netherlands) approved the experimental protocol in compliance with the Dutch law on Animal Experimentation (protocol number 2014125). The experiment was conducted at the Dairy Campus research farm (Lelystad, The Netherlands) between January 27th 2014 and May 9th 2016. The research herd was composed of 400 lactating Holstein cows. For this experiment, cows were selected based on 1) being bred with a Holstein sire, 2) expected calving interval < 490 days, 3) daily milk yield > 16 kg at 90 days before the expected calving date, and 4) no clinical mastitis and preferably SCC < 250,000 cells/mL at final 2 test-days before the conventional drying off day. Cows pregnant with twins were not included in the experiment. Cows were housed in a freestall with a slatted floor and cubicles. Cows were milked twice daily at ~0600 hours and ~1800 hours. The drying off protocol for cows with a 30-d DP consisted of a transition to the dry cow ration at day 7 before drying off and to milking once daily from day 4 before drying-off. At drying-off, no dry cow antibiotics were used.

3.3.2 Experimental design

In total, 130 cows entered the experiment, including 6 cows that entered twice. To obtain a balanced distribution of cows across treatments, cows were blocked according to expected calving date, milk yield in the previous lactation, and parity (2, ≥ 3) in the subsequent lactation. Within each group of 6 cows, 4 cows were assigned randomly to a DP length treatment of 0 days and 2 cows to a DP length treatment of 30 days. Within the group of cows with a 0-d DP, cows were assigned randomly to either a low level of energy in wk 4 – 44, which is based on the requirement for their expected milk yield (**LOW**) or a standard (**STD**) level of energy based on the requirement for the expected milk yield of cows with a 30-d DP. Cows with a 30-d DP were fed a STD level of energy, which is based on the requirement for their expected milk yield. From wk 8 postpartum onwards, cows received either a glucogenic (**G**) or lipogenic (**L**) basal ration. This resulted in the following 3 transition treatments, each with 2 different rations: cows with a 30-d DP and a STD dietary energy level [**30-d DP(STD)**] and fed a G (n = 19) or L (n = 21) ration, cows with a 0-d DP with the same STD dietary energy level [**0-d DP(STD)**] as cows with a 30-d DP and fed a G (n = 18) or L (n = 16) ration, and cows with a 0-d DP with a LOW energy level [**0-d DP(LOW)**] and fed a G (n = 16) or L (n = 20) ration. The experimental period of the current study started at calving and lasted until wk 44 postpartum.

3.3.3 Rations

The ingredient and nutrient composition of the basal rations and of the concentrates is presented in **Table 3.1 and 3.2**, respectively. The basal (early lactation) ration was fed for 7 wks and was described previously (**Van Hoeij *et al.***, 2017). In short, all cows were fed the same basal ration for 7 wks postpartum, including 1 kg/d of standard concentrate in the milking parlor. All cows received 1 kg/d of experimental concentrate from 10 days before the expected calving date. Experimental concentrate supply increased stepwise by 0.3 kg/d from 4 DIM up to an experimental concentrate intake of 8.5 kg/d at 28 DIM for cows receiving the ration with STD energy level [30-d DP(STD) and 0-d DP(STD)], or stepwise by 0.3 kg/d from 4 DIM up to an experimental concentrate intake of 6.7 kg/d at 22 DIM for cows receiving the ration with the LOW energy level [0-d DP(LOW)]. The difference in dietary energy level between the LOW and STD ration was based on previous research where cows with a 0-d DP (35.4 kg fat- and protein- corrected milk (**FPCM**)/d) produced 12% less FPCM between 1 and 14 wk in lactation, than cows with a 30-d DP (40.4 kg FPCM/d) (**Van Knegsel *et al.***, 2014). Cows with a 0-d DP were fed the LOW dietary energy level, which was calculated for the expected milk yield of cows with a 0-d DP, or the STD dietary energy level, which was calculated for the expected milk yield of cows with a 30-d DP. Cows with a 30-d DP were only fed the STD dietary energy level. The experimental concentrate decreased stepwise by 0.5 kg/wk starting in wk 15 to 0 kg/d in wk 31 for cows with a STD dietary energy level. The experimental concentrate decreased stepwise by 0.4 kg/wk starting in wk 15 down to 0 kg/d in wk 31 for cows with a LOW dietary energy level. Experimental concentrate was provided individually over 6 periods within 24 hours by a computerized feeder located in the freestall that was available to all cows at all times (Manus VC5, DeLaval, Steenwijk, the Netherlands). From 8 wks postpartum onwards, the glucogenic and lipogenic basal ration were supplied. The basal rations were calculated to be iso-caloric, within a STD or LOW energy level. Cows had free access to water and basal ration throughout the experiment.

Rations were mixed once daily at ~1000 h and fed twice daily at ~1000 h and ~1700 h. Dry matter content of basal ration was measured daily. Forage samples were taken weekly and stored at −20°C until analysis. Net energy was calculated using the Dutch NE system for lactation (**VEM**) (**Van Es**, 1975), and intestinal digestible protein (**IDP**) and rumen-degradable protein balance (**RDPB)** were calculated according to the Dutch DVE/OEB-system (**Tamminga *et al.***, 1994).

3.3.4 Measurements

Milk yield and milk composition. Milk yield was recorded daily from day of calving until 44 wks postpartum. Milk samples for fat, protein and lactose analysis [(**ISO 9622**, 2013), Qlip, Zutphen, The Netherlands] were collected 4 times per wk (Tuesday afternoon, Wednesday morning, Wednesday afternoon, and Thursday morning) and were analyzed as a pooled sample of 2 morning and 2 afternoon milkings per cow per wk. FPCM was calculated as:

$$\text{FPCM (kg/d)} = [0.337 + 0.116 \times \text{fat content (\%)} + 0.06 \times \text{protein content (\%)}]$$

$$\times \text{ milk yield (kg/d)}$$ (**CVB**, 2011).

Feed intake, body weight, body condition score, and conception. Daily basal ration intake was measured individually using roughage intake control (RIC) troughs and was averaged per wk (Insentec, Marknesse, The Netherlands). The stocking density was 2 cows per trough. Concentrate supply (kg/d) was recorded by a computerized feeder (Manus VC5, DeLaval, Steenwijk, the Netherlands). Body weight was recorded daily before each milking and was averaged per week. Weekly BW gain was calculated. Body condition score was measured every 4 wks by the same person using a 1 to 5 scale (**Ferguson *et al.***, 1994). Cows were inseminated after a voluntary waiting period of 50-d till at least 170 DIM.

Artificial insemination took place 12 h after detection of estrus, which was detected using Lely Qwes-HR Activity Tags (Lely, Maassluis, the Netherlands) mounted on the neck collar.

Pregnancy was checked every 4 wks by a veterinarian for cows inseminated > 30 d before the pregnancy check.

Blood collection. Blood was collected weekly from calving until wk 10 postpartum, and every 2 wks between wk 12 and 44 postpartum. Blood samples were collected after the morning milking and between 3 and 1 hours before the morning feeding. Blood (10 mL) was collected from the coccygeal vein into evacuated EDTA tubes (Vacuette, Greiner BioOne, Kremsmunster, Austria). Blood samples were kept on ice before centrifugation for plasma isolation (3,000 × *g* for 15 min, 4°C). Plasma samples were stored at −20°C. The plasma insulin concentration was measured using kit no. PI-12K from EMD Millipore Corporation (Billerica, MA, USA). The plasma IGF-1 concentration was measured using kit no. A15729 from Beckman Coulter (Fullerton, CA, USA). The plasma GH concentration was measured by radioimmunoassay as described previously (**Vicari *et al.***, 2008).

3.3.5 Calculations

Energy balance was calculated per wk, according to the Dutch NE system for lactation (**Van Es**, 1975), as the difference between intake of VEM and the requirements of VEM for maintenance, milk yield, and pregnancy (1,000 VEM = 6.9 MJ of NE). According to the VEM system, the daily requirement for maintenance is 42.4 VEM/kg$^{0.75}$ BW per day, the requirement for milk yield is 442 VEM/kg FPCM, and the daily requirement for pregnancy is 450, 850, 1500, and 2700 VEM for month 6, 7, 8, and 9 of pregnancy, respectively. Energy intake and EB are expressed in kJ/ kg$^{0.75}$ BW per day (**Van Es**, 1975). Energy efficiency was calculated as:

Energy efficiency = E_L / NE_I,

where E_L = milk energy output (kJ/d) and NE_I = NE intake (kJ/d).

Table 3.1: Ingredient and nutrient composition of the average basal ration fed for 7 weeks postpartum (R-7wk), and of the average glucogenic (G) and the lipogenic (L) basal ration at standard energy level (STD) or reduced energy level (LOW) fed from week 8 to 44 postpartum

	R-7wk	G-LOW	G-STD	L-LOW	L-STD
Ingredient (g/kg DM)					
Grass silage[1]	437	162	175	571	619
Corn silage[2]	338	519	616	0	0
Sugar beet pulp	97	0	0	206	217
Dried sugar beet pulp[3]	0	0	0	60	69
Soybean meal[4]	84	150	144	61	67
Wheat straw	20	152	49	81	8
Urea	4	1	1	3	3
Vitamins and minerals	20	16	15	18	17
Nutrient composition (g/kg DM)					
DM (g/kg product)	418	514	459	460	433
Crude ash	82	64	62	92	94
Crude protein	137	131	133	139	148
Crude fat	33	28	30	30	31
NDF	419	414	372	461	433
ADF	237	241	213	253	232
ADL	20	22	18	24	19
Starch	124	182	216	7	7
Sugar	62	38	39	85	91
IDP[5]	79	77	81	84	91
RDPB[6]	7.8	11.8	9.3	1.1	1.8
NE (MJ/kg DM)[7]	6.4	6.1	6.5	6.3	6.5

[1]Chemical composition of grass silage (g/kg DM, unless otherwise stated): DM 410 g/kg product, crude ash 111, crude protein 140, crude fat 42, NDF 458, ADF 258, ADL 17, starch 6, sugar 123, intestinal digestible protein 70, rumen-degradable protein balance 5.7, NE 6.4 MJ/kg DM. [2]Chemical composition of corn silage (g/kg DM, unless otherwise stated) DM 354 g/kg product, crude ash 49, crude protein 66, crude fat 30, NDF 383, ADF 218, ADL 18, starch 344, sugar 3, intestinal digestible protein 48, rumen-degradable protein balance -36, NE 6.8 MJ/kg DM. [3]Dried sugar beet pulp was fed in the lipogenic rations between May 4th 2015 and May 9th 2016. [4]Soybean meal was treated with formaldehyde to make it rumen protected. [5]Intestinal digestible protein, calculated according to the Dutch DVE/OEB-system (**Tamminga *et al.*,** 1994). [6]Rumen-degradable protein balance, calculated according to the Dutch DVE/OEB-system (**Tamminga *et al.***, 1994). [7]Net energy, calculated according to the Dutch VEM-system (**Van Es**, 1975). NE, IDP and RDPB values for grass silage, corn silage, and sugar beet pulp were based on near-infrared spectrometry (Blgg AgroXpertus, Wageningen, the Netherlands). Values for IDP, RDPB and NE of soybean meal were obtained from the manufacturer (Agrifirm, Apeldoorn, The Netherlands). Values for wheat straw and urea were obtained from CVB Feed Tables 2011 (**CVB**, 2011).

Table 3.2: Ingredient and nutrient composition of the standard and experimental concentrate

Ingredient (g/kg)	Standard concentrate	Experimental concentrate
Palmkernel, expeller	131	179
Corn	206	44
Lupine		26
Sugar beet pulp (max 12% sugar)	63	
Citric pulp	137	120
Wheat	62	128
Wheat meal		9
Barley	20	
Soybean hulls		169
Soybean meal	34	54
Soybean meal (rumen protected)	11	9
Rapeseed meal	113	45
Rapeseed meal (rumen protected)	81	70
Molasses-cane	29	36
Molasses-beet	26	
Protamylasse[1]	14	
Beet vinasses[2]	34	
Citrocol[3]	20	35
Linseed oil		9.1
Palm oil	2.3	3.5
Calcium carbonate	2.6	2.1
Sodium chloride	3.4	4.4
Magnesium oxide	3.9	4.1
Urea	3.9	2.7
Vitamin E	0.6	0.9
Selenium	1.8	1.8
Methionine source[4]	0.4	0.5
Nutrient composition (g/kg product)		
DM (g/kg product)	883	892
Crude protein	175	171
Ash	65	59
Crude fat	38	41
NDF	221	338
ADF	136	201
ADL	31	41
Starch	221	118
Sugar	80	77
IDP[5]	105	123
RDPB[6]	17	16
NE (MJ/kg DM)[7]	7.5	7.5

[1]Protamylasse is potato juice concentrate (Avebe, Veendam, The Netherlands). Chemical composition of protamylasse (g/kg DM, unless otherwise stated): DM 548 g/kg product, crude ash 290, sugar 57, crude protein 327, IDP 52, RDPB 228, NE 6.2 MJ/kg DM. [2]Chemical composition of beet vinasses (g/kg product): DM 663, crude ash 174, crude protein 214, sugar 37, NDF 2, ADF 1, IDP 34, RDPB 148, NE 4.1 MJ/kg. [3] Citrocol was used as a binding agent in the rations. [4]Methionine as 2-hydroxy-4-methylthiobutanoic acid isopropyl ester (MetaSmart dry, Adisseo, Antony, France). [5]Intestinal digestible protein, calculated according to the Dutch DVE/OEB-system (**Tamminga *et al.***, 1994). [6]Rumen-degradable protein balance, calculated according to the Dutch DVE/OEB-system (**Tamminga *et al.***, 1994). [7]Net energy, calculated according to the Dutch VEM-system (**Van Es**, 1975). Values for IDP, RDPB and NE were obtained from the manufacturer (Agrifirm, Apeldoorn, The Netherlands).

For the estimation of lactation curve characteristics, Wood's equation [1] was used with FPCM yield (kg/d) as dependent variable (**Wood**, 1967). In Wood's incomplete gamma function, *a* is a scaling factor associated with the initial milk yield, *b* is the inclining slope up to peak yield, *c* is the declining slope towards the end of lactation, and *t* is time expressed in days after calving. Estimates of *a*, *b*, and *c* were obtained from Wood's equation by fitting weekly milk production data from the lactation curve of 305-d for each cow in a non-linear regression model [PROC NLIN, SAS version 9.3] (**SAS Institute Inc.**, 2011). Variables from Wood's equation were excluded from analyses for 4 cows because *b* was negative for these animals. Persistency of peak milk yield (*S*) was calculated using equation [2]. Day of peak milk yield was calculated using equation [3]. Peak milk yield was calculated using equation [1] with t = day of peak milk yield. The relative rate of decline of milk yield at the midway point between day of peak milk yield and end of lactation was calculated using equation [4], in which tf is the 305th day in milk (**Dijkstra *et al.***, 2010).

$$\text{FPCM (kg/d)} = at^{b}\, e^{-ct} \qquad [1]$$

$$S = \ln c^{-(b+1)} \qquad [2]$$

$$\text{Day of peak milk yield} = b/c \qquad [3]$$

$$\text{Relative rate of decline (/d)} = 2bc/(b + c\, tf) - c \qquad [4]$$

3.3.6 Statistical analyses

For the current study, 20 of 130 cows were excluded from the dataset because of an unplanned DP for cows assigned to a 0-d DP (n = 4), due to being fed an incorrect concentrate level postpartum (n = 5), due to stealing from a different basal ration than assigned to (n = 3), or having less than 200 DIM of milk yield records (n = 8). The final dataset consisted of full lactation data of 110 cows (53 with parity 2 and 57 with parity ≥ 3). Preliminary analysis showed that repeated use of the 6 cows in this experiment did not affect the results of this study. Statistical analyses were performed using repeated measures analyses in a mixed linear model (PROC MIXED) with cow as the repeated subject. A first-order autoregressive covariance matrix was the best fit according to Akaike's corrected information criterion and was used to account for within-cow variation. Results are presented as the LSmeans with their SE. The natural logarithm was calculated for plasma GH concentration to approximate normality.

Milk production, milk composition, feed intake, EB, BW, BW gain, and plasma hormones were analyzed in a mixed linear model with fixed effects of ration (G or L), transition treatment [0-d DP(LOW), 0-d DP(STD), 30-d DP(STD)], parity (2, ≥ 3), wk after calving (wk 1 through 44), and their two-way interactions included in the model (Model 1). Plasma insulin, IGF-1, and GH concentration were measured once every 2 wks between wk 11 – 44 and were averaged per 2 wks. Body condition score was analyzed with model 1, with the exception of the fixed effect of month after calving instead of wk after calving.

Variables *a*, *b*, *c*, *S*, day of peak lactation, peak milk yield, the relative rate of decline in milk yield, total lactation yield, and days to conception were analyzed in a mixed linear model, with fixed effects of ration (G or L), transition treatment [0-d DP(LOW), 0-d DP(STD), 30-d DP(STD)], postpartum parity (2, ≥ 3), and their two-way interactions included in the model (Model 2). The proportion of cows pregnant within 44 wks was defined as the proportion of cows that was successfully inseminated within 44 wks of lactation. Pregnancy was analyzed using model 2 with a binary distribution and the default logit link function in a mixed linear regression model (PROC GLIMMIX). The number of services per pregnancy (**Fetrow *et al.***, 1990) was analyzed using model 2. To evaluate the correlation of lactation curve characteristics with EB and plasma hormones, a Pearson correlation (PROC CORR) was used. The average EB, plasma insulin, IGF-1 or GH concentration between wk 1 – 7 and wk 8 – 44 were analyzed separately with *a*, *b*, *c*, *S*, and the relative rate of decline in milk yield. Significance was declared at $P < 0.05$ and trends at $0.05 \leq P < 0.10$.

3.4 Results

3.4.1 Milk production

Milk, lactose, and protein yield were greater for cows fed a G ration than for cows fed an L ration ($P < 0.05$), whereas ration did not affect fat yield (**Table 3.3**). Milk yield and composition did not differ between cows with a LOW or STD energy level after a 0-d DP. Milk, FPCM, lactose, and protein yield were greater for cows with a 30-d DP than for cows with a 0-d DP during wk 1 through 44 of lactation ($P < 0.01$). Fat yield was greater for cows with a 30-d DP, than for cows with a 0-d DP ($P < 0.01$), but was not different between cows with a 0-d DP(LOW) and 30-d DP(STD) in wk 2, 3, and 4 of lactation, and between cows with a 0-d DP(STD) and 30-d DP(STD) in wk 2 through 9 of lactation ($P \geq 0.05$).

Milk lactose content was greater in cows with parity 2 (4.53 ± 0.02 %), compared with cows with parity ≥ 3 (4.46 ± 0.02 %) ($P < 0.01$).

An interaction of ration with transition treatment (i.e. 0-d DP(LOW), 0-d DP(STD) or 30-d DP(STD) was present for milk lactose content (**Figure 3.1a**) and milk fat content (**Figure 3.1b**). Compared with an L ration, a G ration resulted in a greater milk lactose content for cows with a 0-d DP(LOW) ($P <$ 0.05), but did not affect milk lactose content for cows with other transition treatments ($P \geq 0.05$). A G ration resulted in a lower milk fat content, compared with an L ration, for cows with a 0-d DP(STD) or 30-d DP(STD) ($P < 0.01$), but did not affect milk fat content for cows with a 0-d DP(LOW) ($P \geq 0.05$). The interaction of ration and parity indicated a greater milk fat content (**Figure 3.1c**) for cows with parity 2 compared with parity ≥ 3 when fed an L ration ($P < 0.05$), but not when fed a G ration ($P \geq 0.05$). An interaction of transition treatment with parity was present for milk fat (**Figure 3.1d**) and protein content (**Figure 3.1e**). Cows with parity 2 in transition treatment 0-d DP(LOW) had a greater milk fat content than cows with parity ≥ 3 ($P < 0.05$), while an effect of parity was not present for the other transition treatments ($P \geq 0.05$). Milk protein content was lower for cows with parity ≥ 3 after a 0-d DP(LOW) or 0-d DP(STD) compared with cows with parity 2 ($P < 0.05$), and did not differ between parities after a 30-d DP(STD) ($P \geq 0.05$).

An interaction of transition treatment with wk after calving was present for milk yield, FPCM yield (**Figure 3.2a**), lactose yield (**Figure 3.2b**), fat yield (**Figure 3.2c**), and protein yield (**Figure 3.2d**) ($P < 0.05$). An interaction of parity with wk after calving was present for milk lactose (**Figure 3.2e**) and protein content (**Figure 3.2f**) ($P < 0.05$).

Table 3.3: Milk yield and milk composition during week 1 to 44 of lactation in cows after a 0 or 30 days dry period. Cows received a glucogenic (G) or lipogenic (L) ration in wk 8 – 44. Cows with a 0-d DP were provided either a low [0-d DP(LOW)] dietary energy level, or the standard [0-d DP(STD)] dietary energy level that cows with a 30-d DP received [30-d DP(STD)]

	Ration[1]			Transition treatment[2]				P-values[3]									
	G	L	SEM	0-d DP (LOW)	0-d DP (STD)	30-d DP (STD)	SEM	R	T	P	W	R × T[4]	R × P	T × P[5]	R × W	T × W	P × W
Cows (n)	53	57		36	34	40											
Milk yield (kg/d)	27.7	25.1	0.73	24.2[a]	24.7[a]	30.3[b]	0.88	0.02	<0.01	0.06	<0.01	0.62	0.77	0.06	0.34	<0.01	0.13
FPCM (kg/d)	29.8	28.1	0.79	27.1[a]	27.4[a]	32.3[b]	0.95	0.13	<0.01	0.21	<0.01	0.45	0.41	0.14	0.84	<0.01	0.68
Lactose (%)	4.52	4.47	0.02	4.48	4.46	4.55	0.03	0.14	0.05	0.01	<0.01	0.03	0.35	0.41	0.99	0.97	0.04
Fat (%)	4.57	4.86	0.05	4.83[a]	4.76[a]	4.54[b]	0.06	<0.01	<0.01	<0.01	<0.01	<0.01	0.03	0.04	0.85	0.11	0.12
Protein (%)	3.86	3.96	0.05	4.02[a]	3.99[a]	3.72[b]	0.06	0.18	<0.01	0.01	<0.01	0.54	0.36	0.05	0.84	0.58	<0.01
Lactose (kg/d)	1.26	1.16	0.03	1.09[a]	1.11[a]	1.38[b]	0.04	<0.01	<0.01	0.15	<0.01	0.44	0.68	0.10	0.45	<0.01	0.05
Fat (kg/d)	1.23	1.19	0.03	1.14[a]	1.15[a]	1.34[b]	0.04	0.44	<0.01	0.29	<0.01	0.17	0.21	0.18	0.82	<0.01	0.83
Protein (kg/d)	1.04	0.97	0.02	0.95[a]	0.96[a]	1.10[b]	0.03	0.04	<0.01	0.34	<0.01	0.46	0.44	0.19	0.16	<0.01	0.12

[a-c] Values within transition treatment with different superscript letters differ (*P* < 0.05). [1]From week 8 postpartum onwards, cows received a basal ration with either glucogenic (G) or lipogenic (L) energy sources.[2]Transition treatment: Cows with a 0-d DP were fed a glucogenic or lipogenic ration with either a low [0-d DP(LOW)] energy level, based on their requirement for their expected milk yield, or a standard [0-d DP(STD)] energy level based on the expected milk yield of cows with a 30-d DP [30-d DP(STD)]. [3]R = Ration, T = transition treatment, P = Parity; W= Week after calving. [4] The interaction of transition treatment and ration was present for milk lactose content and milk fat content. Lactose (%): 0-d DP(LOW) × G: 4.55 ± 0.04, 0-d DP(LOW) × L: 4.41 ± 0.04, 0-d DP(STD) × G: 4.43 ± 0.04, 0-d DP(STD) × L: 4.49 ± 0.04, 30-d DP(STD) × G: 4.58 ± 0.04, 30-d DP(STD) × L: 4.51 ± 0.04. Fat (%): 0-d DP(LOW) × G: 4.84 ± 0.08, 0-d DP(LOW) × L: 4.80 ± 0.08, 0-d DP(STD) × G: 4.60 ± 0.08, 0-d DP(STD) × L: 4.92 ± 0.08, 30-d DP(STD) × G: 4.23 ± 0.08, 30-d DP(STD) × L: 4.81 ± 0.07. [5]Interactions of transition treatment with parity (T × P) (*P* < 0.05) are shown in Figure 3.1.

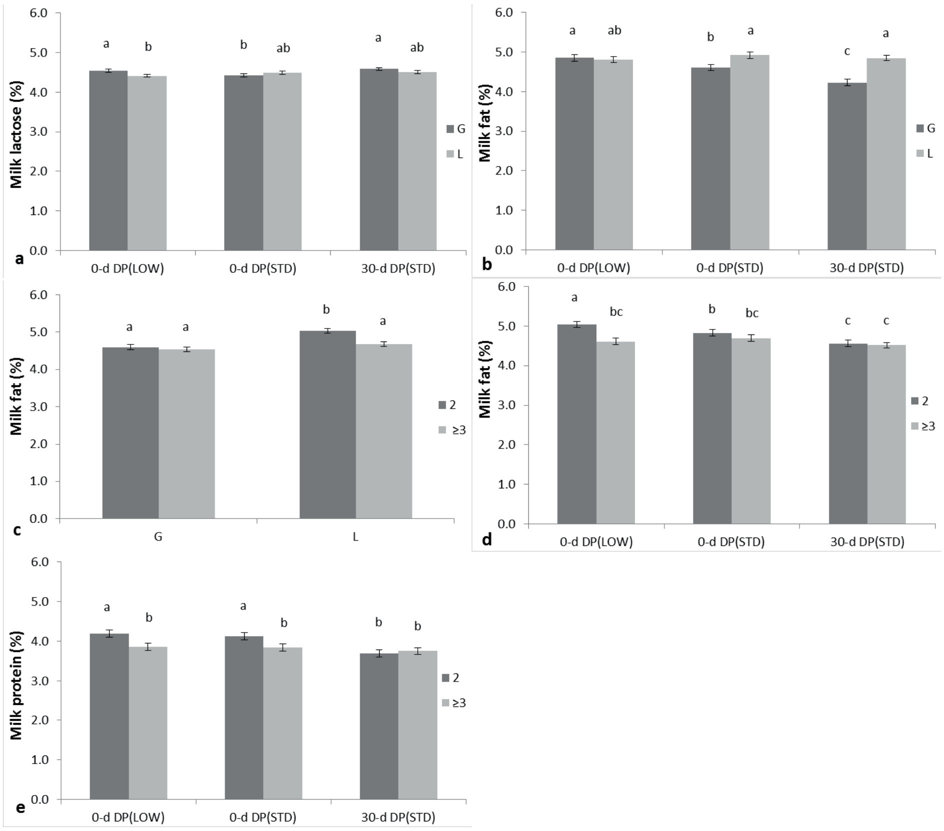

Figure 3.1: The effect of the interaction between transition treatment (cows with a 0-d DP fed a low energy level [0-d DP(LOW)], based on their requirement for their expected milk yield, or a standard [0-d DP(STD)] energy level that cows with a 30-d DP [30-d DP(STD)] received based on the requirement for their expected milk yield) and postpartum ration [glucogenic (G) in dark grey or lipogenic (L) in light grey] fed in wk 8 – 44, for milk lactose content (a), milk fat content (b), the interaction between ration and parity for milk fat content (c), and the interaction between transition treatment and parity (2 in dark grey or ≥ 3 in light grey) for milk fat content (d) and milk protein content (e). [a-c]Values with different letters differ (*P* < 0.05). LSM ± SEM.

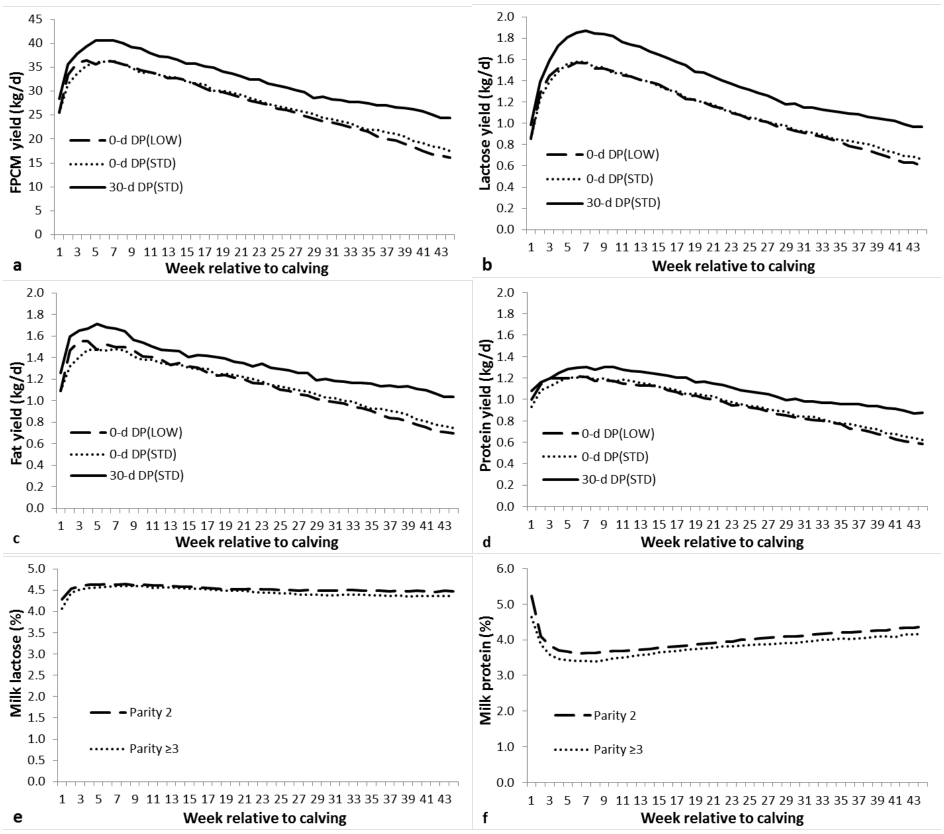

Figure 3.2: Yield (kg/d) of FPCM (a), lactose (b), fat (c), and protein (d) in cows with different DP lengths and dietary energy level (cows with a 0-d DP were fed a glucogenic or lipogenic ration in wk 8 – 44 with a low [0-d DP(LOW)] energy level, based on their requirement for their expected milk yield, or a standard [0-d DP(STD)] energy level that cows with a 30-d DP [30-d DP(STD)] received based on the requirement for their expected milk yield), and milk lactose (e) and protein (f) content for cows with different parities (2 or ≥ 3).

3.4.2 Dry matter intake, energy balance, and hormones

Energy intake and the plasma IGF-1 concentration were greater for cows fed a G ration, compared with cows fed an L ration ($P < 0.05$) (**Table 3.4**). However, EB was not affected by ration. In line with the experimental contrast made, an interaction was present between transition treatment and week for concentrate intake. Concentrate intake was lower in cows with a 0-d DP(LOW), than in cows with a 0-d DP(STD) or 30-d DP(STD) from wk 4 until 30 in lactation ($P < 0.01$). Energy intake and EB were lower for cows with 0-d DP(LOW), compared with cows with a 0-d DP(STD). Dietary energy level did not affect BW, BCS or plasma insulin, IGF-1 or GH concentration for cows with 0-d DP, but cows with a 0-d DP(LOW) had a lower weekly BW gain than cows with a 0-d DP(STD). Omitting the dry period reduced energy intake, energy efficiency, and the plasma GH concentration (only for cows with a 0-d DP(STD)), and increased the EB, BW, weekly body weight gain, and the plasma insulin and IGF-1 concentration [only for cows with a 0-d DP(STD)], compared with a DP of 30-d ($P < 0.05$). Cows with parity 2 had a lower BW (661 ± 8 vs. 716 ± 8 kg), and a greater weekly body weight gain (2.3 ± 0.1 vs. 1.3 ± 0.1 kg/wk) and plasma IGF-1 concentration (167.7 ± 5.2 vs. 147.8 ± 5.2 ng/mL), compared with cows with parity ≥ 3 ($P < 0.01$).

Footnotes for table3.4

[a-c] Values within transition treatment with different superscript letters differ ($P < 0.05$). [1]From week 8 week postpartum onwards, cows received a basal ration with either glucogenic (G) or lipogenic (L) energy sources. [2]Transition treatment: Cows with a 0-d DP were fed a glucogenic or lipogenic ration with either a low [0-d DP(LOW)] energy level, based on their requirement for their expected milk yield, or a standard [0-d DP(STD)] energy level based on the expected milk yield of cows with a 30-d DP [30-d DP(STD)]. [3]R = ration, T = transition treatment, P = parity, W = week after calving. [4]Energy efficiency = E_L / NE_I, where E_L = milk energy output (kJ/d) and NE_I = NE intake (kJ/d). [5]BCS; analyzed with a repeated measures effect of month after calving with cow as subject, instead of week after calving. [6]The interaction of transition treatment and ration was present for DMI, basal ration intake, BCS, and the plasma insulin and growth hormone concentration. DMI: 0-d DP(LOW) × G: 21.9 ± 0.25, 0-d DP(LOW) × L: 19.9 ± 0.23, 0-d DP(STD) × G: 21.4 ± 0.24, 0-d DP(STD) × L: 20.6 ± 0.24, 30-d DP(STD) × G: 21.8 ± 0.24, 30-d DP(STD) × L: 20.9 ± 0.22. Basal ration intake: 0-d DP(LOW) × G: 18.3 ± 0.25, 0-d DP(LOW) × L: 16.3 ± 0.24, 0-d DP(STD) × G: 17.2 ± 0.25, 0-d DP(STD) × L: 16.3 ± 0.25, 30-d DP(STD) × G: 17.6 ± 0.24, 30-d DP(STD) × L: 16.6 ± 0.22. BCS: 0-d DP(LOW) × G: 2.91 ± 0.12, 0-d DP(LOW) × L: 3.13 ± 0.1, 0-d DP(STD) × G: 3.29 ± 0.11, 0-d DP(STD) × L: 2.79 ± 0.11, 30-d DP(STD) × G: 2.74 ± 0.11, 30-d DP(STD) × L: 2.78 ± 0.10. Plasma insulin: 0-d DP(LOW) × G: 23.48 ± 1.66, 0-d DP(LOW) × L: 23.48 ± 1.61, 0-d DP(STD) × G: 29.88 ± 1.55, 0-d DP(STD) × L: 20.61 ± 1.36, 30-d DP(STD) × G: 18.34 ± 1.44, 30-d DP(STD) × L: 17.71 ± 1.19. Plasma growth hormone: 0-d DP(LOW) × G: 3.71 ± 0.20, 0-d DP(LOW) × L: 3.51 ± 0.20, 0-d DP(STD) × G: 2.98 ± 0.19, 0-d DP(STD) × L: 3.70 ± 0.16, 30-d DP(STD) × G: 3.62 ± 0.17, 30-d DP(STD) × L: 4.08 ± 0.13.

Table 3.4: Dry matter intake, energy balance (EB), and plasma metabolites during week 1 to 44 of lactation in cows after a 0 or 30 days dry period. Cows received a glucogenic (G) or lipogenic (L) ration in wk 8 – 44. Cows with a 0-d DP were provided either a low [0-d DP(LOW)] dietary energy level, or the standard [0-d DP(STD)] dietary energy level that cows with a 30-d DP received [30-d DP(STD)]

	Ration[1]			Transition treatment[2]				*P*-values[3]									
	G	L	SEM	0-d DP (LOW)	0-d DP (STD)	30-d DP (STD)	SEM	R	T	P	W	R × T[6]	R × P	T × P	R × W	T × W	P × W
Cows (n)	53	57		36	34	40											
DMI (kg DM/d)	21.7	20.4	0.14	20.8	20.9	21.3	0.2	<0.01	0.08	<0.01	<0.01	0.04	0.02	0.55	0.05	0.03	0.09
Concentrate intake (kg DM/d)	4.0	4.0	0.01	3.6[a]	4.2[b]	4.2[b]	0.01	0.14	<0.01	0.18	<0.01	0.34	0.47	0.07	0.29	<0.01	0.67
Basal ration intake (kg DM/d)	17.7	16.4	0.14	17.2	16.7	17.1	0.17	<0.01	0.07	<0.01	<0.01	0.05	0.02	0.60	0.04	0.03	0.06
Energy intake ($kJ/kg^{0.75}\cdot d$)	1061	1021	9	990[a]	1039[b]	1094[c]	11	<0.01	<0.01	0.18	<0.01	0.18	0.14	0.02	0.34	<0.01	0.10
Energy output in milk ($kJ/kg^{0.75}\cdot d$)	685	651	19	616[a]	626[a]	762[b]	23	0.20	<0.01	0.73	<0.01	0.39	0.55	0.03	0.76	<0.01	0.81
EB ($kJ/kg^{0.75}\cdot d$)	50	48	10	51[a]	92[b]	4[c]	12	0.89	<0.01	0.56	<0.01	0.59	0.98	0.04	0.57	0.18	0.07
Energy efficiency[4]	0.64	0.63	0.02	0.60[a]	0.59[a]	0.70[b]	0.02	0.66	<0.01	0.92	<0.01	0.39	0.94	0.09	0.73	0.33	<0.01
BW (kg)	692	683	8	699[a]	699[a]	665[b]	10	0.44	0.02	<0.01	<0.01	0.59	0.96	0.16	0.69	<0.01	<0.01
BW gain (kg/wk)	1.9	1.8	0.2	1.9[a]	2.6[b]	1.0[c]	0.2	0.44	<0.01	<0.01	<0.01	0.40	0.51	0.04	0.58	<0.01	<0.01
BCS[5]	2.99	2.90	0.06	3.04[a]	3.02[a]	2.77[b]	0.08	0.29	0.03	0.08	<0.01	<0.01	0.26	0.11	0.16	0.30	0.13
Insulin (μU/mL)	23.9	20.6	1.0	23.5[a]	25.2[a]	18.0[b]	1.2	0.01	<0.01	0.79	<0.01	<0.01	0.28	0.12	0.36	0.18	0.66
IGF-1 (ng/mL)	164.8	150.7	5.1	159.7[ab]	170.7[a]	142.9[b]	6.2	0.05	<0.01	<0.01	<0.01	0.13	0.79	0.14	0.80	0.42	0.19
Growth hormone (μg/L)	3.47	3.77	0.11	3.62[ab]	3.23[a]	4.01[b]	0.14	0.06	<0.01	0.30	<0.01	<0.01	0.55	0.14	0.96	0.08	0.99
Days to conception	100	108	7	84[a]	114[b]	113[b]	9	0.45	0.03	0.02		<0.01	0.36	0.36			
Services per pregnancy	2.82	3.00	0.33	2.80	2.76	3.18	0.40	0.68	0.71	0.86		0.59	0.22	0.94			
Pregnant within 44 wks	88%	96%		92%	91%	93%		1.00	0.99	0.99		0.99	0.99	0.99			

Dry matter intake (**DMI**), basal ration intake, BCS, and plasma insulin and GH concentration were affected by an interaction of ration with transition treatment. Cows with a 0-d DP(LOW) or 30-d DP(STD) fed a G ration had a greater DMI (**Figure 3.3a**) and basal ration intake than cows fed an L ration, ($P <$ 0.05). A G ration, compared with an L ration, increased BCS (**Figure 3.3b**) and the plasma insulin concentration (**Figure 3.3c**) of cows with a 0-d DP(STD), and decreased the plasma GH concentration (**Figure 3.3d**) of cows with a 0-d DP(STD) and a 30-d DP(STD), but did not affect cows with other transition treatments. Energy intake, energy output in milk, and EB were affected by an interaction of transition treatment with parity, and DMI and basal ration intake were affected by an interaction of ration with parity. Cows with parity 2 had greater energy intake (**Figure 3.3e**) and energy output in milk (**Figure 3.3f**), but a lower EB (**Figure 3.3g**), than cows with parity ≥ 3, after a 30-d DP ($P < 0.05$), but parity did not affect the EB of cows with a 0-d DP. The DMI (**Figure 3.3h**) and basal ration intake were affected by an interaction of ration with parity.

The interaction of transition treatment with week after calving was present for DMI (**Figure 3.4a**), concentrate intake, basal ration intake, energy intake (**Figure 3.4b**), energy output in milk (**Figure 3.4c**), BW (**Figure 3.4d**), and weekly BW gain ($P < 0.05$), but not for EB (**Figure 3.4e**), or for the plasma insulin (**Figure 3.4f**), IGF-1 (**Figure 3.4g**) or GH concentration (**Figure 3.4h**) ($P \geq 0.05$). The interaction of ration with wk after calving was present for DMI (**Figure 3.4i**), and basal ration intake ($P < 0.05$). The interaction of parity with wk after calving was present for energy efficiency, BW, and weekly BW gain ($P <$ 0.01).

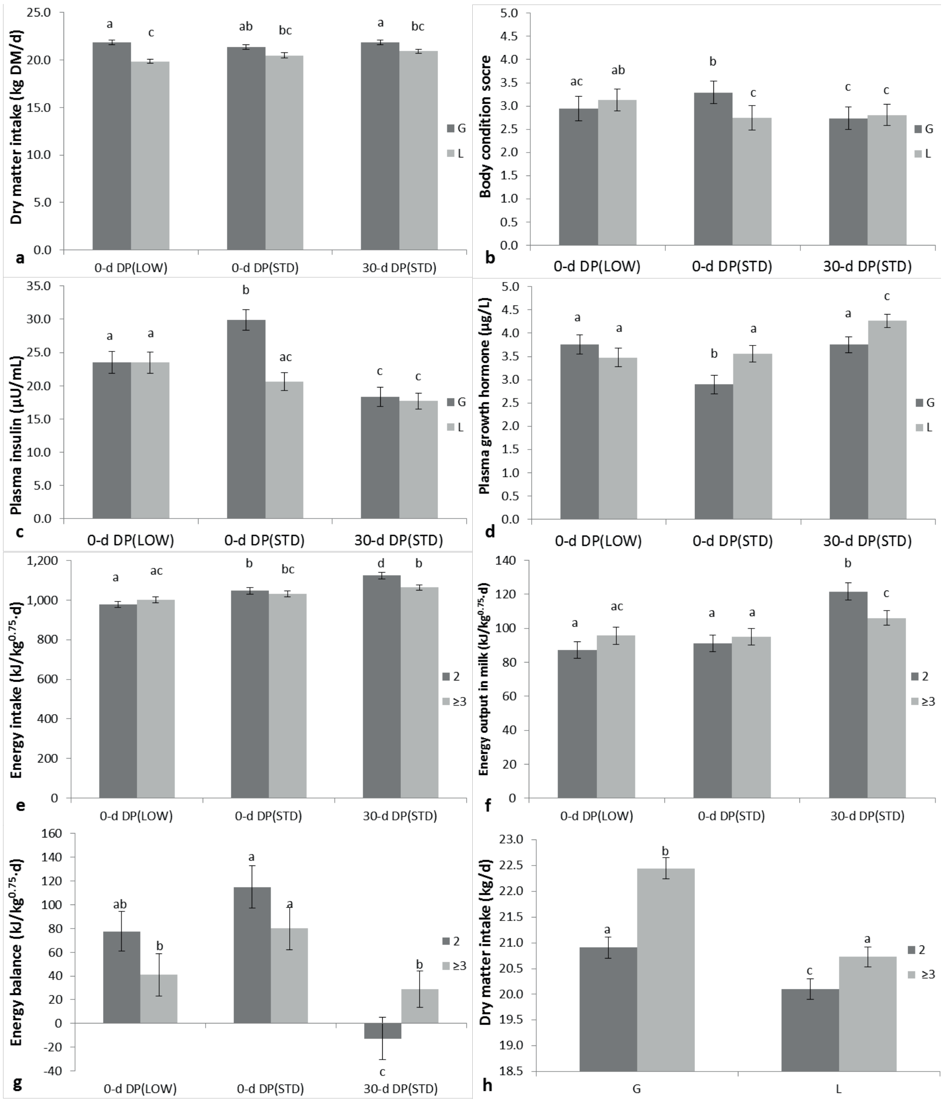

Figure 3.3: The effect of the interaction between transition treatment (cows with a 0-d DP fed a low energy level [0-d DP(LOW)], based on their requirement for their expected milk yield, or a standard [0-d DP(STD)] energy level that cows with a 30-d DP [30-d DP(STD)] received based on the requirement for their expected milk yield) and postpartum ration [glucogenic (G) in dark grey or lipogenic (L) in light grey] fed in wk 8 – 44, or parity (2 in dark grey or ≥ 3 in light grey) for dry matter intake (a), body condition score (b), plasma insulin concentration (c), plasma growth hormone concentration (d), energy intake (e), energy output in milk (f), energy balance (g), and the interaction between ration and parity for DMI (h). Values with different letters (a-c) differ ($P < 0.05$). Results were presented as LSM ± SEM.

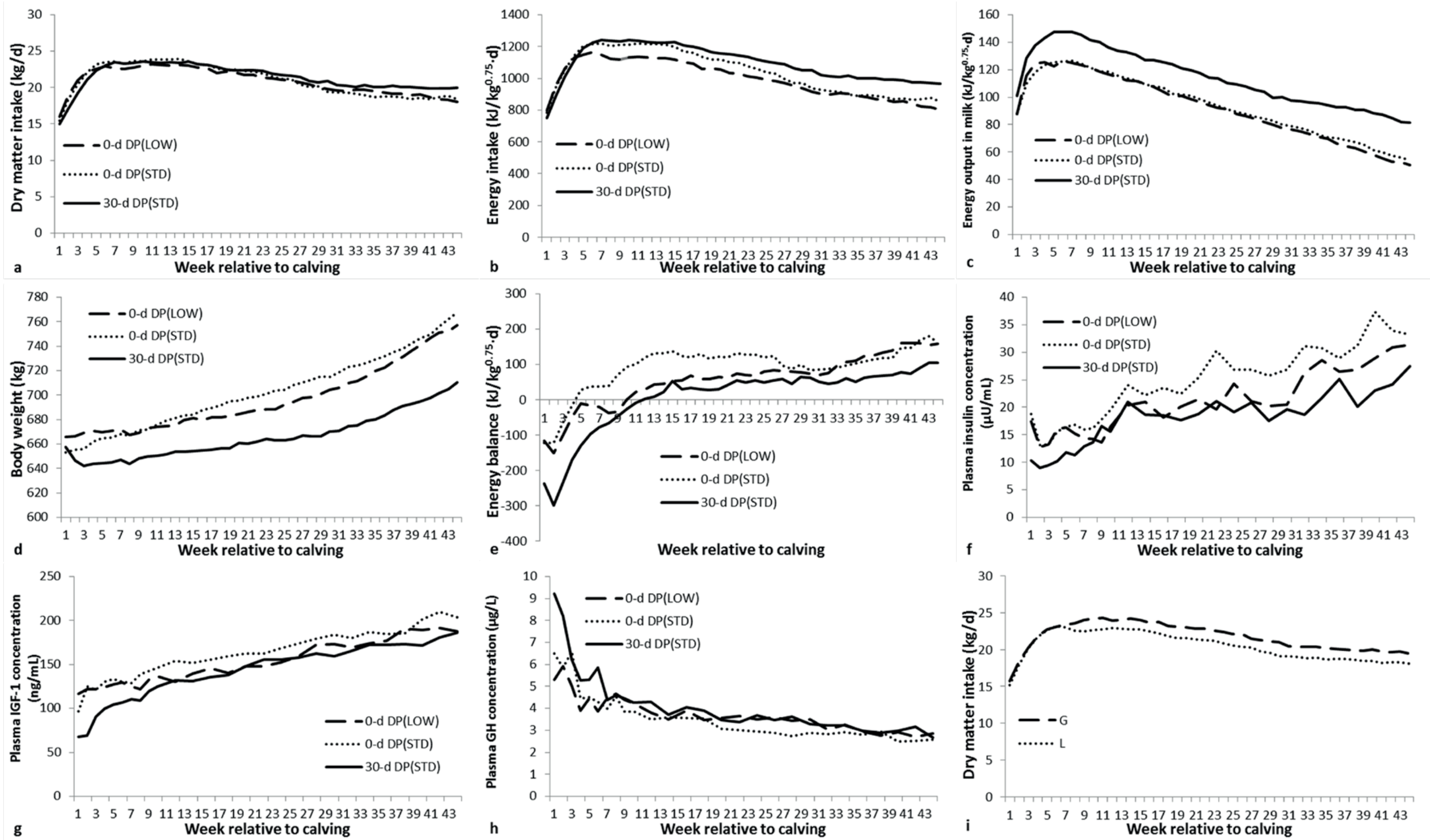

Figure 3.4: Dry matter intake (a), energy intake (b), energy output in milk (c), body weight (d), energy balance (e), plasma insulin (f), plasma IGF-1 (g) and plasma GH (h) for cows with different DP lengths and dietary energy level (cows with a 0-d DP were fed a glucogenic or lipogenic ration with a low [0-d DP(LOW)] energy level, based on their requirement for their expected milk yield, or a standard [0-d DP(STD)] energy level that cows with a 30-d DP [30-d DP(STD)] received based on the requirement for their expected milk yield), and dry matter intake (i) for cows with different postpartum rations [glucogenic (G) or lipogenic (L)] fed in wk 8 – 44.

3.4.3 Lactation curve characteristics

Ration did not affect initial FPCM yield (*a*), the decreasing slope of the lactation curve (*c*), lactation persistency (*S*), and the relative rate of decline of FPCM yield midway between peak lactation and 44 wks in lactation (**Table 3.5**). An interaction was present between ration and transition treatment on the increasing slope of the lactation curve (*b*) ($P = 0.01$). A G ration resulted in a greater increasing slope (*b*) in cows with a 0-d DP(STD), but in a lower *b* in cows with a 30-d DP(STD), while it did not affect cows with a 0-d DP(LOW) (**Figure 3.5a**). Dietary energy level did not affect initial FPCM yield (*a*), the decreasing slope of the lactation curve (*c*), lactation persistency (*S*), and the relative rate of decline of FPCM yield. Initial FPCM yield (*a*), peak FPCPM yield, and total predicted lactation yield were greater for cows with a 30-d DP than in cows with a 0-d DP ($P < 0.05$). The increasing (*b*) and decreasing slope (*c*) of the lactation curve, and the relative rate of decline in FPCM yield were lower for cows with a 30-d DP than for cows with a 0-d DP ($P < 0.05$). Cows with parity 2 had lower peak FPCM yield than cows with parity ≥ 3 (35.1 ± 0.8 vs. 38.7 ± 0.8 kg FPCM/d) ($P < 0.01$).

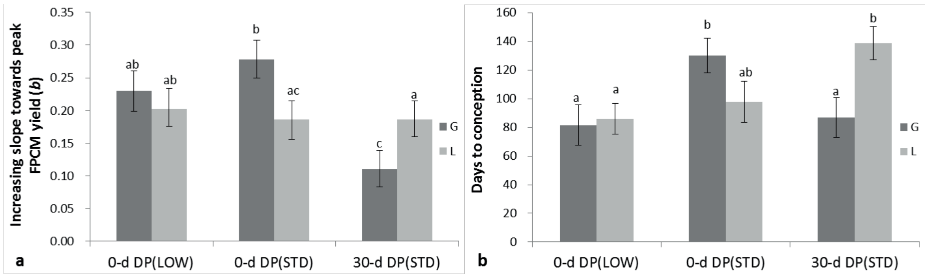

Figure 3.5: The effect of the interaction between transition treatment (cows with a 0-d DP fed a low energy level [0-d DP(LOW)], based on their requirement for their expected milk yield, or a standard [0-d DP(STD)] energy level that cows with a 30-d DP [30-d DP(STD)] received based on the requirement for their expected milk yield) and postpartum ration [glucogenic (G) in dark grey or lipogenic (L) in light grey] fed in wk 8 – 44, for the parameter representing the increasing slope towards peak FPCM yield (a) and days to conception (b). Values with different letters (a-c) differ ($P < 0.05$). Results were presented as LSM ± SEM.

3.4.4 Conception

The number of services per pregnancy and the proportion of cows pregnant within 44 wks of lactation were not affected by ration, transition treatment, parity or any of the interactions (**Table 3.4**). Days to conception was affected by an interaction between ration and transition treatment. Cows with a 0-d DP(STD) fed a G ration and cows with a 30-d DP fed an L ration had a greater number of days to conception than cows in other groups (**Figure 3.5b**). Cows with a 30-d DP(STD) or 0-d DP(STD) had more days to conception than cows with a 0-d DP(LOW) (P = 0.03). Cows with parity ≥ 3 (116 ± 8 days) had a greater number of days to conception than cows with parity 2 (91 ± 7 days) (P = 0.02).

3.4.5 Correlations between lactogenic hormones and lactation curve characteristics

In wk 1 – 7, negative correlations of EB, plasma insulin and IGF-1 concentration were present with initial milk yield (*a*). Positive correlations of EB, plasma insulin and IGF-1 concentration were present with the increasing (*b*) and decreasing slope (*c*) of the lactation curve (**Table 3.6a**). Additionally, positive correlations of EB and the plasma IGF-1 concentration were present with persistency (*S*), and negative correlations of EB and the plasma insulin concentration were present with the relative rate of decline in FPCM yield. A positive correlation of the plasma GH concentration with initial milk yield (*a*), and a negative correlation of the plasma GH concentration with the increasing (*b*) slope of the lactation curve were present. In wk 8 – 44, negative correlations of EB, plasma insulin and IGF-1 concentration were present with initial milk yield (*a*) and the relative rate of decline in FPCM yield. Positive correlations of EB, plasma insulin and IGF-1 concentration were present with the increasing (*b*) and decreasing slope (*c*) of the lactation curve, (**Table 3.6b**). There was also a positive correlation of the plasma GH concentration with initial milk yield (*a*), and a negative correlation of the plasma GH concentration with the increasing (*b*) slope of the lactation curve.

Table 3.5: Lactation curve characteristics during week 1 to 44 of lactation in cows after a 0 or 30 days dry period. Cows received a glucogenic (G) or lipogenic (L) ration in wk 8 – 44. Cows with a 0-d DP were provided either a low [0-d DP(LOW)] dietary energy level, or the standard [0-d DP(STD)] dietary energy level that cows with a 30-d DP received [30-d DP(STD)]

	Ration[1]			Transition treatment[2]				*P*-values[3]					
	G	L	SEM	0-d DP (LOW)	0-d DP (STD)	30-d DP (STD)	SEM	R	T	P	R × T	R × P	T × P
Cows (n)	51	55		35	34	37							
a^4	22.5	22.8	1.3	21.0[a]	19.4[a]	27.6[b]	1.6	0.87	<0.01	0.76	0.08	0.19	0.08
b^4	0.216	0.192	0.016	0.216[a]	0.232[a]	0.149[b]	0.021	0.54	0.01	0.60	0.01	0.28	0.08
c^4	0.0041	0.0042	0.0003	0.0047[a]	0.0046[a]	0.0031[b]	0.0003	0.88	<0.01	0.19	0.10	0.33	0.26
S^4	6.75	6.63	0.06	6.60	6.74	6.73	0.07	0.14	0.26	0.11	0.33	0.58	0.75
Relative rate of decline (/d)[5]	-0.0030	-0.0031	0.00021	-0.0035[a]	-0.0033[a]	-0.0023[b]	0.00026	0.66	<0.01	0.16	0.22	0.43	0.44
Peak milk yield (kg FPCM/d)	37.6	36.0	0.8	35.9[a]	35.3[a]	39.2[b]	1.0	0.17	0.02	<0.01	0.73	0.44	0.64
Day of peak milk yield	52	47	3	47	52	49	3	0.18	0.56	0.34	0.10	0.69	0.59
Total lactation yield, predicted[6] (kg FPCM)	9117	8591	232	8266[a]	8409[a]	9887[b]	284	0.11	<0.01	0.26	0.49	0.40	0.31

[a-c] Values within transition treatment with different superscript letters differ ($P < 0.05$). [1]From week 8 week postpartum onwards, cows received a basal ration with either glucogenic (G) or lipogenic (L) energy sources. [2]Transition treatment: Cows with a 0-d DP were fed a glucogenic or lipogenic ration with either a low [0-d DP(LOW)] energy level, based on their requirement for their expected milk yield, or a standard [0-d DP(STD)] energy level based on the expected milk yield of cows with a 30-d DP [30-d DP(STD)]. [3]R = ration, T = transition treatment, P = parity. [4]*a, b, c, S* represent initial fat and protein corrected milk (FPCM) yield (kg/d), the increasing slope, the decreasing slope, and persistency according to Wood's lactation curve, respectively. [5]Relative rate of decline midway between peak lactation and 44 weeks in lactation calculated according to Dijkstra et al. (2010). [6]Predicted total lactation yield (from calving until 305 DIM) was modelled according to Wood (1967).

Table 3.6: Pearson correlation coefficients (P < 0.05) of average lactation curve characteristics, plasma hormones, milk yield, and energy balance in wk 1 – 7 (a) and wk 8 – 44 (b)

a	*a*[1]	*b*[1]	*c*[1]	*S*[1]	Rd[1]
EB (kJ/kg$^{0.75}$·d) [2]	-0.65**	0.47**	0.30**	0.30**	-0.22*
Insulin (μU/mL)	-0.51**	0.40**	0.37**	Ns	-0.33**
IGF-1 (ng/mL)[2]	-0.45**	0.37**	0.20*	0.29**	Ns
GH (μg/L)[2]	0.27**	-0.25*	Ns	Ns	Ns

b	*a*[1]	*b*[1]	*c*[1]	*S*[1]	Rd[1]
EB (kJ/kg$^{0.75}$·d) [2]	-0.40**	0.46**	0.60**	Ns	-0.63**
Insulin (μU/mL)	-0.37**	0.39**	0.48**	Ns	-0.50**
IGF-1 (ng/mL)[2]	-0.29**	0.32**	0.27**	Ns	-0.25**
GH (μg/L)[2]	0.24*	-0.20*	Ns	Ns	Ns

**P* < 0.05, ***P* < 0.01, Ns = not significant. [1]Initial milk yield (*a*), the increasing slope of the lactation curve (*b*), the declining slope of the lactation curve (*c*), persistency (*S*) were calculated according to Wood (1967), and the relative rate of decline at midway point between peak and end of lactation (Rd) was calculated according to Dijkstra et al. (2010). [2]EB = energy balance, IGF-1 = insulin-like growth factor-1, GH = growth hormone.

3.5 Discussion

3.5.1 Milk yield, DMI, and energy balance

A G ration fed from wk 8 to 44 in lactation increased milk, lactose and protein yield, and decreased milk fat content, compared with an L ration. The lower fat content in milk of cows with a G ration may be a result of dilution due to the greater milk yield, because fat yield was not different between cows with a G or L ration. Overall, FPCM yield was not different between cows with a G or L ration. Earlier studies also found an increase in milk fat content and fat yield with a more lipogenic ration in early lactation, compared with a glucogenic ration (**Van Knegsel *et al.***, 2007a; **Garnsworthy *et al.***, 2009; **Mahjoubi *et al.***, 2009). In the current study, the L ration was characterized by a much greater proportion of beet pulp and grass silage, and no corn silage, resulting in a greater availability of lipogenic precursors, than the G ration. In cows with a G ration, the greater milk, lactose and protein yield may result from the greater availability of glucogenic precursors compared with cows with an L ration. In earlier studies, however, a glucogenic ration in early lactation did not result in an increase in milk, lactose or protein yield, compared with a more lipogenic ration (**Van Knegsel *et al.***, 2007a; **Garnsworthy *et al.***, 2008; **Garnsworthy *et al.***, 2009). This difference in effect of a glucogenic ration on milk yield and composition among studies could possibly be explained by the stage of lactation: in the current study the contrast in dietary energy source was compared during mid and late lactation, while in the earlier studies the lipogenic and glucogenic diets were fed during the first 8 wks (**Van Knegsel *et al.***, 2007a), between wk 6 and 10 wks of lactation, or during the first 17 wks of lactation (**Garnsworthy *et al.***, 2008; **Garnsworthy *et al.***, 2009). In contrast with mid- or late- lactation, cows in early lactation usually mobilize energy from body reserves, which mainly consists of lipogenic precursors, and may have a relative shortage of glucogenic precursors for fat oxidation, resulting in no extra partitioning of glucogenic nutrients towards the mammary gland, when a glucogenic diet is fed (**Davis and Collier**, 1985). Moreover, in the current study cows fed the G ration had a greater DMI, compared with cows fed the L ration. Also in earlier studies, feeding beet pulp reduced DM intake, milk production and BCS in mid-lactation cows, in comparison with feeding grain (**Voelker and Allen**, 2003). In another study, beet pulp decreased BCS and plasma glucose but increased milk fat and energy content in late lactation (**Mahjoubi *et al.***, 2009). The greater observed DMI and milk production and reduced milk fat content of cows fed a G ration compared with an L ration is in line with the changes observed upon replacing grass silage with corn silage in a previous study (**Abrahamse *et al.***, 2008). In the present experiment, where the contrast between glucogenic and lipogenic nutrients was introduced after 49 DIM, no effects on EB were observed, since the extra energy (+4%) consumed with a G ration, compared with an L ration, led to a numerical increase (+5%) in milk energy output. Additionally, in the current study, the greater DMI of cows with a G ration may result from the greater DM content of this ration, which may have increased DMI and energy intake, compared with the L ration. A low DM content of a ration may decrease DMI (**Zom *et al.***, 2012).

3

The lower dietary energy level fed from calving till wk 44 in lactation for cows after a 0-d DP did not affect milk energy output, BW, or BCS, but did decrease EB and weekly BW gain. Previous research showed that in heifers, additional energy intake through concentrate increased peak milk yield and BW (**Broster *et al.***, 1975). Another study showed that lower energy intake, through less concentrate, decreased energy-corrected milk, EB, and BCS (**Reist *et al.***, 2003). Additionally, a previous study on extending the lactation in a pasture-based system showed that cows fed a high energy level had greater milk yield and BCS at the end of lactation, compared with cows fed a control ration (**Grainger *et al.***, 2009). Comparison of our findings with these previous studies suggest that, in our experiment, cows with a 0-d DP(LOW) did receive sufficient energy for body maintenance and milk production. It should be noted that the contrast in energy intake between LOW and STD groups was smaller than expected. In line with previous studies e.g. (**Dieho *et al.***, 2016), cows offered a lower amount of concentrate increased their intake of the basal ration, herewith partly compensating for the lower intake of energy with concentrate. Cows with a 0-d DP(STD) had a lower BW at calving, but weekly BW gain of cows with a 0-d DP(STD) was greater as a result of a greater energy intake resulting in similar average BW and BCS during the 44 wk experimental period compared with cows with a 0-d DP(LOW). Weekly BW gain may be a more accurate measure for fattening than average BW or BCS for lactation. Dietary energy level, therefore, can be reduced for cows with a 0-d DP, to aim for zero EB, without detrimental effects on metabolic status or milk production, and may reduce fattening and feeding costs.

Cows with a 0-d DP had a 5 kg lower daily FPCM production (27.2 vs. 32.3 ± 0.8 kg FPCM/d; wk 1 - 44) than cows with a 30-d DP. This is in line with previous work where cows with a 0-d DP (35.4 kg FPCM/d) had a 5 kg lower FPCM production than cows with a 30-d DP (40.4 kg FPCM/d) in wk 1 - 14 (**Van Knegsel *et al.***, 2014) or in wk 1 – 44 (**Kok *et al.***, 2016). The consistency in milk production difference between cows with a 0-d or 30-d DP suggests that a DP length predisposes for a certain milk production capacity during the subsequent lactation.

3.5.2 Lactogenic hormones

Cows fed a G ration had a greater plasma insulin concentration than cows fed the L ration in the 0-d DP(STD) treatment, but not in the other transition treatments. Cows fed a G ration tended to have a greater IGF- 1 concentration, and a lower GH concentration (the latter only in STD but not in LOW ration), compared with cows fed an L ration. The tendency to a greater plasma IGF - 1 concentration for cows fed the G ration indicates a more coupled somatotropic axis (**Butler *et al.***, 2003; **Wathes *et al.***, 2007), resulting in a greater negative feedback on GH release from the pituitary gland (**Lucy**, 2008). Furthermore, as insulin and GH have antagonistic roles, a greater plasma insulin and lower GH concentration in mid- and late- lactation can be expected to result in fewer nutrients partitioned towards the mammary gland, resulting in a reduced milk yield (**Hart**, 1983). However, in the current study, greater milk yield was observed in cows with a G ration, compared with cows with an L ration. Cows with a G or an L ration had a positive EB, so it can be hypothesized that the somatotropic axis was coupled for both rations, and that feed intake and milk yield are closely linked (**Lucy *et al.***, 2009). The greater milk yield in cows with a

G ration was, therefore, more likely a result of greater DMI, than of a difference in the plasma insulin, IGF-1 or GH concentration, compared with cows with an L ration.

Reducing dietary energy level for cows with a 0-d DP did not affect lactogenic hormone concentration. Reducing dietary energy level decreased energy intake and EB, but this effect was possibly not large enough to affect concentrations of lactogenic hormones. In several earlier studies, a reduction of dietary energy level through a lower concentrate level was related with a lower a lower plasma glucose, insulin, and IGF-1 concentration (**Reist *et al.***, 2003), while another study reported that the plasma insulin concentration was not affected by a lower concentrate level (**Kokkonen *et al.***, 2004). In a previous study, in wk 1 – 16 of lactation, North-American Holstein cows with a low supplementation additional to pasture had lower milk yield, plasma IGF-1 concentration, tended to have a greater plasma GH concentration, but the plasma insulin concentration was not affected, compared with cows with a high supplementation (**Lucy *et al.***, 2009). Differences in lactogenic hormone concentrations within studies are likely related with the difference in dietary energy level between treatment groups.

Cows with a 30-d DP had a lower plasma insulin and IGF-1 concentration, and greater GH concentration, compared with cows with a 0-d DP. Previous studies reported a lower insulin (**Andersen *et al.***, 2005; **Chen *et al.***, 2015) and IGF-1 (**Chen *et al.***, 2015) concentration for cows with a short DP, compared with no DP. The difference in lactogenic hormone concentrations between DP lengths reflects the EB of cows with a 30-d DP compared with cows with a 0-d DP, and the degree of coupling of the somatotropic axis (**Butler *et al.***, 2003).

In general, the plasma GH concentration had a low correlation with measures of lactation persistency. Similarly, in an earlier study, the plasma GH concentration was only slightly related with lactation persistency (**Sorensen and Knight**, 2002). Administration of exogenous GH was reported to increase lactation persistency to a greater extent (**Van Amburgh *et al.***, 1997; **Bauman**, 1999). The difference in effect of endogenous and exogenous GH on lactation persistency could be related with a difference in dose between endogenously synthesized versus exogenously administered GH. In a previous study, exogenous administration of bST resulted in a 2.4 times increase of the plasma GH concentration (14.2 vs 6.0 ng/mL) and increased milk yield up to 7 kg/d compared with cows not treated with bST (**Bilby *et al.***, 2006), while in the current study, an L ration increased the plasma GH concentration with 1.1 times compared with a G ration. The effect of GH could also be related with the complex relation of exogenous or endogenous GH with IGF-1 as mediator in the somatotropic axis and may depend on differences in the plasma IGF-1 concentration, the local mammary IGF-1 concentration, the presence of IGF-1 receptors, and types of and presence of insulin-like growth factor binding proteins (**Capuco *et al.***, 2003).

3.5.3 Lactation curve characteristics

We hypothesized that dietary energy source affects lactation curve characteristics through effects of energy source on the plasma insulin, IGF-1 and GH concentrations. An L ration was expected to result in reduced plasma insulin and IGF-1, and increased plasma GH concentration, due to a lower availability of glucogenic precursors (**Voelker and Allen**, 2003; **Mahjoubi *et al.***, 2009). Indeed, cows fed the L ration after wk 8 in lactation had a greater plasma GH concentration in the STD groups, as discussed above, but this was not related with effects on characteristics of the lactation curve. Lack of effect of an L ration on lactation curve characteristics could be due to the lower DMI of these cows. The lower DMI of cows with an L ration resulted in a lower milk production than for cows with a G ration, despite GH levels being greater, and insulin and IGF-1 levels being lower in cows with an L ration, than in cows with a G ration. The lack of effect of dietary energy source on lactation curve characteristics could also be related with introduction of ration contrast in wk 8, and not from calving onwards. A previous study shows that feeding a G or L concentrate from calving to 100 DIM affected day of peak milk yield in an interaction with parity, but peak milk yield or lactation persistency were not affected by dietary energy source (**Chen *et al.***, 2016a).

The lower dietary energy level for cows with a 0-d DP did not affect lactation curve characteristics, which is in line with a previous study (**Broster *et al.***, 1975). Dietary energy level negatively affects persistency in case of underfeeding (**Chilliard**, 1992). Feeding the energy level for cows with a 30-d DP to cows with a 0-d DP did not affect persistency in the current study. In a previous study, feeding the energy level for cows with a 60-d DP to cows with a 0-d DP did seem to negatively affect persistency, and resulted in spontaneous drying off (**Chen *et al.***, 2016a). Additionally, a previous study on extending the lactation in a pasture-based system showed that a greater proportion of cows fed a high energy level dried off spontaneously at the end of lactation, compared with cows fed a control ration (**Grainger *et al.***, 2009).

Our hypothesis was that not only dietary energy source, but also DP length affects lactation curve characteristics. Cows with a 30-d DP had a higher initial milk yield, lower increasing and declining slope of the lactation curve, and greater total milk yield. In cows with a 30-d DP, the decreasing slope of the lactation curve (c) and the relative rate of decline (Rd), as measures for persistency, were smaller, indicating greater persistency than in cows with a 0-d DP. This is in line with a previous study that showed greater persistency in cows after a short DP, compared with cows with an omitted DP (**Mantovani *et al.***, 2010), but is in contrast to another study that showed no effect of DP length on persistency (**Chen *et al.***, 2016a). Lactation persistency in the different studies was measured using different variables, which may explain the somewhat contrasting results. During the dry period, renewal and regeneration of secretory udder cells occurs (**Capuco *et al.***, 1997; **Martignani *et al.***, 2014), which may help to explain the difference in persistency between 0-d DP and 30-d DP in the present study. Lack of sufficient time to renew secretory cells in cows with a 0-d DP may have resulted in elevated numbers of senescent cells with a lower secretory capacity, which may have been detrimental to milk yield at any time in lactation, including lactation persistency. Grummer and Rastani (2004) hypothesized that the better EB during early lactation for cows with a 0-d DP potentially enables the cow to increase regeneration of udder cells during lactation, resulting in a better lactation persistency. However, in the current study the greater milk yield and lower EB in cows with a 30-d DP were associated with a lower plasma IGF-1, greater plasma GH concentration and greater nutrient partitioning towards milk yield in these cows, compared with cows with a 0-d DP (**Lucy *et al.***, 2009).

Considering dietary energy source and level, and DP length, changes in the plasma GH, IGF-1, and insulin concentrations due to differences in DP length were associated with changes in milk production related to DP length. Thus, the greater magnitude of milk production response to DP length treatment may be related with the fact that a greater plasma GH concentration for cows with a 30-d DP was associated with an improved lactation persistency, whereas a greater plasma GH concentration for cows with an L ration was not associated with an increase in milk yield or improved lactation persistency. It could also be

3

hypothesized that this difference in the effect of dietary energy source versus DP length treatment on lactation persistency and hormone concentrations could be related to the observed responses in EB. Dry period length did affect EB in this study, while dietary energy source did not. Further studies are needed to clarify the difference in effect of management strategies on the relation of the plasma GH concentration with milk yield and lactation persistency.

3.5.4 Parity and pregnancy

Other known cow factors that influence lactation persistency are parity and pregnancy. In the current study, the plasma IGF-1 concentration was greater in cows with parity 2, compared with cows with parity ≥ 3, which is in line with a previous study (**Miller *et al.***, 2006). Insulin-like growth factor 1 plays an important role in cell survival and as an anti-apoptotic factor in mammary tissue, and may, therefore, result in greater cell survival and greater persistency in cows with parity 2 than in cows with parity ≥ 3 (**Flint and Knight**, 1997). In contrast, the plasma GH concentration was not different between parity groups in the current study. In line with the plasma GH concentration, lactation curve characteristics were not affected by parity, while previous studies reported greater persistency in primiparous cows compared with multiparous cows (**Gengler**, 1996; **Rehn *et al.***, 2000; **Ehrlich**, 2011). However, to our knowledge, previous studies did not reported on the plasma GH concentration of cows with different parity.

In the current study, cows with a 0-d DP(LOW) were pregnant earlier than cows with a 30-d DP(STD) or a 0-d DP(STD). Cows with a 0-d DP(LOW) had a greater EB than cows with a 30-d DP(STD), which may be beneficial for reproduction in early lactation (**Butler and Smith**, 1989). However, cows with a 0-d DP(STD) had an even greater EB, though the number of days to conception was larger than for the 0-d DP(LOW) cows. Previous research showed that cows with a 0-d DP had a greater EB and had an earlier resumption of cyclicity postpartum than cows with a short or conventional DP (**Gümen *et al.***, 2005; **Chen *et al.***, 2015). In the current study, cows with a 0-d DP(LOW), received more straw (i.e, more lipogenic components), which was associated with a numerically lower plasma insulin concentration despite a positive EB compared with cows with a 0-d DP(STD). A low plasma insulin concentration, combined with a positive EB, was earlier related with more medium-sized follicles, less small-sized follicles, and an earlier postovulatory increase of progesterone, resulting in a greater mean plasma progesterone concentration between 3 – 5 d post-ovulation, compared with cows with a greater plasma insulin concentration (**Garnsworthy *et al.***, 2008). Vice versa, cows with a 0-d DP(STD) conceived later than cows with a 0-d DP(LOW) and had a numerically greater plasma insulin concentration in the current study. A greater plasma insulin concentration has been associated with decreased oocyte cleavage rate (**Fouladi-Nashta and Campbell**, 2006) and more small size follicles that will not grow to ovulating follicles and can be considered as a measure of reduced fertility (**Garnsworthy *et al.***, 2008).

A reduced interval from calving to pregnancy may have reduced persistency later on in lactation in cows with a 0-d DP(LOW), compared with cows with a 30-d DP(STD). Pregnancy from 3.5 to 6 months onwards negatively influences milk yield and lactation persistency through feto-placental estrogen that strongly stimulates apoptosis of mammary epithelial cells which decreases both cell number and secretory capacity (**Olori *et al.***, 1997; **Brotherstone *et al.***, 2004; **Yart *et al.***, 2012). Decrease of lactation persistency is related with increasing plasma estrogen, produced by the feto-placental unit (**Thatcher *et al.***, 1980; **Bachman *et al.***, 1988). Estrogen stimulates apoptosis of mammary epithelial cells that decreases both cell number and secretory capacity and results in decreased milk synthesis (**Yart *et al.***, 2013; **Martignani *et al.***, 2014).

3.6 Conclusions

A 0-DP resulted in a lower FPCM yield, greater EB, and greater plasma insulin and IGF-1 concentration, but decreased lactation persistency, compared with a 30-d DP. Feeding a ration with a reduced energy level for cows with a 0-d DP, did not affect FPCM yield and lactation persistency, but reduced the EB and weekly body weight gain, which implies a lower chance for fattening of cows with a 0-d DP. Feeding a more glucogenic ration, compared with a more lipogenic ration, did not affect EB or lactation persistency of cows with different dry period lengths, although milk yield, DMI, and plasma insulin and IGF-1 concentration were greater, and plasma GH concentration tended to be lower.

3.7 Acknowledgements

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CHAPTER 4

Relationship between metabolic status and behavior in dairy cows in early lactation

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4.1 Abstract

The aim of this study was to analyze the relation of metabolic status, based on plasma metabolites, with feeding behavior, lying behavior, and daily motion and steps of dairy cows in wk 4 postpartum after a 0-d or 30-d DP. Data of 81 Holstein-Friesian cows were collected using computerized feeders, accelerometers, and analyses of EDTA plasma samples for free-fatty acid (**FFA**), β-hydroxybutyrate (**BHB**), glucose, insulin, insulin-like growth factor 1 (**IGF-1**), and growth hormone (**GH**) concentrations. Dairy cows had either a dry period of 30 days (**30-d DP**), or a dry period of 0 days (**0-d DP**). Cluster analyses for metabolic status based on plasma metabolite and metabolic hormone concentrations resulted in 4 clusters of cows with good, average, poor, or very good metabolic status. Cows with a poor or average metabolic status had greater fat- and protein- corrected milk (**FPCM**) yield than cows with a good, or very good metabolic status. Furthermore, cows with a poor metabolic status had a lower DMI, daily number of visits, and lower EB than cows with an average or good metabolic status. In contrast, cows with a poor metabolic status had more visits to the feeder than cows with a very good metabolic status. Cows with a very good metabolic status were all cows with a 0-d DP. These cows had less visits to the feeder which is likely related with the lower energy demand for the 12 kg lower daily FPCM yield compared with cows with a poor metabolic status. Feeding rate, daily meal time, lying bouts, steps and motion were not related with metabolic status. In conclusion, better metabolic status in dairy cows in early lactation was associated with a greater DMI, increased feeding activity, and more time spent lying, compared with a poor metabolic status. Lying time can be measured using sensor technology and can be a practical tool to monitor metabolic status in dairy cows in early lactation.

Keywords: dry period length, continuous milking, feeding behavior, lactogenic hormones, sensor technology

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4.2 Introduction

Changes in lying, walking, and feeding behavior of dairy cows may be an indicator for metabolic status or diseases (**Edwards and Tozer**, 2004) such as lameness (**González *et al.***, 2008), metritis (**Urton *et al.***, 2005), or a displaced abomasum (**Van Winden *et al.***, 2003). Disease incidence is high in early lactation and diseases include ketosis, retained placenta, metritis, mastitis, milk fever, displaced abomasum, lameness, and impaired fertility (**Drackley**, 1999; **Ingvartsen *et al.***, 2003). Disease in early lactation may affect milk production performance in later lactation including total lactation yield (**Fourichon *et al.***, 1999).

Diseases in early lactation are often related with a negative energy balance (**EB**) (**Collard *et al.***, 2000; **Ingvartsen *et al.***, 2003). A negative EB is associated with insufficient energy intake for lactation, and is related with level of milk yield and dry matter intake (**DMI**) (**Lean *et al.***, 1992; **Maselyne *et al.***, 2017). A negative EB results in lower plasma glucose, insulin, and insulin-like growth factor 1 (**IGF-1**) concentrations, and greater plasma free-fatty acid (**FFA**), β-hydroxybutyrate (**BHB**) and growth hormone (**GH**) concentrations (**Fenwick *et al.***, 2008).

Plasma metabolites and hormones can be an indicator for metabolic status (**Butler *et al.***, 2003), but blood sampling is more invasive than monitoring behavioral observations. Behavioral observations such as feeding time and feeding rate, rumination time, and steps are increasingly collected using sensors to indicate, for instance, heat or the moment of calving (**Reith *et al.***, 2014). Better insight in the relation of metabolites and metabolic hormones with behavior may improve interpretation of behavior with respect to metabolic status in early lactation. Previous studies reported that cows with a lower plasma FFA concentration had a greater walking activity postpartum, compared with cows with a greater plasma FFA concentration (**Adewuyi *et al.***, 2006). In addition, cows with ketosis had lower feed intake, feeding time, meal time, and less meals and visits than cows without ketosis (**González *et al.***, 2008). To our knowledge, the relation between behavior and other plasma metabolites and metabolic hormones was not described previously.

Energy balance can be improved in dairy cows in early lactation by shortening (**30-d**) or omitting (**0-d**) the dry period (**DP**), compared with a more conventional DP (60-d) (**Rastani *et al.***, 2005; **Van Knegsel *et al.***, 2014). The dry period is a non-lactating period for dairy cows which allows renewal of udder cells before the next calving and start of the subsequent lactation (**Rastani *et al.***, 2005). Postpartum, cows with a 0-d DP had a greater plasma glucose concentration, and a lower FFA concentration than cows with a 30-d DP (**Andersen *et al.***, 2005; **De Feu *et al.***, 2009; **Bernier-Dodier *et al.***, 2010). Additionally, cows with a 0-d DP spent more time lying in early lactation than cows with a 30-d DP (**Kok *et al.***, 2016). The created variation in metabolic status through application of different DP lengths may help unravel relationships between metabolic status and behavior.

The aim of this study was to analyze the relation of metabolic status, based on plasma metabolites and hormones, with feeding behavior and daily motion and steps of dairy cows in wk 4 postpartum after a 0-d or 30-d DP.

4.3 Materials and methods

4.3.1 Animals and housing

The Institutional Animal Care and Use Committee of Wageningen University & Research approved the experimental protocol in compliance with the Dutch law on Animal Experimentation (protocol number 2014125). The experiment was conducted at the Dairy Campus research herd (Lelystad, The Netherlands) between January 27th 2014 and August 26th 2015. The research herd was composed of 400 lactating Holstein cows. Cows were selected based on 1) being bred with a Holstein sire, 2) expected calving interval <490 days, 3) daily milk yield >16 kg at 90 days before the expected calving date, and 4) no clinical mastitis or high SCC (≥250,000 cells/mL) at final two test-days before drying off. All cows were housed in the same freestall with concrete slatted floors, and cubicles (1.25 m × 2.20 m) fitted with rubber mattresses (4 cm thick) covered with sawdust. The stocking density was maintained at 7 m^2 per cow, with one cow per cubicle. Cows were milked twice daily at ~06:00 hours and ~17:00 hours. Lying behavior and steps of cows were recorded for 6 complete days (Friday till Wednesday) in wk 4 after calving, because of limited sensor availability and changing of sensors on Thursdays.

4.3.2 Experimental design

In total, 130 cows entered the experiment, including 6 cows that entered twice. To obtain a balanced distribution of cows across treatments, cows were blocked according to expected calving date, milk yield in the previous lactation, and parity (2, ≥ 3) in the subsequent lactation. Within each group of 3 cows, 2 cows were assigned randomly to a DP length treatment of 0 days (0-d DP) and 1 cow to a DP length treatment of 30 days (30-d DP). Within the group of cows with a 0-d DP, cows were assigned randomly to either a low level of concentrate based on the energy requirement for their expected milk yield (**LOW**) or a standard (**STD**) level of concentrate based on the energy requirement for the expected milk yield of cows after a 30-d DP (**Van Knegsel *et al.***, 2014). Cows with a 30-d DP were fed a STD level of energy, which is based on the requirement for their expected milk yield. This resulted in the following 3 treatment groups: cows with a 30-d DP fed the STD level of concentrate required for their expected milk yield [**30-d DP(STD)**], cows with a 0-d DP fed the same STD concentrate level as cows with a 30-d DP [**0-d DP(STD)**], and cows with a 0-d DP fed a LOW concentrate level [**0-d DP(LOW)**]. Preliminary analyses showed no effect of concentrate level within 0-d DP treatment on feeding behavior or steps and motion. Concentrate level was therefore excluded from further analyses in this study. Lying behavior, steps, and motion were only measured for 81 of 130 cows. The final dataset for the current study consisted of milk production, EB, body weight, feed intake, plasma metabolite and metabolic hormone concentrations, and lying behavior and steps data of 81 unique cows in wk 4 postpartum (n = 53 for 0-d DP and n = 28 for 30-d DP). Basal lactation ration, concentrate composition, and feeding strategy were reported previously (**Van Hoeij *et al.***, 2017)

4.3.3 Measurements

Milk yield and milk composition. Milk yield was recorded daily from 4 weeks prepartum until 7 weeks postpartum. Milk samples for fat, protein and lactose analysis [(**ISO 9622**, 2013), Qlip, Zutphen, The Netherlands] were collected four times per week (Tuesday afternoon, Wednesday morning, Wednesday afternoon, and Thursday morning) and were analyzed as a pooled sample per cow per week.

FPCM was calculated as:

FPCM = (0.337 + 0.116 × fat content + 0.06 × protein content) × milk yield (**CVB**, 2011).

Feed intake, body weight, body condition score and energy balance. Basal lactation ration was provided and its daily intake was measured individually using roughage intake control (RIC) troughs (Insentec, Marknesse, The Netherlands). The stocking density was 2 cows per trough. The actual quantity of concentrate dispensed (kg/d) was recorded by the computerized feeder (Manus VC5, DeLaval, Steenwijk, the Netherlands). Body weight (BW) was recorded daily before each milking and averages per week.

Energy balance was calculated per week according to the Dutch NE system for lactation (VEM) (**Van Es**, 1975), as the difference between energy intake and energy requirements for maintenance and milk yield (1,000 VEM = 6.9 MJ of NE). According to the VEM system, the daily requirement for maintenance is 42.4 VEM/kg$^{0.75}$ BW per day and the requirement for milk yield is 442 VEM/kg FPCM (**Van Es**, 1975). Energy intake and EB are expressed in kJ/ kg$^{0.75}$ BW per day (**Van Es**, 1975).

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Blood collection and analysis. Blood was collected on Thursday in wk 4 postpartum. Blood samples were collected after the morning milking and between 3 and 1 hours before the morning feeding. Blood (10 mL) was collected from the coccygeal vein into evacuated EDTA tubes (Vacuette, Greiner BioOne, Kremsmunster, Austria). Blood samples were kept on ice before centrifugation for plasma isolation (3,000 × *g* for 15 min, 4°C). Plasma samples were stored at −20°C. Concentrations of FFA and BHB were measured enzymatically using kit no. 994–75409 from Wako Chemicals (Neuss, Germany) and kit no. RB1007 from Randox Laboratories (Ibach, Switzerland), respectively (**Graber *et al.***, 2012). The plasma glucose concentration was measured using kit no. 61269 from BioMerieux (Marcy l'Etoile, France) (**Graber *et al.***, 2012). The plasma insulin concentration was measured using kit no. PI-12K from EMD Millipore Corporation (Billerica, MA, USA). The plasma IGF-1 concentration was measured using kit no. A15729 from Beckman Coulter (Fullerton, CA, USA). The plasma GH concentration was measured by radioimmunoassay as described previously (**Vicari *et al.***, 2008).

Behaviors. Measurement of behaviors was described earlier by (**Kok *et al.***, 2016). In short, feeding behavior was measured in wk 4 postpartum using RIC feeders. For each visit to a feeder, RIC feeders recorded cow identity, the start time and end time (hh:mm:ss) of the visit, and the start weight and end weight of the feed in the feeder to the nearest 0.1 kg. Visits were clustered into meals based on the interval length between visits (**Yeates *et al.***, 2001; **Tolkamp *et al.***, 2002), with a threshold of 20.9 minutes between meals (**Kok *et al.***, 2016). Feeding behaviors used for analyses were the average daily duration of meals (meal time, min/d), average daily number of visits (visits, n/d), the average daily number of meals (meals, n/d), and the secondary variables daily feed intake (kg DM/d) and feeding rate (kg/min) were derived from these variables. Means of feeding behavior characteristics per cow per day were used for analyses.

Lying behavior and steps and motion were recorded in wk 4 postpartum during 6 consecutive days with triaxial accelerometers (IceQube, IceRobotics, South Queensferry, UK). Lying behavior is recorded when the hind leg is in a horizontal position; the step count measures the number of times the animal lifts its leg up and places it back down again. Lying bouts < 33 seconds were discarded as erroneous (**Kok *et al.***, 2015). The step count was used as indicator for walking activity. Motion is a measure of the overall acceleration measured by the sensor in all three axes. Sensors were attached to the hind leg and detached on Thursdays between 10.00 h and 12.00 h. Means of lying bouts, lying time, steps, and motion per cow per day were used for the analysis in wk 4 of lactation.

4.3.4 Statistical analyses

The natural logarithm of the plasma FFA, BHB, and GH concentration were calculated to approximate normal distribution of these variables and were used in all statistical analyses. To analyze correlations of plasma metabolites and hormones FFA, BHB, glucose, insulin, IGF-1, and GH, with DMI, feeding behavior, steps, and motion, a Pearson correlation was used [PROC CORR; SAS 9.3, SAS Institute Inc. (2011)]. Pearson correlation analysis was also used to analyze correlations among the different plasma metabolites and hormones.

Because in the current study most metabolites and hormones were correlated (**Table 4.1b**), as a second step, cluster analysis with the Ward method was performed using plasma FFA, BHB, glucose, insulin, IGF-1, and GH concentration (PROC CLUSTER). Based on the cubic clustering criterion, pseudo F statistic, and pseudo t-squared, cows were clustered in 4 clusters using the tree procedure (PROC TREE).

To evaluate the plasma concentration of metabolites and hormones, FPCM yield (kg/d), EB (kJ/kg$^{0.75}$·d), and BW for cows with different metabolic status, a general linear model was used (PROC GLM). The independent variable was cluster.

To evaluate DMI, feeding behavior, steps, and motion for cows with different metabolic status, the general linear model was extended with DP length (0-d DP or 30-d DP) and parity (2 or ≥3) as dependent variables. Two-way interactions with cluster were excluded from analyses due to the unbalanced number of cows per DP length or parity within and among clusters. The model was analyzed using a backward elimination procedure with a stay-in P-value of <0.05 in the type III Wald test.

4.4 Results

4.4.1 Correlations between plasma metabolites and hormones, and behavioral traits

Average daily FPCM yield was negatively correlated with plasma glucose, insulin, and IGF-1, and positively with plasma FFA, BHB, and GH ($P < 0.05$) (**Table 4.1a**). Dry matter intake was negatively correlated with plasma FFA and GH, and positively with plasma glucose and IGF-1 ($P < 0.05$). Energy balance was negatively correlated with the plasma FFA, BHB and GH, and positively with plasma glucose, insulin, and IGF-1 ($P < 0.05$). Feeding rate was negatively correlated with plasma GH, and positively with plasma glucose ($P < 0.05$). Number of meals per day was negatively correlated with plasma FFA and GH. Number of visits to the feeder was negatively correlated with plasma FFA and BHB, and positively with plasma glucose and IGF-1 ($P < 0.05$). Meal time and lying time were negatively correlated with plasma FFA, and lying time was positively correlated with plasma IGF-1 ($P < 0.01$). Steps and motion were negatively correlated with plasma FFA and BHB ($P < 0.05$).

The plasma FFA concentration was negatively correlated with the plasma glucose, insulin, and IGF-1, and positively with the plasma BHB and GH (P < 0.01) (**Table 4.1b**). The plasma BHB concentration was negatively correlated with the plasma glucose and insulin concentration (P < 0.05). The plasma glucose concentration was negatively correlated with the plasma GH concentration, and positively with the plasma insulin and IGF-1 concentration (P < 0.05). The plasma IGF-1 concentration was negatively correlated with the plasma GH concentration (P < 0.01).

Table 4.1: Pearson correlation coefficients (P < 0.05) of plasma metabolites with feeding behavior, steps, and motion (a). Pearson correlation coefficients (P < 0.05) among plasma FFA, BHB, glucose, insulin, IGF-1, and GH concentrations (b)

a)	FFA (mmol/L)[1]	BHB (mmol/L)[1]	Glucose (mmol/L)	Insulin (μU/mL)	IGF-1 (ng/mL)[1]	GH (μg/L)[1]
FPCM (kg/d)[1]	0.49**	0.31**	-0.37**	-0.47**	-0.41**	0.26*
DMI (kg DM/d)[1]	-0.49**	Ns	0.23*	Ns	0.22*	-0.34**
EB (kJ / $kg^{0.75}$·d)[1]	-0.78**	-0.41**	0.48**	0.33*	0.55**	-0.45**
Feeding rate (kg/min)	Ns	Ns	0.30**	Ns	Ns	-0.25*
Meals (n/d)	-0.34**	Ns	Ns	Ns	0.34**	-0.24*
Visits (n/d)	-0.39**	-0.2*	0.23*	Ns	0.22*	Ns
Meal time (min/day)	-0.38**	Ns	Ns	Ns	Ns	Ns
Lying time (hrs/d)	-0.43**	Ns	Ns	Ns	0.32**	Ns
Lying bouts (n/d)	Ns	Ns	Ns	Ns	Ns	Ns
Steps (n/d)	-0.32**	-0.25*	Ns	Ns	Ns	Ns
Motion	-0.37**	-0.28*	Ns	Ns	Ns	Ns

b)	FFA (mmol/L)[1]	BHB (mmol/L)[1]	Glucose (mmol/L)	Insulin (μU/mL)	IGF-1 (ng/mL)[1]	GH (μg/L)[1]
FFA[1]		0.33**	-0.48**	-0.40**	-0.52**	0.40**
BHB[2]			-0.52**	-0.24*	Ns	Ns
Glucose				0.39**	0.53**	-0.27*
Insulin					Ns	Ns
IGF-1						-0.39**

*P < 0.05, **P < 0.01, Ns = not significant. [1]FFA= free fatty acids, analyzed using the natural logarithm of FFA, BHB= β-hydroxybutyrate, analyzed using the natural logarithm of BHB, IGF-1 = insulin-like growth factor 1, GH = bovine growth hormone, analyzed using the natural logarithm of GH, FPCM = fat- and protein- corrected milk, DMI = dry matter intake, EB = energy balance.

4.4.2 Relation between metabolic status and feeding behavior, steps, and motion.

Cows were clustered for metabolic status based on their plasma FFA, BHB, glucose, insulin, IGF-1, and GH concentration in wk 4 postpartum. This resulted in 4 groups of cows with a good, average, poor, or very good metabolic status. Cows with a good, average, or poor metabolic status had a 0-d or 30-d DP length, and parity 2 or ≥3 (**Table 4.2**). All cows with a very good metabolic status had a 0-d DP. Cows with a good or very good metabolic status had low plasma FFA (**Figure 4.1a**), and greater plasma glucose (**Figure 4.1c**) than cows with average or poor metabolic status ($P < 0.01$) (**Table 4.3**). Cows with an average metabolic status had lower plasma FFA and greater plasma glucose than cows with a poor metabolic status. Cows with a good or very good metabolic status tended to have a lower plasma BHB (**Figure 4.1b**) and greater plasma insulin (**Figure 4.1d**) than cows with a poor metabolic status ($P < 0.10$). Plasma IGF-1 increased with metabolic status (adjusted R^2 was 0.88, $P < 0.01$; **Figure 4.1e**). Cows with a very good metabolic status had lower plasma GH (**Figure 4.1f**) concentration than cows with an average or poor metabolic status ($P < 0.05$). Cows with a very good metabolic status had lower FPCM yield than cows with a good, average, or poor metabolic status, and cows with a good metabolic status had lower FPCM yield than cows with average or poor metabolic status ($P < 0.01$). Energy balance increased with metabolic status ($P < 0.01$). Body weight did not differ between cows with different metabolic status.

Table 4.2: Number of cows in different clusters based on the plasma FFA, glucose, and IGF-1 concentration

		Metabolic status			
Dry period length	Parity	Good	Average	Poor	Very good
0-d DP	2	14	10	0	4
0-d DP	≥3	6	14	3	2
30-d DP	2	3	6	2	0
30-d DP	≥3	2	11	4	0
Total cows (n =)		25	41	9	6

4

Table 4.3: Plasma metabolite and metabolic hormone concentrations in wk 4 postpartum for cows with different metabolic status after a 0 or 30 days dry period. Metabolic status was based on the plasma FFA, BHB, glucose, insulin, IGF-1, and GH concentration. Values are presented as LSM + SE

	Adjusted R^2	Metabolic status				SEM	P- Value
		Good	Average	Poor	Very good		
Cows		25	41	9	6		
FFA (mmol/L)[2]	0.33	0.10^{a} (0.07 – 0.13)	0.16^{b} (0.13 – 0.20)	0.46^{c} (0.29 – 0.75)	0.07^{a} (0.04 – 0.13)		<0.01
BHB (mmol/L)[2]	0.08	0.63^{a} (0.52 – 0.7)	0.72^{ab} (0.61 – 0.84)	1.00^{b} (0.73 – 1.38)	0.59^{a} (0.40 – 0.87)		0.08
Glucose (mmol/L)	0.26	3.96^{a}	3.72^{b}	3.21^{c}	4.1^{a}	0.9	<0.01
Insulin (μU/mL)	0.09	14.9^{a}	15.0^{a}	7.7^{b}	20.0^{a}	1.9	0.07
IGF-1 (ng/mL)[2]	0.88	151^{a}	101^{b}	52^{c}	214^{d}	3	<0.01
GH(μg/L)[2]	0.24	3.81^{ab} (3.15 – 4.59)	4.52^{a} (3.94 – 5.19)	4.68^{a} (3.49 – 6.28)	2.61^{b} (1.82 – 3.74)		0.03
FPCM[3]	0.11	35.6^{a}	39.0^{b}	38.8^{b}	25.8^{c}	1.2	<0.01
EB (kJ / $kg^{0.75}$·d)[3]	0.34	6^{a}	-124^{b}	-242^{c}	190^{d}	32	<0.01
Body weight (kg)	0.03	662	674	638	676	13	0.43

[a-d]Values with different superscripts differ ($P < 0.05$). [2]FFA = Free fatty acids, BHB = β-hydroxybutyrate, IGF-1 = insulin-like growth factor 1, GH = growth hormone. FFA, BHB, and GH were log transformed for analyses, but are shown as actual values with confidence interval. [3]FPCM = fat- and protein- corrected milk. EB = energy balance.

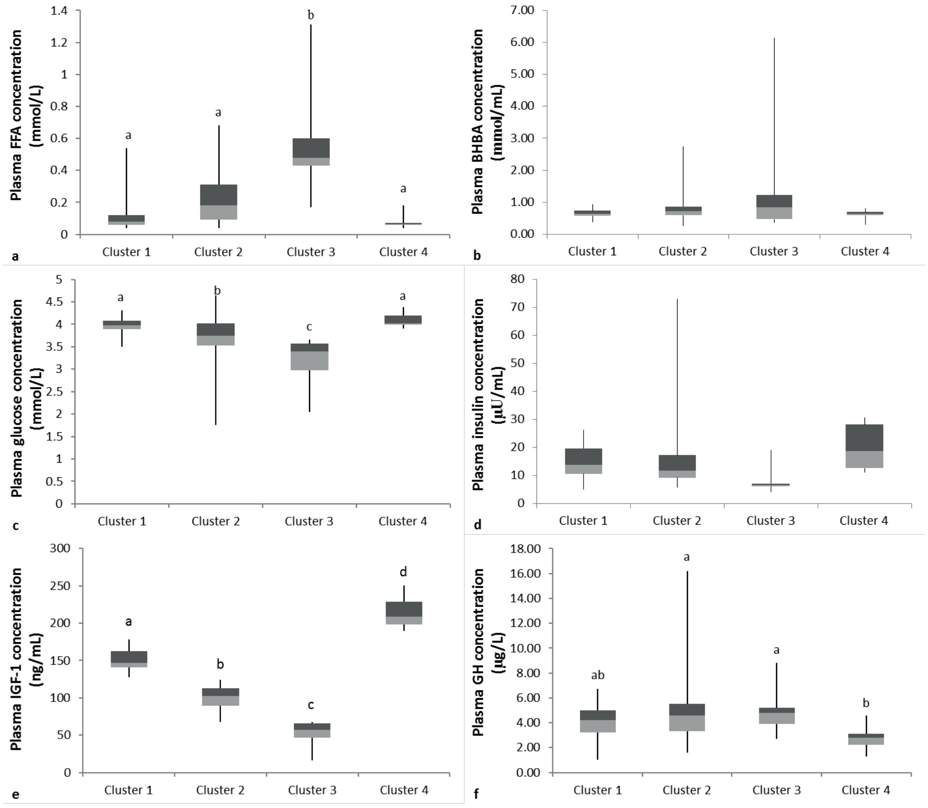

Figure 4.1 a-f: The plasma FFA (a), BHB (b), glucose (c), insulin (d), insulin-like growth factor-1 (e), and growth hormone (f) concentration for cows clustered for metabolic status based on plasma FFA, BHB, glucose, insulin, IGF-1, and GH concentration. Values with different symbols differ (P < 0.05).

Cows with good or average metabolic status had greater DMI and basal ration intake than cows with a poor metabolic status (P < 0.01) (**Table 4.4**). Cows with a good metabolic status had more meals per day than cows with an average or poor metabolic status (P < 0.05). Cows with a good metabolic status had more visits to the feeder than cows with a poor or very good metabolic status, and cows with an average metabolic status had more visits to the feeder than cows with a very good metabolic status (P < 0.05). Cows with a good or very good metabolic status had longer lying time than cows with an average or poor metabolic status (P < 0.05). Irrespective of metabolic cluster, cows with a 0-d DP had greater DMI (21.9 ± 0.3 vs 20.5 ± 0.5 kg/d), basal ration intake (15.0 ± 0.3 vs. 13.5 ± 0.4 kg DM/d), and visits (29.0 ± 1.5 vs 24.2 ± 1.9 /d) than cows with a 30-d DP (P < 0.05). Cows with parity 2 had lower feeding rate (0.20 ± 0.01 vs. 0.24 ± 0.01 kg/min), and more visits to the feeder (29.7 ± 1.7 vs. 23.1 ± 1.6 /d), longer meal time (233 ± 9 vs. 195 ± 9 min/d), and more steps (1280 ± 57 vs 1085 ± 56 /d) and motion (5234 ± 233 vs 4464 ± 230) (P < 0.01) than cows with parity ≥ 3.

Table 4.4: Behavior in wk 4 postpartum of cows with different metabolic status after a 0 or 30 days dry period. Metabolic status was based on the plasma FFA, BHB, glucose, insulin, IGF-1, and GH concentration. Values are presented as LSM + SE

		Metabolic status				SEM	P-value[1]		
	Adjusted R^2	Good	Average	Poor	Very good		C	DP	P
Cows		25	41	9	6				
Dry matter intake (kg DM/d)	0.25	22.3^{a}	22.0^{a}	19.6^{b}	20.8^{ab}	0.5	<0.01	<0.01	0.51
Basal ration intake (kg DM/d)	0.29	15.3^{a}	15.1^{a}	12.5^{b}	13.9^{ab}	0.4	<0.01	<0.01	0.52
Feeding rate (kg/min)	0.17	0.23	0.22	0.20	0.22	0.01	0.89	0.13	<0.01
Meals (n/d)	0.15	8.23^{a}	7.38^{b}	7.18^{b}	8.16^{ab}	0.24	0.03	0.62	0.58
Visits (n/d)	0.32	31.9^{a}	28.4^{ab}	23.5^{bc}	21.9^{c}	1.9	0.02	0.03	<0.01
Meal time (min/d)	0.21	226	222	198	203	10	0.37	0.57	<0.01
Lying time (h/d)	0.12	11.9^{a}	10.9^{b}	10.3^{b}	12.4^{a}	0.4	0.05	0.25	0.28
Lying bouts (n/d)	0.06	13.2	12.0	11.6	13.7	0.7	0.47	0.84	0.21
Steps (n/d)	0.18	1322	1231	1102	1054	64	0.14	0.73	<0.01
Motion	0.19	5485	4945	4410	4509	261	0.18	0.48	0.01

[a-c]Values with different superscripts differ (P < 0.05). [1]C = Cluster, DP = Dry period length, P = Parity. [2]FFA = Free fatty acids, BHB = β-hydroxybutyrate, IGF-1 = insulin-like growth factor 1, GH = growth hormone. FFA, BHB, and GH were log transformed for analyses, but are shown as actual values without SEM. [3]N.s. = not significant.

4.5 Discussion

The aim of this study was to analyze the relation of metabolic status, based on plasma metabolites and metabolic hormones, with feeding behavior, lying behavior, and daily motion and steps of dairy cows in wk 4 postpartum after a 0-d or 30-d DP. First, Pearson correlation analysis was used to correlate metabolites and metabolic hormones with behavior. Especially plasma FFA concentration was related to behavioral indicators. Dry matter intake, number of meals and visits, meal time, lying time, steps, and motion were all lower at a greater plasma FFA concentration. Plasma BHB, glucose, insulin, IGF-1, and GH concentration were related to only 3 or fewer behavioral indicators, and the strength of these correlations was generally lower than between FFA concentration and behavioral indicators. This seems to indicate that especially cows with a greater FFA concentration have a lower feed intake, partly explained by lower feed intake related behavior and lower activity. Our results are in line with previous studies that found that cows with a greater plasma FFA concentration have lower feed intake (**Waterman *et al.***, 1972; **Lean *et al.***, 1992), and lower walking activity (**Adewuyi *et al.***, 2006). Because in the current study most metabolites and hormones were correlated (**Table 4.1b**), as a second step, cluster analysis was performed to cluster cows for metabolic status based on their plasma FFA, BHB, glucose, insulin, IGF-1, and GH concentration. Cows were clustered in 4 groups of cows with a good, average, poor, or very good metabolic status. Variation of plasma IGF-1 concentration between metabolic status clusters was greater than variation within clusters (R^2 = 0.88), whereas for plasma FFA concentration, variation between metabolic status clusters was smaller than variation within clusters. This suggests that plasma IGF-1 concentration largely determined the clustering of cows' metabolic status, while Pearson correlation coefficients indicated that especially plasma FFA concentration determined differences in behavior. The importance of plasma FFA or IGF-1 concentration for clustering in groups of metabolic status among weeks in early lactation may be related with lactation stage and related metabolic status. Plasma FFA concentration is likely more important during nadir EB as an indicator of lipid mobilization, while plasma IGF-1 concentration may be more important during the recovering phase of EB, such as in wk 4 of lactation, as an indicator of increasing glucose availability, and can be hypothesized to indicate recovering carbohydrate metabolism.

To our knowledge no other studies have directly related metabolic status with behavioral parameters. However, previous studies evaluated the relation between disease events in early lactation and metabolic status, and reported plasma metabolites for the diseased and non-diseased cows. These studies reported that cows with a metabolic disease, metritis, or mastitis had lower metabolic status as reflected by a greater plasma FFA, BHB, and haptoglobin, and lower plasma calcium concentration, than non-diseased cows (**Soriani *et al.***, 2012). Additionally, cows in previous studies had lower daily feeding time (**Urton *et al.***, 2005), decreased activity and rumination (**Soriani *et al.***, 2012; **Stangaferro *et al.***, 2016), compared with non-diseased cows. In another study, cows with ketosis had lower feed intake, feeding time, meal time, and less meals and visits than cows without ketosis (**González *et al.***, 2008). The lower reported DMI, and number of meals and visits to the feeder in these earlier studies are in accordance to our findings. On the contrary, in our study steps and motion, and meal time were not different among cows with different metabolic status. It should, however, be noted that cows in previous studies were diseased and likely showed sickness behavior, whereas cows in our study were not clinically diseased but likely showed behavior related with different metabolic status.

In general, cows with a good or very good metabolic status had, or tended to have, a lower plasma FFA, BHB, and GH, and a greater plasma glucose, insulin, and IGF-1 concentration, than cows with an average or poor metabolic status. Cows with a very good metabolic status had different feeding behavior than expected based on behavior of cows with a good, average, or poor metabolic status. Cows with a very good metabolic status had numerically lower DMI and basal ration intake, and less meals and visits, while these behaviors were expected to be increased compared with cows with a good metabolic status. Cows with a very good metabolic status were only 6 cows subjected to the 0-d DP, and the lower feed intake and less visits to the feeder were likely due to the lower energy requirement for the 12 kg lower daily FPCM production of these cows compared with cows with a good or average metabolic status. Conclusions on these cows with a very good metabolic status should be taken with caution due to the low number of animals in this group, large difference in milk yield and unbalanced representation of DP length in this metabolic status group.

When comparing only cows with a good, average, or poor metabolic status in relation to behavior, better metabolic status was related with greater DMI, basal ration intake, number of meals and visits to the feeder, and increased lying time. These behaviors may be reliable indicators for metabolic status. Number of visits to the feeder can be measured as proximity to the feed bunk using sensors, and can be processed into number of meals. However, these sensors are unlikely to be used already in commercial settings. Meanwhile, lying time can be recorded with commercially available sensors that are widely used for estrus detection (**Rutten *et al.***, 2017). Lying time may, therefore, be the most useful indicator for metabolic status. In previous studies, reduced lying time was related with restlessness, likely due to discomfort (**Huzzey *et al.***, 2005), and was related with induced ruminal acidosis (**DeVries *et al.***, 2009), and higher milk production (**Norring *et al.***, 2012; **Kok *et al.***, 2016) and udder pressure (**Bertulat *et al.***, 2017). In the current study, reduced lying time was likely related with discomfort due to a poor metabolic status and possibly increased udder pressure to greater milk yield, compared with cows with a good metabolic status.

Cows with a good, average, or poor metabolic status represented both DP length and parity classes in this study. Irrespective of metabolic status, DP length and parity affected feed intake, number visits to the feeder, and meal time in the current study. Regardless of metabolic status, cows with a 0-d DP had greater DMI and more visits to the feeder than cows with a 30-d DP. This is likely related with the greater plasma glucose, insulin, IGF-1, and lower plasma FFA and BHB concentration (**Van Hoeij *et al.***, 2017), or less or no changes in ration and re-grouping around transition (**Kok *et al.***, 2016) for cows with a 0-d DP compared with cows with a 30-d DP. Regardless of metabolic status, cows with parity 2 had more visits, longer meal time than cows with parity ≥3. These findings are likely related with the greater plasma glucose and IGF-1, and tendency to lower plasma BHB concentration (**Van Hoeij *et al.***, 2017), or a lower feeding rate (**Kok *et al.***, 2016) of cows with parity 2 had than in cows with parity ≥ 3. The model to evaluate the effect of metabolic status on behavior corrected for effects of DP length and parity. Correlations between metabolites and metabolic hormones and behavior were performed without DP length and parity as control variables. Including DP length an parity in a general linear model to evaluate effects of a plasma metabolite on behavior (results not shown) resulted in similar relations between plasma metabolites and behavior compared with the Pearson correlations presented in **table 4.1a**.

In our previous study, EB of cows with a 0-d or 30-d DP was correlated with DMI, meals, and visits to the feeder, lying time, and steps (**Kok *et al.***, 2016). In the current study, the plasma FFA concentration had stronger correlations and the plasma IGF-1 concentration had similar correlations with behavior, compared with correlations between EB and behavior found in our previous study (**Kok *et al.***, 2016). These results imply that the plasma FFA has a stronger relation with behavior than calculated EB. In the current study, in wk 4 of lactation, EB was greater and plasma FFA concentration were lower for cows with a 0-d DP than for cows with a 30-d DP, which is in line with previous studies (**Rastani *et al.***, 2005; **Van Knegsel *et al.***, 2014). The greater negative EB and plasma FFA concentration in cows with a 30-d DP may have resulted in more pronounced behavior compared with cows with a 0-d DP. In earlier studies, EB was even lower and plasma FFA concentration were greater for cows with a conventional DP, than for cows with a short DP (**Klusmeyer *et al.***, 2009). It could be hypothesized that the relation between behavior and plasma metabolites may even be stronger in cows with a conventional DP than in cows with a 0-d or 30-d DP.

The direction of causation between plasma metabolites or metabolic status with behavioral traits in dairy cows is unclear. For example, less feed directed behavior and activity may result in lower DMI, and more body fat mobilization which is related with a greater plasma FFA and BHB concentration and a lower glucose and insulin concentration (**Lean *et al.***, 1992). Vice versa, greater FFA concentration is related with metabolic acidosis and a feeling of discomfort that may decrease appetite and DMI (**Waterman *et al.***, 1972; **Allen *et al.***, 2009).

4.6 Conclusions

Better metabolic status in dairy cows in early lactation was associated with a greater DMI, increased feeding activity, and more time spent lying, compared with a poor metabolic status. Lying time can be measured using sensor technology and can be a practical tool to monitor metabolic status in dairy cows in early lactation.

4.7 Acknowledgements

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CHAPTER 5

Cow characteristics and their association with udder health after different dry period lengths

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5.1 Abstract

Shortening or omitting the dry period (**DP**) in dairy cows is of interest because of potential beneficial effects on energy balance and metabolic status. Reported effects of a short or omitted dry period on udder health are ambiguous. The aims of this study were to evaluate the effect of no DP (0-d), a short DP (30-d), or a conventional DP (60-d) on the occurrence of intramammary infections (**IMI**) during the precalving period, and on somatic cell counts (SCC), elevations of SCC (SCC ≥ 200,000 cells/mL), and clinical mastitis in the subsequent lactation. The study also aimed to analyze which prepartum cow characteristics are associated with udder health after different DP lengths. Holstein-Friesian dairy cows (n = 167) were randomly assigned to a DP length (0, 30, or 60-d). Cows with a 0-d DP had a greater occurrence of chronic IMI and a lower occurrence of cured IMI during the precalving period than cows with a 30-d or 60-d DP. Postpartum average SCC for lactation was greater in cows with a 0-d DP than in cows with a 30-d or 60-d DP. The number of cows with at least 1 elevation of SCC, the number of elevations of SCC per affected cow, the number of cows treated for clinical mastitis, and the number of cases of mastitis per affected cow did not differ among DP lengths. Cow characteristics related to postpartum average SCC for lactation were DP length, parity, and the following interactions: DP length with prepartum elevation of SCC, DP length with fat- and protein-corrected milk (FPCM) reduction between 150 and 67 d prepartum, DP length with parity and with average SCC for lactation, and last FPCM before the conventional drying-off day with average SCC for lactation. Cows with prepartum parity 1 had a lower occurrence of at least 1 elevation of SCC in subsequent lactation compared with cows with parity >2. Last SCC before the conventional drying-off day was positively associated with occurrence of clinical mastitis in the subsequent lactation. In this study, DP length was not a risk factor for either elevation of SCC or occurrence of clinical mastitis in the subsequent lactation. The identified cow characteristics could be used in a decision support model to optimize DP length for individual cows.

Key words: continuous milking, decision making, somatic cell count, clinical mastitis

5

5.2 Introduction

Shortening or omitting the dry period (**DP**) may potentially improve energy balance and reduce the risk of ketosis at the start of the subsequent lactation (**Van Knegsel *et al.***, 2013). The histology of udder tissue at different prepartum time points indicates that a DP of at least 35 d is needed for proliferation and cell turnover to increase the proportion of epithelial cells in the udder for milk synthesis (**Capuco *et al.***, 1997). Milk production does not differ between a 30-d DP or a 60-d DP (**Gulay *et al.***, 2003); however, a 0-d DP allows the udder little or no time to regenerate secretory cells compared with a 60-d DP, which could explain the lower milk yield after a 0-d DP (**Capuco *et al.***, 1997). Lower milk yield requires less energy, which explains the better energy balance and improved metabolic status in early lactation for cows with a 30-d or 0-d DP (**Grummer and Rastani**, 2004; **Watters *et al.***, 2008; **Chen *et al.***, 2015).

The effect of DP length on udder health is unclear. Studies showed that following a 30-d or 0-d DP, SCC in the subsequent lactation tended to decrease (**Gulay *et al.***, 2003; **Pinedo *et al.***, 2011), was not affected (**Rastani *et al.***, 2005; **Church *et al.***, 2008; **Watters *et al.***, 2008; **Bernier-Dodier *et al.***, 2011), or tended to increase compared with a 60-d DP (**Kuhn *et al.***, 2006; **Pezeshki *et al.***, 2007; **Steeneveld *et al.***, 2013). A 30-d DP tended to reduce the number of new IMI postpartum (**Pezeshki *et al.***, 2007; **Pinedo *et al.***, 2011), but other studies indicated no effect of DP length on clinical mastitis in the subsequent lactation compared with a 60-d DP (**Church *et al.***, 2008; **Watters *et al.***, 2008; **Shoshani *et al.***, 2014). Overall, results are ambiguous, and no definite conclusions can be drawn because too little research has been done on the effect of DP length on udder health (**Collier *et al.***, 2012). The effect of DP length on udder health in the subsequent lactation may be related to other cow characteristics such as parity, calving month, occurrence of clinical mastitis in the previous lactation, SCC before drying-off, and milk yield during the days before drying-off (**Green *et al.***, 2007; **Pantoja *et al.***, 2009b). We previously showed that part of the effect of DP length on postpartum milk yield can be explained by differences in parity, milk yield at drying-off, and persistency in the previous lactation (**Steeneveld *et al.***, 2014). It can be hypothesized that the effect of DP length on udder health in the subsequent lactation is also explained by prepartum cow characteristics such as parity, milk production, and SCC. These cow characteristics could be used in a decision support model to optimize DP length for individual cows.

The aims of this study were to evaluate the effect of a 0-d, 30-d, and 60-d DP on IMI during the precalving period on SCC, elevations of SCC, and clinical mastitis in the subsequent lactation. Another aim was to analyze which prepartum cow characteristics are associated with these udder health variables after different DP lengths.

5.3 Materials and methods

5.3.1 Animals, experimental design, and Housing

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Wageningen University & Research as earlier described (**Van Knegsel *et al.***, 2014). The experiment was originally designed to study the effects of DP length and dietary energy source on milk characteristics, energy balance, and plasma metabolites. The experiment was conducted at the Dairy Campus research herd (Lelystad, the Netherlands), with 167 Holstein-Friesian cows (60 primiparous and 107 multiparous) between May 10, 2010, and September 30, 2012. The research herd comprised 400 lactating Holstein cows. Cows for this experiment were selected based on (1) being bred with a Holstein sire, (2) an expected calving interval 16 kg at 90 d before the expected calving date, and (4) no clinical mastitis or high SCC (>250,000 cells/ mL) at 2 test-days before the conventional drying-off day (67 d before the expected calving date). Cows were blocked for prepartum parity (1, 2, or >2), expected calving date, milk production, and BCS. Each block consisted of 6 cows. Within blocks, cows were randomly assigned to a DP length (0, 30, or 60 d) and an early lactation ration (glucogenic or lipogenic) resulting in a 3 × 2 factorial design. Ration was included in the experimental setup because its effect on negative energy balance was evaluated in an earlier study (**Van Knegsel *et al.***, 2014). Ration composition and feeding strategies were described earlier (**Van Knegsel *et al.***, 2014). Briefly, during the precalving period, lactating cows were fed a lactation ration supporting 25 kg of milk, and dry cows were fed a dry cow ration. From 10 d before the expected calving date and onwards, cows of all treatments were fed 1 kg/d of concentrate, which increased stepwise at 0.5 kg/d postpartum until reaching 8.5 kg/d. The basal dry cow and lactation ration consisted of grass silage, corn silage, wheat straw, rapeseed meal or soybean meal, and vitamins and minerals. Rations were formulated to be equal in net energy for lactation, rumen undegradable protein, and rumen degradable protein, as described earlier by Van Knegsel et al. (2014). Preliminary analyses showed no effect of ration on average SCC for lactation or elevations of SCC or clinical mastitis. Ration was therefore excluded from further analyses in this study. Cows were housed in a freestall barn with slatted floors and cubicles. Cows were milked twice daily. The drying-off protocol for cows with a 30-d or 60-d DP consisted of a transition to the dry cow ration at d 7 before the actual drying-off day and transition to milking once daily at d 4 before the actual drying-off day. At drying-off, cows with a 30-d or 60-d DP received intramammary treatment with a dry cow antibiotic (Supermastidol, Virbac Animal Health, Barneveld, the Netherlands). Clinical mastitis cases were diagnosed and recorded by the milkers at the Dairy Campus research herd during the morning or evening milking. A case of clinical mastitis was defined as a treated case of visibly abnormal milk, changes in the udder due to inflammation, or both. All cows with clinical mastitis were treated with antibiotics according to the herd-specific treatment plan based on severity of disease.

5.3.2 Milk Collection and Analysis

Milk yield, milk fat and protein, and SCC for the lactation before the DP of study were available monthly for each cow and were obtained from the Dutch national milk recording system (CRV, Arnhem, the Netherlands). After calving, milk yield, milk fat and protein, and SCC were available weekly until the end of lactation (wk 44 postpartum) for each cow. Milk samples analysis of fat, protein, and SCC (Qlip, Zutphen, the Netherlands) were collected 4 times weekly (Tuesday afternoon, Wednesday morning, Wednesday afternoon, and Thursday morning) and pooled per week.

5.3.3 Statistical analyses

Data Handling. Statistical analyses were performed using SAS version 9.3 (**SAS Institute Inc.**, 2011). The natural logarithm of SCC (LnSCC) was used for analyses to approximate normality. Some prepartum variables were not available for all cows due to missing milk records (**Table 5.1**). The final data set consisted of 1,983 prepartum test-days (median −202; range −496 to −1 DIM) and 7,348 postpartum test-days (median 147; range 1–307 d in milk) of 167 cows between May 7th 2009 and September 30th 2012.

Table 5.1: Number of available observations per prepartum variable for cows with a dry period length of 0, 30 or 60 days

	Dry period length (d)			
	0	30	60	Total
Number of cows	56	55	56	167
Pre calving variable				
Complete lactation pre calving	54	50	52	156
Total lactation yield	56	55	55	166
SCC and milk yield 150 - 67 d	55	55	56	166
Last SCC and last FPCM before DP	54	53	54	161
Post calving variable				
Complete lactation (44 w)	35	36	40	111

Milk Yield and Udder Health Before and After Different DP lengths in Cows of Different Parities. To compare prepartum variables for different DP lengths and to analyze the effect of DP length on postpartum udder health, a mixed linear model (PROC MIXED) was used. The dependent variables in the statistical analysis were total lactation yield (305-d milk production in kg) and average SCC in the complete previous lactation. The dependent variables between 150 and 67 d prepartum were fat- and protein- corrected milk (FPCM) production (kg/d), FPCM reduction (kg), and SCC. The dependent variables on the last test-day before the conventional drying-off day (67 d before the expected calving date) were last FPCM and last SCC. The independent variables were DP length (0, 30, or 60 d), prepartum parity (1, 2, >2), and calving season (meteorological winter, spring, summer, or autumn). The following mixed linear model (PROC MIXED) was used:

$$y_{ijk} = \mu + DP_i + Parity_j + (DPxParity)_{ij} + Season_k + \varepsilon_{ijk} \quad [1]$$

where y_{ijk} indicates the *ijk*-th observation of a continuous prepartum variable. The mean is represented by μ. DP_i indicates the DP length (i = 0-, 30-, or 60-d DP), $Parity_j$ indicates the parity of the cow prepartum (j = parity 1, 2, or >2), $(DP \times Parity)_{ij}$ indicates the interaction between DP and Parity, $Season_k$ indicates the random effect of calving season (k = meteorological winter, spring, summer, or autumn), and the random residual term from a normal distribution is indicated by ε_{ijk}.

For analyses of postpartum SCC between 2 and 44 weeks of lactation, Model [1] was used with a repeated measurement effect consisting of postpartum lactation weeks (*l*) with cows as the subject. A first-order heterogeneous autoregressive covariance matrix (ARh(1)) was the best fit according to Akaike's corrected information criterion and was used to account for within-cow variation. The dependent variable in the statistical analysis was postpartum weekly SCC between 2 and 44 weeks of lactation. The independent variables in Model [2] remained the same as in Model [1], except for the addition of the repeated measurement effect. The following mixed linear model (PROC MIXED) was used:

$$y_{ijkl} = \mu + DP_i + Parity_j + (DPxParity)_{ij} + Season_k + Week_l + \varepsilon_{ijkl,} \quad [2]$$

where y_{ijkl} indicates the *ijkl*-th observation of a postpartum SCC.

For binary variables, Model [1] was used with a binary distribution for the dependent variables in this model and the default logit link function to model the mixed linear regression analysis using PROC GLIMMIX. Dependent binary variables in the complete previous lactation were: at least one elevation of SCC, and at least one case of clinical mastitis for each cow. Dependent postpartum binary variables were: at least one elevation of SCC between 3 and 44 weeks postpartum, and at least one case of clinical mastitis between 1 and 44 wk postpartum for each cow. An elevation of SCC was defined as SCC ≥ 200,000 cells/mL after two previous weeks with SCC < 200,000 cells/mL (**Schukken *et al.***, 2003).

Differences in number of elevations of SCC per cow prepartum, cases of clinical mastitis per cow prepartum, elevations of SCC per cow postpartum, and cases of clinical mastitis per cow postpartum were analyzed using Model [1] with a Poisson distribution for the dependent variables and the default log link function in a mixed linear regression analysis (PROC GLIMMIX).

Occurrence of intramammary infections during different dry period lengths. To evaluate whether new IMI had occurred during the precalving period, the last SCC before 67 days prepartum (median -83 d; range -100 to -68 days prepartum) was compared with the first SCC postpartum, excluding the first week of lactation (median 13 d; range 10 to 23 days postpartum). Cows were considered infected if SCC was ≥ 200,000 cells/mL. Somatic cell count before and after the DP were compared to categorize cows as having a chronic IMI (SCC ≥ 200,000 cells/mL before and after the DP), not infected (SCC < 200,000 cells/mL before and after the DP), having a new IMI (SCC < 200,000 cells/mL before and SCC ≥ 200,000 cells/mL after the DP) or cured (SCC ≥ 200,000 cells/mL before and SCC < 200,000 cells/mL after the DP). The difference in incidence risks of IMI among DP lengths was analyzed using the following logistic regression model [PROC LOGISTIC; model 3]:

$$\text{Logit } P(y_{ij}=1) = \alpha + DP_i + \varepsilon_j, \quad [3]$$

in which the model estimates the probability (P) that the *ij*-th observation of postpartum IMI (y) is 1. Within observations of high SCC prepartum, chronic IMI=1 and cured IMI=0. Within observations of low SCC prepartum new IMI=1 and no IMI=0. Logit is the link function used to model the regression analysis, α is the estimate of the baseline odds of occurrence of IMI, DP_i indicates the DP length (*i*= 0-, 30-, or 60-d DP), and the random residual term from a binary distribution is represented by ε_j.

Association of prepartum milk yield and udder health with postpartum udder health. The association of the prepartum variables and DP length with postpartum udder health was analyzed. To evaluate which cow characteristics are associated with postpartum SCC, a mixed linear model (PROC MIXED) was used. The dependent variable in this statistical analysis was postpartum SCC. For postpartum SCC, the univariable and multivariable model were assessed with the addition of a repeated measurement effect consisting of postpartum lactation weeks with cow as the subject. A first-order heterogeneous autoregressive covariance matrix (ARh(1)) was the best fit according to Akaike's corrected information criterion and was used to account for within-cow variation.

To evaluate which cow characteristics are associated with the binary variables at least one elevation of SCC between 3 and 44 weeks postpartum, and at least one case of clinical mastitis between 1 and 44 weeks postpartum for each cow, a mixed linear regression model (PROC GLIMMIX) was used. The univariable and multivariable model were assessed using a binary distribution for the dependent variables in this model, with a logit link function to model the mixed linear regression analysis. The dependent variables in these two statistical analyses were postpartum elevations of SCC, and postpartum clinical mastitis.

The independent variables in all three statistical analyses were: total lactation yield (305-d milk production in kg) and average SCC for lactation in the complete previous lactation. The independent variables between 150 and 67 days prepartum were: fat- and protein- corrected-milk (FPCM) production (kg/d), FPCM reduction (kg), and SCC. The independent variables at the last test-day before the conventional drying-off day (67 days before the expected calving date) were: last FPCM, and last SCC. The fixed independent variables were DP length (0-d, 30-d, 60-d), prepartum parity (1, 2, >2), and calving season (meteorological winter, spring, summer, or autumn).

First, dependent variables were analyzed with each of the independent variables in a univariable model. Independent variables with $P \leq 0.20$ were included in a multivariable model for each dependent variable. In the multivariable models, variables were analyzed using a backward elimination procedure with a stay-in P-value of ≤ 0.05 in the Wald test, except for the fixed effects described in Models [1] and [2] that remained in the models at all times. After backward elimination, all possible 2-way interactions and 3-way interactions were tested and were maintained in the model when $P \leq 0.05$ in the Wald test and when the interaction lowered Akaike's information criterion of the model ($P \leq 0.05$).

5.4 Results

5.4.1 Udder health and milk production before the conventional drying-off day

Cows with a 0-d DP were on average 385 DIM (± 5.5 DIM) at the day before calving. Cows with a 30-d DP were on average 367 DIM (± 6.4 DIM) at drying-off, and cows with a 60-d DP were on average 335 DIM (± 7.3 DIM) at drying-off. Last milk yield before drying-off was greater in cows with a 60-d DP (9.4 ± 0.5

kg) than in cows with a 30-d DP (7.4 ± 0.5 kg). Milk production in cows with a 0-d DP was 6.8 kg (± 0.5 kg) at the day before calving.

Prepartum average SCC for lactation, occurrence of at least 1 elevation of SCC, and clinical mastitis did not differ among DP lengths (**Table 5.2**). Parity effects, however, were present. Cows with parity 1 had a lower FPCM reduction between 150 and 67 d prepartum ($P < 0.01$) and tended to have a lower occurrence of clinical mastitis ($P = 0.07$) and a lower number of cases of clinical mastitis per treated cow ($P = 0.06$) compared with cows with parity >2. Cows with parity 1 had a lower ($P < 0.01$) total lactation yield, lower SCC at last test-day before the conventional drying-off day, and lower number of elevations of SCC per affected cow, compared with cows with parity 2 or parity >2. Lower parity was associated with lower average SCC for lactation, lower SCC between 150 and 67 d prepartum, and lower number of cows showing at least 1 elevation of SCC ($P < 0.01$). Regardless of DP length or parity, cows with an IMI (SCC ≥ 200,000 cells/mL) on the last test-day before the conventional drying-off day had a greater FPCM reduction between 150 and 67 d prepartum (6.2 ± 0.7 kg), compared with cows that had no IMI on the last test-day (4.4 ± 0.4 kg) ($P = 0.03$).

5.4.2 Udder Health After Different DP Lengths

The actual DP length was 2 ± 1 d for cows with a 0-d DP, 30 ± 1 d for cows with a 30-d DP, and 61 ± 1 d for cows with a 60-d DP (mean ± SEM). Postpartum SCC differed for parity and among DP lengths (**Table 5.2**). The interaction of DP length with parity resulted in the greatest postpartum SCC for cows with parity >2 and a 0-d DP (lnSCC 5.33 ± 0.08) and the lowest postpartum SCC for cows with parity 1 and a 30-d or 60-d DP (lnSCC 4.33 ± 0.08 and 4.38 ± 0.08, respectively) ($P = 0.02$). Lower parity ($P < 0.01$) was associated with a smaller number of cows showing at least 1 elevation of SCC or clinical mastitis in the subsequent lactation.

Table 5.2: Prepartum milk production and udder health variables, and postpartum udder health variables for cows with a 0, 30 or 60 days dry period based on a mixed model with dry period length, parity and their two-way interaction as fixed variables (LSM ± SEM)

	Dry period length (d)				Parity				P-values [1]		
	0	30	60	SEM	1	2	>2	SEM	DP	P	DP * P[2]
Cows (n)	56	55	56		60	53	54				
Prepartum variables[3]											
Total lactation yield (305 days) (kg milk)	9976	9993	10028	156	8989[a]	10310[b]	10698[b]	156	0.97	<0.01	0.21
FPCM[4] reduction between 150 and 67d prepartum (kg)	-6	-5	-4	0.71	-4[a]	-5[ab]	-6[b]	0.71	0.16	<0.01	0.83
FPCM between 150 and 67d prepartum (kg/d)	27	27	27	0.72	27	27	27	0.72	0.80	0.98	0.39
Last FPCM 67 days prepartum (kg/d)	24	25	25	0.75	25	25	24	0.74	0.70	0.41	0.44
Average SCC for lactation[5]	4.25	4.35	4.13	0.09	3.96[a]	4.27[b]	4.60[c]	0.09	0.12	<0.01	0.83
SCC between 150 and 67d prepartum[5]	4.56	4.52	4.46	0.09	3.99[a]	4.56[b]	5.00[c]	0.09	0.72	<0.01	0.38
Last SCC before 67d prepartum[5]	4.73	4.70	4.52	0.11	4.08[a]	4.82[b]	5.06[b]	0.11	0.33	<0.01	0.25
Elevations of SCC (% cows)[6]	41	42	34		20[a]	43[b]	56[c]		0.57	<0.01	0.65
Elevations of SCC (n cases/ affected cow)[7]	1.26	1.30	1.26		1.08[a]	1.30[b]	1.33[b]		0.37	<0.01	0.41
Clinical mastitis (% cows)	25	20	15		10[a]	23[ab]	28[b]		0.37	0.07	n.m.[8]
Clinical mastitis (n cases/affected cow)[7]	1.29	1.18	1.00		1.00[a]	1.17[ab]	1.27[b]		0.18	0.03	n.m.
Postpartum variables (1-44 weeks in lactation)											
Average SCC for lactation[5,7]	5.01[a]	4.68[b]	4.52[c]	0.06	4.49[a]	4.71[b]	5.01[c]	0.06	<0.01	<0.01	0.02
Elevations of SCC (% cows)[6]	79	78	79		67[a]	83[b]	87[c]		0.99	0.03	0.94
Elevations of SCC (n cases/affected cow)[7]	2.30	2.29	1.77		1.83[a]	2.20[b]	2.28[b]		0.22	<0.01	0.62
Clinical mastitis (% cows)	27	25	25		13[a]	30[b]	35[c]		0.95	0.03	0.98
Clinical mastitis (n cases/affected cow)[7]	1.44	1.36	1.21		1.00[a]	1.19[b]	1.63[b]		0.80	<0.01	0.99

Footnotes for Table 5.2

[a-c]Values within dry period length or parity, within a row with different superscripts differ (P<0.05). [1]DP= Dry period length; P= Parity. [2]DP*P= interaction of dry period length with parity. [3]The median and range of prepartum variables between 150 and 67 days: fat- and protein- corrected-milk (FPCM) reduction had a median between -135-d (range -150 to -101 d) and -83-d (range -112 to -67 d), FPCM had a median at -109-d (range -150 to -67 d), and SCC had median at -108-d (range -150 to -67 d). The variables at the last test-day before the conventional drying off day (67 days before the expected calving date): last FPCM had a median at -84-d (range -121 to -67 d), and last SCC had a median at -83-d (range -100 to -68 d). [4] Fat and protein corrected milk. [5]Somatic cell count (SCC) is shown as the natural logarithm of SCC (LnSCC). [6]An elevation of SCC in milk was defined as SCC ≥ 200,000 cells/mL after two previous weeks with SCC < 200,000 cells/mL. [7]For FPCM, SCC, and cases per affected cow, week was included in the model (P<0.01). [8]n.m.: not in model.

5.4.3 Occurrence of IMI During Different DP Lengths

The prevalence of SCC ≥ 200,000 cells/mL on the last test-day before the conventional drying-off day was not different (*P* = 0.75) among DP lengths (**Table 5.3**). At the first test-day after calving, cows with a 0-d DP had a greater prevalence of SCC ≥ 200,000 cells/mL than cows with a 60-d DP (*P* = 0.05). Compared with cows with a 0-d DP, the proportion of cows with a chronically high SCC ≥ 200,000 cells/mL after the DP was lower and the cure rate was greater in cows with a 30-d or 60-d DP (*P* = 0.04). The proportion of cows with a newly high SCC ≥ 200,000 cells/mL or no infection after the DP did not differ among DP lengths (*P* = 0.58).

Table 5.3: Proportion (and number) of cows with a high somatic cell count (SCC) (≥ 200,000 cells/mL) at the last test-day before the conventional drying off day (67 days prepartum) and at the first test-day (10 days postpartum) after a 0, 30 or 60 days dry period

	Dry period length (d)			
	0	30	60	P - value
Total number of cows	54	53	54	
SCC < 200,000 prepartum	81 (44)	75 (40)	78 (42)	
SCC ≥ 200,000 prepartum	19 (10)	25 (13)	22 (12)	0.75
SCC < 200,000 postpartum	74 (40)	87 (46)	89 (48)	
SCC ≥ 200,000 postpartum	26[a] (14)	13[ab] (7)	11[b] (6)	0.09
High SCC prepartum				
Chronic IMI[1]	50[a] (5)	8[b] (1)	8[b] (1)	0.04
Cured IMI[2]	50[a] (5)	92[b] (12)	92[b] (11)	0.04
Low SCC prepartum				
New IMI[3]	20 (9)	15 (6)	12 (5)	0.58
No IMI[4]	80 (35)	85 (34)	88 (37)	0.58

[a-b]Values with the same superscript within a row are not different (*P*>0.05). [1]A chronic intramammary infection (IMI) was defined as SCC ≥ 200,000 cells/mL before and after the dry period (DP). [2]A cured IMI was defined as SCC ≥ 200,000 cells/mL before and SCC < 200,000 cells/mL after the DP. [3]A new IMI was defined as SCC < 200,000 cells/mL before and SCC ≥ 200,000 cells/mL after the DP. [4]No IMI was defined as SCC < 200,000 cells/mL before and after the dry period.

5.4.4 Predictors for SCC, Elevation of SCC, and Clinical Mastitis After Different DP Lengths SCC.

Postpartum average SCC for lactation. Based on the univariable model, 10 prepartum variables were found to be related ($P < 0.20$) to postpartum average SCC for lactation: DP length, parity, clinical mastitis, at least 1 elevation of SCC, total lactation yield, average SCC for lactation, FPCM reduction and SCC between 150 and 67 d prepartum, and last FPCM and last SCC before the conventional drying-off day. After backward elimination, 6 prepartum variables remained in the multivariable model: DP length ($P = 0.52$), parity ($P < 0.01$), at least 1 elevation of SCC ($P = 0.02$), FPCM reduction ($P < 0.01$), last FPCM before the conventional drying-off day ($P < 0.01$), and average SCC in the previous lactation ($P = 0.05$) (**Table 5.4**). Significant 2-way interactions were present of DP length with parity ($P < 0.01$), DP length with elevations of SCC ($P < 0.01$), DP length with FPCM reduction ($P = 0.04$), and last FPCM with average SCC for lactation ($P < 0.01$). A significant 3-way interaction was present of DP length with parity and average SCC for lactation ($P < 0.01$).

Table 5.4: Final multivariable model for prediction of postpartum average somatic cell count (SCC) between week 2 and 44 in lactation for cows with a 0, 30 or 60 days dry period (LSM ± SEM or regression coefficient (β) with standard error). The final multivariable model was based on 11 univariable models, with individual prepartum variables as independent variable, to identify potential predictors for postpartum average SCC

		Postpartum variables	
		SCC[1, 2]	
Prepartum variable	Category	LSM (SEM)	P-value
Dry period length (DP)	0	5.03 (0.08) [a]	0.52
	30	4.56 (0.09) [b]	
	60	4.49 (0.09) [b]	
Parity	Parity 1	4.61 (0.09) [a]	<0.01
	Parity 2	4.58 (0.08) [a]	
	Parity >2	4.88 (0.09) [b]	
DP*Parity	0* 1	5.02 (0.10) [a]	<0.01
	0* 2	4.77 (0.10) [b]	
	0* >2	5.30 (0.11) [c]	
	30* 1	4.42 (0.10) [d]	
	30* 2	4.56 (0.11) [d,e]	
	30* >2	4.69 (0.11) [e]	
	60* 1	4.40 (0.13) [d]	
	60* 2	4.42 (0.10) [d]	
	60* >2	4.65 (0.11) [e]	
Elevation of SCC[3]	0	4.62 (0.08) [a]	0.02
	1	4.77 (0.09) [b]	
DP * Elevation of SCC	0*0	4.76 (0.09) [a]	<0.01
DP * Elevation of SCC	0*1	5.30 (0.10) [b]	
DP * Elevation of SCC	30*0	4.64 (0.10) [a]	
DP * Elevation of SCC	30*1	4.47 (0.11) [c]	
DP * Elevation of SCC	60*0	4.46 (0.09) [c,d]	
DP * Elevation of SCC	60*1	4.52 (0.11) [a,d]	
Lactation week postpartum		β (SE)	<0.01

[a-e]Values with the same superscript per variable are not different (*P*>0.05). [1] Somatic cell count (SCC) is shown as the natural logarithm of SCC (LnSCC). [2]SCC was monitored between 2 and 44 weeks in lactation. [3]An elevation of SCC in milk was defined as SCC in milk ≥ 200,000 cells/mL after two previous weeks with SCC < 200,000 cells/mL.

Table 5.4 is continued on the next page.

Continuation of table 5.4.

Table 5.4: Final multivariable model for prediction of postpartum average somatic cell count (SCC) between week 2 and 44 in lactation for cows with a 0, 30 or 60 days dry period (LSM ± SEM or regression coefficient (β) with standard error). The final multivariable model was based on 11 univariable models, with individual prepartum variables as independent variable, to identify potential predictors for postpartum average SCC

		Postpartum variables	
		SCC[1, 2]	
Prepartum variable	Category	LSM (SEM)	P-value
		β (SE)	<0.01
Last FPCM before 67-d DP	Average 25	-0.19 (0.04)	<0.01
Last FPCM * SCC[1]		0.03 (0.01)	<0.01
FPCM reduction 150 – 67-d	Average -4.77	-0.06 (0.02)	<0.01
DP * FPCM reduction	0	0.03 (0.01) [a,b]	<0.04
DP * FPCM reduction	30	0.05 (0.02) [a]	
DP * FPCM reduction	60	Ref [b]	
Average SCC for lactation[1]	Average 113	-0.70 (0.26)	0.05
DP * average SCC for lactation[1]	0	-0.01 (0.10) [a]	0.18
DP * average SCC for lactation[1]	30	0.41 (0.13) [b]	
DP * average SCC for lactation[1]	60	Ref [a]	
Parity * average SCC for lactation[1]	1	-0.05 (0.10) [a]	<0.01
Parity * average SCC for lactation[1]	2	0.39 (0.11) [b]	
Parity * average SCC for lactation[1]	>2	Ref [a]	
DP*Parity*average SCC for lactation[1]	0*1	0.18 (0.27)	<0.01
DP*Parity*average SCC for lactation[1]	0*2	0.02 (0.27)	
DP*Parity*average SCC for lactation[1]	30*1	-0.08 (0.28)	
DP*Parity*average SCC for lactation[1]	30*2	-0.89 (0.29)	
DP*Parity*average SCC for lactation[1]	0*>2, 30*>2, 60*1, 60*2, 60*>2	Ref	

[a-e]Values with the same superscript per variable are not different (P>0.05). [1] Somatic cell count (SCC) is shown as the natural logarithm of SCC (LnSCC). [2]SCC was monitored between 2 and 44 weeks in lactation. [3]An elevation of SCC in milk was defined as SCC in milk ≥ 200,000 cells/mL after two previous weeks with SCC < 200,000 cells/mL.

Table 5.5: Final multivariable models for prediction of postpartum at least one elevation of SCC between week 3 and 44 in lactation and clinical mastitis between week 1 and 44 in lactation after a 0, 30 or 60 days dry period (values represent occurrence, odds ratio with confidence interval and P-value)

		Postpartum variables			
		Elevation of SCC[2]		Clinical mastitis[3]	
Prepartum variable	Category	Occurrence (%)	P-value	Occurrence (%)	P-value
Dry period length	0	79		27	
	30	78	0.99	25	0.99
	60	79		25	
Parity	Parity 1	67 [a]		13	
	Parity 2	83 [ab]	0.03	30	0.56
	Parity >2	87 [b]		35	
				OR (CI)	
Last SCC 67d[1]				2.04 (1.22 – 3.43)	<0.01

[a-c] Values with the same superscript per variable are not different (P>0.05). [1] Somatic cell count (SCC) is shown as the natural logarithm of SCC (LnSCC). [2] Elevation of SCC was evaluated between 3 and 44 weeks in lactation. An elevation of SCC in milk was defined as at least one elevation of SCC ≥ 200,000 cells/mL after two previous weeks with SCC < 200,000 cells/mL. [3] Clinical mastitis was evaluated between week 1 and 44 in lactation.

Elevation of SCC. Based on the univariable models, 8 prepartum variables were related (P < 0.20) to at least 1 elevation of SCC postpartum: parity, occurrence of clinical mastitis, total lactation yield, average SCC for lactation, FPCM reduction and SCC between 150 and 67 d prepartum, and last FPCM and last SCC before the conventional drying-off day. In the multivariable model only parity (P = 0.03) remained, independent of DP length (**Table 5.5**). Cows with parity 1 showed at least 1 elevation of SCC in the subsequent lactation less often.

Clinical Mastitis. Based on the univariable models 7 prepartum variables were related (P < 0.20) to at least 1 case of clinical mastitis postpartum: parity, clinical mastitis prepartum, total lactation yield, average SCC for lactation, SCC between 150 and 67 d prepartum, and last SCC before the conventional drying-off day. In the multivariable model only last SCC before the conventional drying-off day (P < 0.01) remained, independent of DP length (**Table 5.5**). Cows with a greater last SCC before the conventional drying-off day had 2 times greater odds of having at least 1 case of clinical mastitis in the subsequent lactation.

5.5 Discussion

Postpartum SCC was greater in cows with a 0-d DP than in cows with a 30-d or 60-d DP. The greater postpartum SCC in cows with a 0-d DP in the current study is in contrast with earlier studies of **Gulay *et al.*** (2003) and **Rastani *et al.*** (2005), but it is in line with **Pezeshki *et al.*** (2007) and **Steeneveld *et al.*** (2013). The greater postpartum SCC in cows with a 0-d DP may be the consequence of uncured IMI before calving because of the lack of both a DP and treatment with dry cow antibiotics. Cows with a 0-d DP, however, have lower milk production in the subsequent lactation, resulting in a lower dilution effect, which may also explain the increased SCC (**Steeneveld *et al.***, 2013). Additionally, the udder involutes in cows with a DP compared with cows with a 0-d DP. Udders that are fully involuted are highly resistant to new IMI (**Oliver and Sordillo**, 1989), probably through the increase of immunoglobulins and lactoferrin in lacteal secretions during the DP (**Hurley and Rejman**, 1993). Cows with a 0-d DP or 30-d DP do not have a fully involuted udder during the precalving period and may have a lower concentration of lactoferrin and immunoglobulins in milk. Moreover, milking twice daily instead of once daily in the week before drying off decreased the concentration of lactoferrin in milk (**Newman *et al.***, 2009). Cows with a 30-d DP, but especially cows with a 0-d DP, may therefore be more prone to a greater SCC in the subsequent lactation.

5

It is unknown if the observed high SCC in early lactation in cows with a 0-d DP is actually correlated with intramammary bacterial infections. If the greater postpartum SCC in cows with a 0-d DP is indeed related to bacterial infections, then a 0-d DP would not be an attractive alternative to using dry cow antibiotics to prevent IMI during the precalving period. New IMI during the precalving period in cows with a 0-d DP could also be the result of withholding preventive dry cow antibiotics in these cows compared with cows with a 30-d or 60-d DP. The significance of DP length in 4 interactions in the final model for predicting postpartum SCC could thus be explained by either DP length, use of preventive dry cow antibiotics, or both.

In many countries blanket dry cow treatment with antibiotics is advised, and application of a DP is therefore confounded with the administration of preventive dry cow antibiotics as is omitting the DP altogether without administering preventive dry cow antibiotics. We excluded cows with a SCC

>250,000 on the last test-day before the conventional drying-off day, based on the currently used attention levels for high SCC in multiparous cows in the Netherlands (**Scherpenzeel *et al.***, 2014). Our results suggest that cows with a SCC >200,000 cells/mL on the last test-day should also be treated with antibiotics and have a DP matching with the withdrawal time. In the subsequent lactation, cows with a 30-d DP had a lower SCC than cows with a 0-d DP. In contrast, cows with a 30-d DP had a greater SCC than cows with a 60-d DP in the subsequent lactation. In the current study, a 30-d and 60-d DP was confounded by the use of preventive dry cow antibiotics. To our knowledge, the effect of a 30-d DP without preventive dry cow antibiotics on postpartum SCC has not yet been evaluated.

This suggests that a 0-d DP is not beneficial for cows with an IMI at the last test-day before the conventional drying off day. An IMI before the conventional drying off day has a better chance to cure during a DP treatment with dry cow antibiotics. The greater proportion of chronic IMI in cows with a 0-d DP was related with a lower cure rate of IMI. Compared with previous studies using a 30-d or 60-d DP (**Church *et al.***, 2008) or a 60-d DP (**Pantoja *et al.***, 2009a), the proportion of cows with a chronic IMI was lower, while the proportion of cows with a cured IMI in cows with a 30-d or 60-d DP was greater in the current study. The proportion of cows with a new IMI was either greater (**Church *et al.***, 2008) or smaller (**Pantoja *et al.***, 2009a), and the proportion of cows with no high SCC was smaller (**Church *et al.***, 2008) or not different (**Pantoja *et al.***, 2009a) in cows with a 30-d or 60-d DP in this study. These differences in the effect of DP length among different studies, may not only be explained by the effect of DP length itself, but also by management factors, such as experimental set-up and herd management, or different definitions for IMI among studies.

Factors relevant for udder health in the subsequent lactation include interactions of DP length with udder health variables, parity, and milk production. To identify cow characteristics that can be used by farmers to optimize DP length for individual cows, prepartum variables were selected that can be calculated on every farm with milk production recording. The prepartum variable at least one elevation of SCC was based on monthly test-day SCC. Results of the current study indicate that postpartum SCC is affected by at least one elevation of SCC in the previous lactation. The prepartum variable FPCM reduction between 150 and 67 days prepartum, as a measure for persistency, was negatively correlated with postpartum SCC. Less persistent cows had higher SCC postpartum, which could be explained by the higher prevalence of IMI (SCC ≥ 200,000 cells/mL) at the last test-day before the conventional drying off day. Milk production at drying off as such, was not included in the model because it was not comparable among groups with different DP lengths in our study. The interaction of DP length with prepartum FPCM reduction revealed that a 60-d DP was beneficial for postpartum SCC. In cows with a FPCM reduction >5 kg between 150 and 67 days prepartum, postpartum SCC was lowest when they had a 60-d DP, compared with a 0-d or 30-d DP. Additionally, it is practical to dry off a cow with a large reduction of FPCM before the conventional drying off day at 60-d prepartum. From the three-way interaction of DP length with parity and prepartum average SCC for lactation it appears that the effect of DP length on postpartum average SCC depends on parity and prepartum SCC. The identified characteristics can be used in a decision support model to optimize DP length for individual cows, which may support the farmer in the optimal DP strategy for individual cows.

Other management measures to reduce the risk of new IMI during the precalving period include cow-level measures, such as dry cow antibiotics and internal teat sealants, and herd-level measures, such as housing, hygiene, and to a certain extent feeding (**Green *et al.***, 2008). Preventive use of dry cow antibiotics has long been an important management measure to prevent new IMI during the precalving period, but it is no longer allowed in several European countries, including the Netherlands (**Scherpenzeel *et al.***, 2014). Other management factors to prevent new IMI during the precalving period, such as shortening or omitting the DP, have become more important. Simultaneously, management measures such as environmental factors that influence udder health also require attention. Environmental factors such as experimental set-up, housing (tie-stall vs. freestall, bedding, and grazing) and herd management factors (i.e., hygiene measures, protocols, and personnel) differ among herds and experiments, and they contribute to variation among herds and experiments (**Green *et al.***, 2008). Therefore, including knowledge of management measures on herd level is important in the DP approach for individual cows. Management factors should thus be included in a decision support model to optimize DP strategies for individual cows, but could not be determined from our study, because it was performed in only one herd.

The number of cows with at least one elevation of SCC or with at least one case of clinical mastitis postpartum was not different among DP lengths, despite the greater postpartum average SCC for lactation in cows with a 0-d DP. To our knowledge, studies that report the effect of omitting the DP on mastitis incidence are limited (**Rastani *et al.***, 2005; **Schlamberger *et al.***, 2010) and studies that report the effect of DP length on SCC elevation in the subsequent lactation are absent. Across studies, it is difficult to draw definite conclusions on consequences of shortening (**Pezeshki *et al.***, 2007; **Watters *et al.***, 2008; **Santschi *et al.***, 2011) or omitting (**Rastani *et al.***, 2005) the DP for mastitis. In several studies, among which the current study, animal numbers are limited. Moreover, lack of clear or consistent effects is possibly also due to variation among studies with respect to definition of mastitis, and disease status at the start of the experiment.

In our study, a greater last SCC before the conventional drying off day was associated with a 2 times greater risk of at least one case of clinical mastitis in the subsequent lactation. In previous studies high SCC (≥ 200,000 cells/mL) was associated with a 2 times greater risk of clinical mastitis in the same lactation (**Van den Borne *et al.***, 2011) and a 4.2 times greater risk of clinical mastitis in next lactation (**Pantoja *et al.***, 2009b). The low odds for at least one case of clinical mastitis or at least one elevation of SCC postpartum, compared with **Pantoja *et al.*** (2009b), is probably due to the low prepartum occurrence of clinical mastitis and elevations of SCC in our study. **Pantoja *et al.*** (2009b) reported a 25% occurrence of clinical mastitis in previous lactation with 1.4 cases per cow, while the current study reports a 20% occurrence of clinical mastitis in previous lactation, with 1.16 cases/cow. Selection for low SCC at two test-days prepartum resulted in a low last SCC before the conventional drying off day. Cows with a SCC ≥ 250,000 cells/mL at two test-days before the conventional drying off day, likely had an IMI, and were therefore not included in our study. These cows should be treated with dry cow antibiotics in order to cure IMI. From this study it appears that a 60-d DP is also beneficial in cows with SCC > 200,000 at the last test-day before the conventional drying off day. On the one hand, a 30-d DP could be beneficial in cows with a low reduction or increase of FPCM between 150 and 67 days prepartum. On the other hand, a 0-d DP could be beneficial in cows with a low reduction or increase of FPCM between 150 and 67 days prepartum, without an elevation of SCC in previous lactation.

5.6 Conclusions

A 0-d DP resulted in a greater SCC in the subsequent lactation, compared with a 30-d or 60-d DP. A 30-d or 0-d DP did not affect the occurrence of at least one elevation of SCC ≥ 200,000 cells/mL, or the occurrence of clinical mastitis postpartum, compared with a 60-d DP. A 0-d DP is disadvantageous in cows with a SCC ≥ 200,000 at the last test-day before the conventional drying off day, because these cows were found to have a greater occurrence of chronic IMI and a lower cure rate during the precalving period, than cows with a 30-d or 60-d DP. Other cow characteristics than DP length, that predict postpartum udder health are parity, prepartum elevations of SCC, average prepartum SCC for lactation, FPCM reduction between 150 and 67 days prepartum, and last FPCM and last SCC before the conventional drying off day.

5.7 Acknowledgments

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CHAPTER 6

Udder health of dairy cows fed different dietary energy levels after a short or no dry period without use of dry cow antibiotics

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6.1 Abstract

Reports on effects of length of dry period (**DP)** without dry cow antibiotics on udder health is scarce. Additionally, effects of a reduced dietary energy level for the lower milk production of cows with a 0-d DP on udder health have not been studied yet. The aim of this study was to compare the effect of a 0-d or 30-d DP without use of dry cow antibiotics on udder health during the peripartum period and subsequent lactation and to evaluate relationships between udder health and metabolic status of dairy cows. In wk 5 before the expected calving date, Holstein-Friesian dairy cows (N = 123) were assigned randomly to 2 DP lengths [0-d DP (n = 81) or 30-d DP (n = 42)]. Postpartum, Holstein-Friesian dairy cows (N = 110) were assigned randomly to 3 transition treatments, viz. a 30-d DP with a standard energy level required for expected milk yield [**30-d DP(STD**), n = 40)], a 0-d DP with the same STD energy level as cows with a 30-d DP [**0-d DP(STD**), n = 34)], and a 0-d DP with a low energy level [**0-d DP(LOW**), n = 36)]. The prevalence of quarters with elevated somatic cell count (**SCC**) (SCC ≥ 200,000 cells/mL), and the prevalence of mastitis pathogens in quarter milk samples in wk 5 prepartum and in wk 1 and 5 postpartum, did not differ between DP lengths. During wk 1 – 44 of lactation, cows with a 0-d DP had a greater SCC than cows with a 30-d DP independent of energy level. The difference in SCC between DP lengths disappeared after it was corrected for milk yield. During wk 1 – 44 of lactation, the occurrence of at least 1 elevation of SCC (≥ 200,000 cells/mL) was not different among transition treatments. Cows with a 0-d DP(STD), but not cows with a 0-d DP(LOW), were more likely to obtain a case of clinical mastitis at any time in lactation than cows with a 30-d DP(STD). Postpartum, greater SCC was related with lower fat- and protein- corrected milk (**FPCM**) yield and energy intake, and greater energy balance (**EB**) and plasma insulin. In conclusion, a 30-d DP without dry cow antibiotics lowered SCC, compared with a 0-d DP, but not when SCC was corrected for difference in milk yield. Dry period length did not affect udder health in the dry period and in early lactation, but cows with a 0-d DP fed a standard energy level tended to have a greater hazard for a case of clinical mastitis than cows with a 30-d DP during the complete lactation.

Key words: continuous milking, decision making, antibiotic use, dietary energy source

6

6.2 Introduction

The dry period (**DP**) is an important period in the dynamics of intramammary infections (**IMI**) in dairy cows (**Green *et al.***, 2005; **Pantoja *et al.***, 2009; **Scherpenzeel *et al.***, 2014). Management of the DP with respect to udder health aims at both curing existing IMI and reducing the risk of new IMI during the DP (**Collier *et al.***, 2012). Management measures to reduce the risk of new IMI during the DP include herd level measures such as housing, hygiene, and nutrition, and cow level measures such as use of dry cow antibiotics or internal teat sealants, or both (**Annen *et al.***, 2004; **Green *et al.***, 2007). Preventive use of dry cow antibiotics is, however, not allowed in several European countries among which the Netherlands (**Scherpenzeel *et al.***, 2014; **Dutch M. o E. a.**, 2017).

Shortening or omitting the DP has been hypothesized to affect udder health in dairy cows, although reported effects are ambiguous. Compared with a conventional DP length (range 49 till 60-d), a short (range 28 till 35-d) or omitted DP (0-d) resulted in a decrease in somatic cell count (**SCC**) in the subsequent lactation (**Gulay *et al.***, 2003; **Pinedo *et al.***, 2011), no difference in SCC (**Rastani *et al.***, 2005; **Church *et al.***, 2008; **Watters *et al.***, 2008; **Bernier-Dodier *et al.***, 2011; **Santschi *et al.***, 2011), or an increase in SCC (**Kuhn *et al.***, 2006; **Pezeshki *et al.***, 2007; **Steeneveld *et al.***, 2013; **Van Hoeij *et al.***, 2016). A short DP length tended to reduce the number of IMI postpartum in some studies (**Pezeshki *et al.***, 2007; **Pinedo *et al.***, 2011), compared with a conventional DP length. Other studies indicated no effect of shortening (**Church *et al.***, 2008; **Watters *et al.***, 2008; **Shoshani *et al.***, 2014; **Van Hoeij *et al.***, 2016) or omitting (**Van Hoeij *et al.***, 2016) the DP on clinical mastitis in the subsequent lactation, compared with a conventional DP length. To our knowledge, all studies on the effect of DP length on udder health were carried out using cows that were treated with dry cow antibiotics at drying off. A greater SCC after a DP without use of dry cow antibiotics could be the result of non-cured IMI that were present before calving, or to new IMI that were acquired during the DP. It is unknown if the observed greater SCC in early lactation in cows with a 0-d DP, compared with cows with a 30-d DP, as observed in our previous study (**Van Hoeij *et al.***, 2016), is actually related with an increase in IMI, or with non-infectious processes such as cell proliferation (**Fitzgerald *et al.***, 2007) or difference in milk yield (**Green *et al.***, 2006a).

Metabolic status may negatively impact udder health (**Ingvartsen and Moyes**, 2013; **Mayasari *et al.***, 2016). Cows with a more negative energy balance (**NEB)** in the period up to 9 wks postpartum had lower plasma concentrations of natural antibodies (**Van Knegsel *et al.***, 2007a), which are regarded as an innate first line of defense against pathogens. Increased lipid accumulation in liver and plasma NEFA concentrations are associated with a greater risk for ketosis (**Drackley**, 1999). Cows with subclinical ketosis during the first 2 wks postpartum had a 9.5 times greater odds of developing clinical mastitis during that period than cows without subclinical ketosis (**Suthar *et al.***, 2013). Moreover, post-partum ketotic cows, with a high β-hydroxybutyrate (**BHB**) and high free fatty acid (**FFA**) concentration, and a low plasma glucose concentration, had more severe mastitis, indicated by greater bacterial growth in the quarter inoculated with *Escherichia coli*, than non-ketotic cows in wk 3 – 6 of lactation (**Kremer *et al.***, 1993). In contrast to previous studies reporting on the relation between ketosis and udder health, to our knowledge, direct relations between calculated EB or related plasma metabolites and udder health in a complete lactation are not available.

Feeding an increased dietary energy level positively affected the energy balance (**EB**) and metabolic status in dairy cows in early lactation (**Reist *et al.***, 2003; **Van Knegsel *et al.***, 2007b). Also shortening or omitting the DP improved metabolic status of dairy cows in early lactation, compared with a conventional DP of 8 wks (**Rastani *et al.***, 2005; **Van Knegsel *et al.***, 2014). So far, effects of DP length on milk yield and EB in the subsequent lactation were always evaluated with a lactation ration containing the same energy level for all cows. It can be hypothesized that, due to the lower milk yield, a lower dietary energy level might reduce the risk of fattening after peak milk yield and is beneficial for the net herd results. Recently, we reported that reducing the dietary energy level for cows with 0-d DP reduced the EB in early lactation, but did not affect milk yield, compared with cows that received a standard concentrate level (**Van Hoeij *et al.***, 2017b). It is unclear what the effects of DP length without use of dry cow antibiotics, and feeding different dietary energy levels during mid and late lactation, including their potential interaction, on udder health are.

The first aim of this study was to compare the effect of a 0-d or 30-d DP without use of dry cow antibiotics on udder health across the DP and the subsequent lactation (up to 44 wks). The second aim was to evaluate effects of dietary energy level in wk 4 – 44 of lactation on udder health of cows with different dry period lengths. The third aim was to evaluate associations between udder health and metabolic status of dairy cows.

6.3 Materials and methods

6.3.1 Animals and housing

The Institutional Animal Care and Use Committee of Wageningen University & Research approved the experimental protocol in compliance with Dutch law on Animal Experimentation (protocol number 2014125). The experimental design, DP lengths, and dietary contrasts were described previously (**Van Hoeij *et al.***, 2017a). For this experiment, cows were selected based on 1) being bred with a Holstein sire, 2) expected calving interval < 490 days, 3) daily milk yield > 16 kg at 90 days before the expected calving date, and 4) cows had no clinical mastitis and preferably a SCC < 250,000 cells/mL at the final 2 test-days before randomization. Cows were housed in a freestall with a slatted floor and cubicles. Cows were milked twice daily at ~0600 hours and ~1800 hours. The experimental period of the current study started in wk 5 prepartum and lasted until wk 44 postpartum.

6.3.2 Experimental design

In total, 130 cows entered the experiment, including 6 cows that entered twice. To obtain a balanced distribution of cows across treatments, groups were made of 3 cows with similar expected calving date, milk yield and SCC in the previous lactation, and postpartum parity (2, ≥ 3). Within each group of 3 cows, 2 cows were assigned randomly to a DP length of 0 days and 1 cows to a DP length of 30 days. Within the group of cows with a 0-d DP, cows were assigned randomly to either a low level of energy (**LOW**) in wk 4 – 44 of lactation, which is based on the requirement for their expected milk yield of cows with a 0-d DP, or a standard (**STD**) level of energy in wk 4 – 44 of lactation based on the requirement for the expected milk yield of cows with a 30-d DP (**Van Knegsel *et al.***, 2014). Cows with a 30-d DP were fed a STD level of energy, which is based on the requirement for their expected milk yield. This resulted in the following 3 treatments: cows with a 30-d DP and a STD dietary energy level [**30-d DP(STD)**], cows with a 0-d DP with the same STD dietary energy level as cows with a 30-d DP [**0-d DP(STD)**], and cows with a 0-d DP with a LOW energy level [**0-d DP(LOW)**]. From wk 8 postpartum onwards, cows were assigned randomly to either a glucogenic (**G**) or lipogenic (**L**) basal ration. Ration was not included in the manuscript because it was only introduced in mid lactation and other factors could have affect udder health as well, which makes distinction between factors impossible. An overview of feeding of cows with different DP lengths in different lactation stages is presented in Table 1. For evaluation of udder health across the DP, 7 of 130 cows were excluded from the dataset due to an unplanned DP for cows assigned to a 0-d DP (n = 4), due to severe claw problems (n = 1) or acute death peripartum (n = 2). The dataset for udder health across the DP consisted of data of 123 cows (58 with parity 2 and 65 with parity ≥ 3). For evaluation of udder health during the complete subsequent lactation, 20 of 130 cows were excluded from the dataset because of an unplanned DP for cows assigned to a 0-d DP (n = 4), due to being fed an incorrect concentrate level in wk 4 – 44 of lactation (n = 5), due to stealing from a different basal ration than assigned to (n = 3) or having less than 200 DIM of milk yield records (n = 8, including 1 cow with severe claw problems and 2 cows with acute death peripartum. The complete lactation dataset consisted of full lactation data of 110 cows (53 with parity 2 and 57 with parity ≥ 3).

The drying off protocol for cows with a 30-d DP consisted of a transition to the dry cow ration at day 7 before drying off and transition to milking once daily at day 4 before drying-off. At drying-off, no dry cow antibiotics were used. Clinical mastitis cases were diagnosed and treated by the milkers of the research herd. A case of clinical mastitis was defined as a treated case of visibly abnormal milk, changes in the udder due to inflammation, or both. All cows with clinical mastitis were treated with antibiotics according to the herd-specific treatment plan based on severity of disease.

6.3.3 Rations

Rations prepartum and in early lactation, and evaluation of feed intake and EB have been described in detail by **Van Hoeij *et al.*** (2017b). Briefly, prepartum, cows with a 0-d DP received a lactation ration that consisted of grass silage, corn silage, sugar beet pulp, soybean meal, wheat straw, urea, and vitamins and minerals (6.4 MJ net energy for lactation (**NE_L**)/kg DM). Lactating cows received 1 kg/d of standard concentrate in the milking parlor. Prepartum, cows with a 30-d DP received a ration that consisted of grass silage, corn silage, rapeseed meal, wheat straw, urea, and vitamins and minerals (5.4 MJ NE_L/kg DM).

Lactation rations were described previously in detail by **Van Hoeij *et al.*** (2017a). In short, all cows were fed the same lactation basal ration for first 7 wks in lactation, as well as 1 kg/d of standard concentrate in the milking parlor. All cows received experimental concentrate between 10 days before the expected calving date and wk 31 in lactation. After build-up of concentrate level, cows fed a STD energy level (30-d DP(STD) and 0-d DP(STD)) received experimental concentrate at a maximum of 8.5 kg/day, and cows fed a LOW energy level (0-d DP(LOW)) at maximum 6.7 kg/day. Cows had free access to water and the dry cow ration or the lactation basal ration throughout the experiment.

6.3.4 Measurements

Milk yield, milk composition, feed intake and energy balance. Milk yield was recorded daily from 5 wks prepartum until 44 wks postpartum. Milk samples for fat, protein, lactose, and SCC analysis [(**ISO 9622**, 2013), Qlip, Zutphen, The Netherlands] were collected four times per wk (Tuesday afternoon, Wednesday morning, Wednesday afternoon, and Thursday morning) and were analyzed as a pooled sample of 2 morning and 2 afternoon milkings per cow per wk. Fat- and protein- corrected milk (**FPCM**) yield was calculated as:

FPCM (kg/d) = [(0.337 + 0.116 × fat content (%) + 0.06 × protein content (%)] × milk yield (kg/d) (**CVB**, 2011).

Feed intake and energy balance was determined as described by **Van Hoeij *et al.*** (2017a). Briefly, daily individual basal ration intake was measured using roughage intake control troughs and was averaged per week), and concentrate supply was recorded by a computerized feeder. Body weight was recorded daily before each milking and was averaged per week. Energy balance was calculated per week as the difference between NEL intake and the NEL requirements for maintenance, milk yield, and pregnancy.

Bacterial culturing. Quarter milk samples for culturing were taken aseptically by trained milkers in wk 5 prepartum, and wk 1 and wk 5 postpartum (**NMC**, 1999). Immediately after collection, the milk samples were frozen at -20°C until processing at the laboratory. Processing occurred within 6 months after collection of the samples. From a total of 1476 samples (123 cows × 4 quarters × 3 time points), 131 samples were not taken due to a dry cow or quarter or were unsuitable for analysis due to abnormal composition.

For bacterial culturing, quarter samples were thawed at room temperature and 10 µl was streaked onto a 6% sheep blood agar plate. Plates were incubated at 37°C and bacterial growth was assessed both after 24 and 48 h of incubation. An IMI was defined as presence of 1 or more colonies of the same type. Identification of bacteria was done by inspection of the colony morphology and Matrix Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) using the MALDI Biotyper 3.0 (Bruker Daltonics, Bremen, Germany) (**Barreiro *et al.***, 2010). Samples were considered contaminated when 2 or more colonies of > 2 different types other than *Staphylococcus aureus*, *Streptococcus agalactiae*, or *α-hemolytic streptococci* were present, without distinct excess of 1 type of colony. The proportion of contaminated samples was 13.5% (182 out of 1345 available samples). A quarter in which *E. coli*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, or *Trueperella pyogenes* was cultured, was defined as infected with a major udder pathogen. All other culture positive quarters, unless considered contaminated, were defined as infected with a minor udder pathogen (*Corynebacterium* spp., Coagulase-negative staphylococci). Bacterial results were classified in three groups: no growth, minor udder pathogens, and major udder pathogens.

Blood collection and analyses. Blood collection and analyses were described previously by **Van Hoeij *et al.*** (2017a). In short, blood was collected weekly from calving until wk 10 postpartum, and every 2 wks between wk 12 and 44 postpartum. Blood (10 mL) was collected from the coccygeal vein or artery into evacuated EDTA tubes (Vacuette, Greiner BioOne, Kremsmunster, Austria). Blood was analyzed for the plasma FFA, BHB, glucose concentration in wk 1 – 7 , and for the plasma insulin, IGF-1, and growth hormone (GH) concentration in wk 1 – 44.

6.3.5 Statistical analyses

Statistical analyses were performed using SAS version 9.3 (**SAS Institute Inc.**, 2011). The natural logarithm of SCC (cells x 103 /ml) (LnSCC) was used for analyses to approximate normality. Significance of treatment effects was declared at $P < 0.05$ and trends at $0.05 \leq P < 0.10$.

Occurrence of intramammary infections across different dry period lengths. Definition of intramammary infections across different DP lengths was based on either evaluation of 1) SCC or 2) bacterical culture. For evaluation 1, prepartum and postpartum SCC in composite samples were compared. For both cows with a 0-d or 30-d DP, the last SCC before the drying off wk for cows with a 30-d DP (median -51 d; range -66 to -37 days prepartum) was compared with the first SCC postpartum obtained after the first 10 DIM (median 13 DIM; range 10 to 16 DIM). Somatic cell count was considered elevated when SCC was ≥ 200,000 cells/mL (**Schukken *et al.***, 2003). Somatic cell counts before and after calving were compared to categorize cows in classes as having a chronically high SCC (SCC ≥ 200,000 cells/mL before and after calving), cured high SCC (SCC ≥ 200,000 cells/mL before and SCC < 200,000 cells/mL after calving), newly high SCC (SCC < 200,000 cells/mL before and SCC ≥ 200,000 cells/mL after calving) or no high SCC (SCC < 200,000 cells/mL before and after calving). For evaluation 2, bacterial culture in wk 5 prepartum was compared with bacterial culture in wk 1 postpartum to assess if quarters got infected with a mastitis pathogen across the DP. A quarter was regarded infected when bacterial culture was positive, regardless of the type of mastitis pathogen. The difference in incidence of postpartum high SCC or incidence of a positive bacterial culture (binary, 0 or 1) between DP lengths was analyzed within cows with low or high SCC, or without growth or positive bacterial culture (binary, 0 or 1) before the DP, using a logistic regression model (Model 1) [PROC LOGISTIC].

$$\text{Logit } P(y_{ij=1}) = \alpha + DP_i + \varepsilon_j, \qquad [1]$$

Model 1 estimates the probability (P) that the ijth observation of postpartum high SCC or positive bacterial culture (y) is 1. Within observations of high SCC or positive bacterial culture prepartum, chronic high SCC or IMI = 1, and cured high SCC or IMI = 0. Within observations of low SCC or without bacterial growth prepartum, new high SCC or IMI = 1, and no high SCC or IMI = 0. Logit is the link function of the mean of the response variable, α is the estimate of the baseline odds of occurrence of high SCC or IMI, DP_i indicates the DP length (i = 0 or 30-d), and the random residual term from a binary distribution is represented by ε_j.

To evaluate changes in presence of a mastitis pathogen across the DP, presence of a bacterial mastitis pathogen (without growth, culture of a minor mastitis pathogen, or culture of a major mastitis pathogen) in prepartum (wk 5) and postpartum (wk 1), quarter milk samples were compared using Model 1 with a multinomial distribution and cumlogit link. The incidence of postpartum bacterial culture was assessed within observations of bacterial culture prepartum (without growth, minor or major mastitis pathogen).

The incidences of quarters infected with different types of bacterial mastitis pathogens and the SCC in individual quarter milk samples from cows with a 0 or 30 days DP in wk 5 prepartum, and wk 1 and wk 5 postpartum were evaluated using a mixed linear model [PROC MIXED; Model 2)]. Fixed effects included in the model were: transition treatment [in wk 5 prepartum and wk 1 postpartum: 0-d or 30-d DP; in wk 5 postpartum: 0-d DP(LOW), 0-d DP(STD), or 30-d DP(STD)], postpartum parity (2, or ≥ 3), and wk after calving (wk 5 prepartum, or wk 1 or 5 postpartum), and their two-way interactions.

Transition treatment on udder health. Weekly SCC and SCC corrected for milk yield (kg milk/d) were analyzed using repeated measures analysis in a mixed linear model (PROC MIXED) (model 3) with cow (random effect) as the repeated subject. Fixed effects of transition treatment [0-d DP(LOW), 0-d DP(STD), 30-d DP(STD)], postpartum parity (2, ≥ 3), wk after calving (wk 1 through 44), and their two-way interactions were included in the model. To obtain SCC corrected for milk yield, average weekly milk yield (kg milk/d) was included in the model for SCC corrected for milk yield (**Steeneveld *et al.***, 2013). To account for the effect of season on variation in SCC (**Bodoh *et al.***, 1976; **Green *et al.***, 2006b; **Olde Riekerink *et al.***, 2007), calving season (meteorological winter, spring, summer or autumn) was included as a random effect. A first-order autoregressive covariance matrix was the best fit according to Akaike's corrected information criterion and was used to account for within-cow variation.

For the binary variables 'at least 1 elevation of SCC between 3 and 44 wks postpartum' and 'at least 1 case of clinical mastitis between 1 and 44 wks postpartum', for each cow a binary distribution and the default logit link function was used to model the mixed linear regression analysis (PROC GLIMMIX) (Model 4). Fixed effects of transition treatment [0-d DP(LOW), 0-d DP(STD), or 30-d DP(STD)], postpartum parity (2, or ≥ 3), and their two-way interactions were included in the model. An elevation of SCC was defined as SCC ≥ 200,000 cells/mL after two previous wks with SCC < 200,000 cells/mL (**Schukken *et al.***, 2003). Postpartum elevations of SCC and cases of clinical mastitis per cow were analyzed between transition treatment groups using Model 4, with a Poisson distribution for the dependent variables and the default log link function in a mixed linear regression analysis (PROC GLIMMIX).

To evaluate survival time to first elevation of SCC or first case of clinical mastitis after different DP lengths, a survival analysis (PROC LIFETEST) was used to obtain Kaplan-Meier curves (Model 5). For cows that did not have an elevation of SCC or a case of clinical mastitis within 44 wks postpartum, the survival time was censored at 44 wks postpartum. To evaluate statistical differences of Kaplan-Meier curves among transition treatments, a Cox proportional hazards model (PROC PHREG) was used.

The association of postpartum udder health with milk production and metabolic status. For the association of udder health with metabolic status postpartum, data on FPCM yield, energy intake, EB, and plasma FFA, BHB, glucose, and IGF-1 concentration in wk 1 – 7 (**Van Hoeij *et al.***, 2017b), and on FPCM yield, energy intake, EB, and plasma insulin, IGF-1, and GH concentration in wk 1 – 44 (**Van Hoeij *et al.***, 2017a) from previous studies were used. In addition, GH concentrations in wk 1 – 7 were not reported in **Van Hoeij *et al.*** (2017b).

To evaluate the relationship of postpartum weekly SCC with metabolic status in wk 1 – 7 and wk 1 – 44, a mixed linear model (PROC MIXED) (Model 6) was used including week as repeated measurement effect with cow as the repeated subject. A first-order autoregressive covariance matrix was the best fit according to Akaike's corrected information criterion and was used to account for within-cow variation. The independent variables were transition treatment [0-d DP(LOW), 0-d DP(STD), or 30-d DP(STD)], parity (2 or ≥ 3), and one of the following metabolic or milk production variables: weekly FPCM yield, EB, energy intake, weekly plasma insulin, IGF-1, GH , FFA, BHB, glucose concentration to assess the coefficient estimate (β) of the variable. Plasma insulin, IGF-1 and GH were available weekly from wk 1 – 7 and biweekly from wk 8 – 44 in lactation. Plasma FFA, BHB, and glucose were available weekly from wk 1 – 7 in lactation. The natural logarithm was used for plasma FFA, BHB, and GH concentration to approximate normality.

To evaluate changes in metabolic status of cows in the 3 wks preceding an elevation of SCC or a case of clinical mastitis, a mixed linear model (Model 7) was used including week as repeated measurement effect with cow as the repeated subject. Cows with an elevation of SCC or case of clinical mastitis in wk 1 – 3 of lactation or without an elevation of SCC or case of clinical mastitis in lactation were not included in the dataset for this analysis. In wk in wk 4 – 7, 10 cows had at least 1 elevation of SCC and 3 cows had at least 1 case of clinical mastitis. In wk 4 – 44, 59 cows had at least 1 elevation of SCC and 21 cows had at least 1 case of clinical mastitis. A first-order autoregressive covariance matrix was the best fit according to Akaike's corrected information criterion and was used to account for within-cow variation. The dependent variable in these statistical analyses was one the following metabolic or milk production variables: weekly FPCM yield, EB, energy intake, plasma insulin, IGF-1, GH, FFA, BHB, or glucose concentration in the last 3 wks before the first elevation of SCC or the first case of clinical mastitis within wk 4 – 44 of lactation. Preliminary analyses showed that dependent variables were not different among wks prior to the last 3 wks before the first elevation of SCC or the first case of clinical mastitis. The independent postpartum variables included in the model were transition treatment [0-d DP(LOW), 0-d DP(STD), 30-d DP(STD)], parity (2 or ≥ 3), and week before elevation of SCC or case of clinical mastitis (week −3, −2, or −1).

6.4 Results

6.4.1 Prepartum and postpartum milk yield

Prepartum FPCM yield was 13.9 kg/d for cows with a 0-d DP and 0 kg/d for cows with a 30-d DP in wk 4 – 1 prepartum (**Van Hoeij *et al.***, 2017b). Postpartum FPCM yield was 27.1 kg/d for cows with a 0-d DP(LOW), 27.4 kg/d for cows with a 0-d DP(STD), and 32.3 kg/d for cows with a 30-d DP(STD) in wk 1 – 44 (**Van Hoeij *et al.***, 2017a).

6.4.2 Occurrence of intramammary infections during different dry period lengths

Based on weekly composite milk samples, the prevalence of SCC ≥ 200,000 cells/mL at the last test-day before 37 days prior to the expected calving date (median 51 d prepartum), and at the first test day after 10 DIM, did not differ between DP lengths ($P \geq 0.05$) (**Table 6.1a**). Similarly, the proportion of cows with a chronically, cured, new, or no high SCC in weekly composite milk samples did not differ between DP lengths ($P \geq 0.05$). Based on bacterial culture of quarter milk samples, the proportion of quarters without growth, or with minor or major mastitis pathogens in wk 1 postpartum, within prepartum bacterial culture classes, did not differ between cows with 0-d or 30-d DPL ($P \geq 0.05$) (**Table 6.1b**).

Table 6.1: Mean incidence (%) and number (within brackets) of a) cows with a low somatic cell count (SCC) (< 200,000 cells/mL) or high SCC (≥ 200,000 cells/mL) at the last test-day before the drying off day for cows with a 30-d DP (median 51 days prepartum) and at first test-day (median 13 days postpartum) and b) the mean incidence (%) of quarters without growth or with positive bacterial culture before drying off (wk 5 prepartum) and in early lactation (wk 1 postpartum) after a 0 or 30 days dry period (DP)

a)	Dry period length (d)		
	0-d DP	30-d DP	P - value
Total number of cows	81	42	
	% (n)	% (n)	
SCC < 200,000 prepartum	84 (68)	74 (31)	
SCC ≥ 200,000 prepartum	16 (13)	26 (11)	0.18
SCC < 200,000 postpartum	72 (58)	69 (29)	
SCC ≥ 200,000 postpartum	28 (23)	31 (13)	0.77
High SCC prepartum			
Chronic high SCC	38 (5)	36 (4)	
Cured high SCC	62 (8)	64 (7)	0.92
Low SCC prepartum			
New high SCC	26 (18)	29 (9)	
No high SCC	74 (50)	71 (22)	0.77

b)			Wk 1 postpartum			
Wk 5 prepartum	Dry period length	Prepartum[1] (%)	Without growth (%)	Minor (%)	Major (%)	*P* – value Dry period length
Without growth						
	0-d	62	61	26	13	0.77
	30-d	60	65	27	8	
Minor mastitis pathogen						
	0-d	31	51	42	7	0.76
	30-d	31	57	43	0	
Major mastitis pathogen						
	0-d	7	42	35	23	0.87
	30-d	9	23	23	54	

[1]The proportion of quarters without growth, or a minor or major mastitis pathogen in wk -5 relative to calving was not different between dry period lengths ($P = 0.43$).

6.4.3 Bacterial infections with different types of mastitis pathogens across different dry period lengths

Based on bacterial culture of quarter milk samples, the proportion of quarters without growth, or with minor or major mastitis pathogens, did not differ between cows with 0-d or 30-d DPL in wk 5 prepartum or wk 1 postpartum, or among transition treatments in wk 5 postpartum ($P \geq 0.05$) (**Table 6.2**). An interaction of parity with week relative to calving was present for the proportion of quarters without growth, or infected with a minor or major mastitis pathogen ($P < 0.01$) (**Figure 6.1**). In general, differences between parities were greatest in wk 5 prepartum, and smallest in wk 5 postpartum.

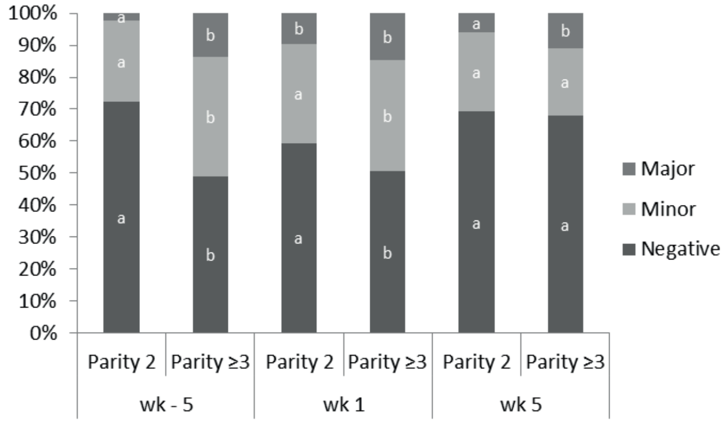

Figure 6.1: Interaction of parity with week relative to calving (wk 5 prepartum, or wk 1 or 5 postpartum) for the proportion of quarters without growth or infected with a minor or major mastitis pathogen. Values within pathogen with different superscript letters differ ($P < 0.05$).

Table 6.2: Mean incidence (%) (and number of observations) of different classes of bacterial culture (without growth, minor pathogen, major pathogen) in individual quarter milk samples from cows with a 0 or 30 days dry period in sampling week 5 wks prepartum, 1 wk postpartum or 5 wks postpartum

		Sampling week												
		5 wks prepartum		1 wk postpartum		5 wks postpartum			P-value[1]					
		0-d DP	30-d DP	0-d DP	30-d D	0-d DP (LOW)	0-d DP (STD)	30-d DP (STD)	T	P	W	T × P	T × W	P × W
Without growth														
Quarters without bacterial growth	%	62 (170)	60 (82)	56 (147)	56 (71)	71 (95)	68 (72)	67 (80)	0.12	<0.01	<0.01	0.09	0.75	<0.01
Positive bacterial culture														
Quarters with a minor mastitis pathogen	%	31 (83)	31 (43)	32 (85)	35 (45)	21 (28)	21 (22)	27 (33)	0.38	<0.01	0.03	0.06	0.55	<0.01
Quarters with a major mastitis pathogen	%	7 (19)	9 (13)	12 (33)	9 (12)	8 (11)	11 (12)	6 (7)	0.12	<0.01	0.07	0.20	0.60	0.09
Total	n	272	138	265	128	134	106	120						

[1]T = Treatment (0-d DP(LOW), 0-d DP(STD), or 30-d DP(STD)); P = Parity; W = Sampling week. Transition treatment: Cows with a 0-d DP were fed either a low [0-d DP(LOW)] energy level, based on the requirement for their expected milk yield, or a standard [0-d DP(STD)] energy level based on the expected milk yield of cows with a 30-d DP [30-d DP(STD)].

6

6.4.4 Udder health for different dry period lengths and dietary energy levels

Cows with a 0-d DP had a greater SCC postpartum than cows with a 30-d DP ($P < 0.01$), but not when SCC was corrected for milk yield ($P = 0.13$) (**Table 6.3**).

Table 6.3: Postpartum (1-44 wks in lactation) somatic cell count variables for cows with a 0 or 30 days dry period (LSM ± SEM). Cows with a 0-d DP were provided either a low [0-d DP(LOW)] or standard [0-d DP(STD)] energy level that cows with a 30-d DP received [30-d DP(STD)]

	Treatment[1]				P-values[2]					
	0-d DP (LOW)	0-d DP (STD)	30-d DP (STD)	SEM	T	P	W	T × P	T × W	P × W
Cows (n)	36	34	40							
Average SCC[3]	4.88[a]	5.0[a]	4.58[b]	0.23	<0.01	<0.01	<0.01	0.17	0.18	0.97
Average SCC corrected for milk yield[3,2]	4.60	4.78	4.72	0.08	0.13	<0.01	<0.01	0.74	0.22	0.98
Elevations of SCC (% cows)[5,6]	53	56	53		0.85	0.02	N.M.[7]	0.20	N.M.	N.M.
Elevations of SCC (no. episodes/affected cow)[6]	2.5	1.9	2.1		0.29	0.04	N.M.	0.06	N.M.	N.M.
Clinical mastitis (% cows treated)[6]	31	35	18		0.16	0.06	N.M.	N.M.	N.M.	N.M.
Clinical mastitis (no. episodes/cow treated)[6,7]	1.1	1.3	1.1		0.72	0.37	N.M.	N.M.	N.M.	N.M.

[a-b]Values within transition treatment within a row with different superscript letters differ ($P < 0.05$). [1]Transition treatment: Cows with a 0-d DP fed either a low [0-d DP(LOW)] energy level, based on the requirement for their expected milk yield, or a standard [0-d DP(STD)] energy level based on the expected milk yield of cows with a 30-d DP [30-d DP(STD)]. [2]T = transition treatment, P = Parity; W= Week after calving. [3]SCC is shown as the natural logarithm of SCC (LnSCC). For SCC, wk was included in the model. [4]For SCC corrected for milk yield, weekly kg of milk was included in the model. The P-value for milk yield (kg/d) in the model was < 0.01. [5]An elevation of SCC in milk was defined as SCC in milk ≥ 200,000 cells/mL after two previous wks with SCC < 200,000 cells/mL. [6]N.M., not in model. [7]A case of clinical mastitis was defined as a treated case of mastitis based on of visibly abnormal milk, changes in the udder due to inflammation, or both.

The proportion of cows that had an elevation of SCC within 44 wks of lactation was 53%, 56%, and 53% for cows with a 0-d DP(LOW), 0-d DP(STD), and 30-d DP(STD), respectively. Curves for survival time for no elevation of SCC did not differ among transition treatments (P = 0.89) (**Figure 6.2a**). The proportion of cows that had a case of clinical mastitis within 44 wks of lactation was 31%, 35%, and 18% for cows with a 0-d DP(LOW), 0-d DP(STD), and 30-d DP(STD), respectively. Curves for survival time for no case of clinical mastitis were not different among transition treatments (P = 0.26) (**Figure 6.2b**). However, cows with a 0-d DP(STD) tended to be more likely to obtain a case of clinical mastitis at any time in lactation (Hazard Ratio 2.17, confidence interval 0.85 – 5.05) compared with cows with a 30-d DP(STD) (P < 0.10), but did not have a greater hazard for a case of clinical mastitis than cows with a 0-d DP(LOW) (Hazard Ratio 1.17, confidence interval 0.36 – 1.91; P ≥ 0.10).

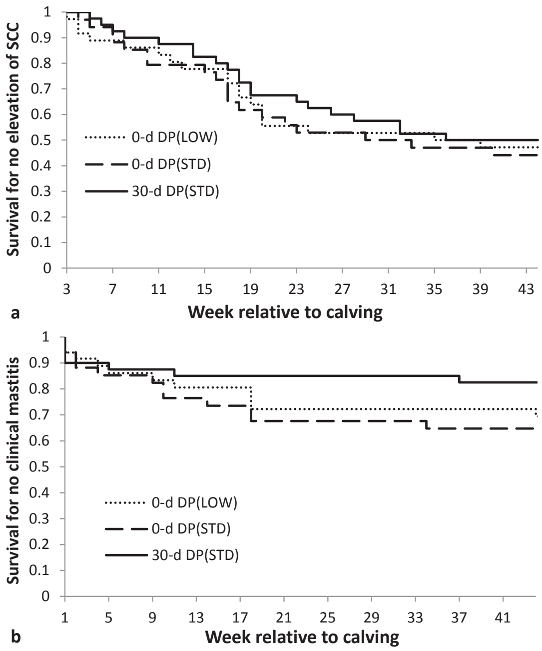

Figure 6.2 a-b: Kaplan-Meier survival curves. The survival function is (a) the time of survival for no elevation of SCC ≥ 200,000 cells/mL after two previous wks SCC < 200,000 cells/mL, and (b) the time of survival for no case of clinical mastitis in wk 1 – 44 in lactation following a 0-d dry period (DP) and a low energy level based on the requirement for their expected milk yield [0-d DP(LOW)], a 0-d DP and standard energy level [0-d DP(STD)] or a 30-d DP and a standard energy level based on the requirement for their expected milk yield [30-d DP(STD)].

6

6.4.5 The association of postpartum udder health with milk production characteristics and metabolic status

The plasma GH concentrations in wk 1 – 7 were 4.23 (3.82 – 4.68) μg/L, 4.47 (4.03 – 4.95) μg/L, and 5.35 (4.86 – 5.89) μg/L for cows with a 0-d DP(LOW), 0-d DP(STD), or 30-d DP(STD), respectively, and were lower for cows with a 0-d DP than for cows with a 30-d DP ($P < 0.01$). In wk 1 – 7 postpartum, ≥ was negatively related with FPCM yield, and positively related with EB and plasma insulin concentration ($P < 0.01$), and tended to be negatively related with energy intake ($P < 0.10$) (**Table 6.4**). In wk 1 – 44 of lactation, SCC was negatively related with FPCM yield and energy intake, and positively related with plasma insulin concentration ($P < 0.01$), and tended to be negatively related with EB ($P < 0.10$).

Table 6.4: Somatic cell count (SCC) related to FPCM yield, energy balance, energy intake, the plasma insulin, IGF-1, GH, FFA, BHB, and glucose concentration in wk 1 – 44 of lactation (a). Values represent regression coefficients (β, with SEM)

	SCC[1] β
Wk 1 – 7 of lactation	
FPCM[2] (kg/d)	-0.059 (0.008)***
Energy balance[3] (kJ/kg$^{0.75}$·d)	1.56×10^{-3} (0.32×10^{-3}) ***
Energy intake (kJ/kg$^{0.75}$·d)	-0.90×10^{-3} (0.44×10^{-3}) *
Insulin (μU/mL)	0.02 (0.003)***
IGF-1 (ng/mL)	0.76×10^{-3} (0.87×10^{-3})
GH[4] (μg/L)	-0.10 (0.06)
FFA[4,5] (mmol/L)	-0.03 (0.05)
BHB[4,5] (mmol/L)	-0.09 (0.08)
Glucose[5] (mmol/L)	0.03 (0.06)
Wk 1 – 44 of lactation	
FPCM[2] (kg/d)	-0.046 (0.004)***
Energy balance[3] (kJ/kg$^{0.75}$·d)	-0.24×10^{-3} (0.14×10^{-3}) †
Energy intake (kJ/kg$^{0.75}$·d)	-1.08×10^{-3} (0.15×10^{-3}) ***
Insulin (μU/mL)	0.005 (0.001)***
IGF-1 (ng/mL)	0.35×10^{-3} (0.36×10^{-3})
GH[4] (μg/L)	-0.02 (0.03)

* P <0.10, ** P < 0.05, *** P < 0.01.

Cows that had at least 1 elevation of SCC in wk 4 – 44 postpartum had a greater EB in wk 2 than in wk 3 prior to SCC elevation, had a greater energy intake in wk 2 than in wk 3 or wk 1 prior to SCC elevation ($P < 0.01$), and tended to have a greater plasma FFA concentration in wk 2 than in wk 1 prior to SCC elevation ($P < 0.10$) (**Table 6.5a**). In cows that had at least 1 case of clinical mastitis in wk 4 – 44 postpartum, EB was greater in wk 1 than in wk 2, and greater in wk 2 than in wk 3 prior to week of first case of clinical mastitis ($P < 0.01$) (**Table 6.5b**).

Table 6.5: FPCM yield, energy balance, energy intake, and the plasma insulin, IGF-1, GH, FFA, BHB, and glucose in the last 3 wks before the first elevation of SCC (a) or first case of clinical mastitis (b) within wk 4 – 44 of lactation. Values represent LSmeans ± SEM

a	Week before first elevation of SCC[5]					P-value
	Wk -3	Wk -2	Wk -1	Week of elevation of SCC[6]	SEM	Week pre diagnosis
FPCM[1,7] (kg/d)	30.9^{ab}	30.8^{a}	30.1^{b}	29.8	1.1	0.11
Energy balance[2,7] ($kJ/kg^{0.75}$·d)	44^{a}	67^{b}	57^{ab}	68	22	<0.01
Energy intake[7] ($kJ/kg^{0.75}$·d)	1089^{a}	1109^{b}	1081^{a}	1085	17	<0.01
Insulin[7] (µU/mL)	21.3	20.9	21.5	23.7	2.0	0.91
IGF-1[7] (ng/mL)	141	150	143	156**	9	0.18
GH[3,7] (µg/L)	3.55	3.45	3.11	3.16	2.83 – 4.01	0.40
FFA[3,4] (mmol/L)	0.14^{ab}	0.16^{a}	0.13^{b}	0.10	0.09 – 0.22	0.09
BHB[3,4] (mmol/L)	0.62	0.68	0.69	0.66	0.52 – 0.86	0.74
Glucose[4] (mmol/L)	3.80	3.81	3.76	3.75	0.12	0.94

b	Week before first case of clinical mastitis					P-value
	Wk -3	Wk -2	Wk -1	Week of clinical mastitis[6]	SEM	Week pre diagnosis
FPCM[1,8] (kg/d)	32.2	32.0	31.5	29.1***	1.9	0.42
Energy balance[2,8] ($kJ/kg^{0.75}$·d)	7^{a}	26^{a}	60^{b}	57	45	<0.01
Energy intake[8] ($kJ/kg^{0.75}$·d)	1071	1094	1108	1048***	33	0.20
Insulin[8] (µU/mL)	17.0	15.8	19.3	23.0	2.1	0.14
IGF-1[8] (ng/mL)	125	138	138	129	16	0.40
GH[3,8] (µg/L)	5.00^{a}	3.91^{ab}	3.21^{b}	4.64†	3.09 – 5.68	0.11
FFA[3,4] (mmol/L)	0.12	0.11	0.08	0.07	0.05 – 0.23	0.22
BHB[3,4] (mmol/L)	0.52	0.60	0.62	0.63	0.34 – 0.98	0.64
Glucose[4] (mmol/L)	3.89	3.88	3.95	4.15	0.15	0.15

a-cValues among wks prediagnosis differ ($P < 0.05$). * P <0.10, ** P < 0.05, *** P < 0.01; Value in wk of elevation of SCC or clinical mastitis is different from wk prediagnosis (wk -1). [1]Somatic cell count (SCC) is shown as the natural logarithm of SCC (LnSCC). [2]Fat- and protein- corrected milk (FPCM). [3]Energy balance calculated according to Van Es (1975). [4]Analyzed as the natural logarithm of the FFA, BHB, or GH concentration and presented with confidence interval between brackets. [5]Plasma FFA, BHB, and glucose concentration was not measured after wk 7 in lactation. Between wk 4 – 7 of lactation, 10 cows had a first elevation of SCC and 4 cows had a first case of clinical mastitis. [6]An elevation of SCC in milk was defined as SCC in milk ≥ 200,000 cells/mL after two previous wks with SCC < 200,000 cells/mL. [7]Value for week of elevation of SCC or of clinical mastitis was not included for statistical analyses. [8]Mean wks in lactation was 18 for first elevation of SCC. Between wk 4 – 44, 59 elevations of SCC occurred. [9]Mean wks in lactation was 14 for first case of clinical mastitis. Between wk 4 – 44, 21 cases of clinical mastitis.

6.5 Discussion

Dry period length

In the current study no dry cow antibiotics were used in cows with a 30-d DP, which is in contrast to previous studies that evaluated the effect of DP length on udder health (**Rastani *et al.***, 2005; **Watters *et al.***, 2008; **Santschi *et al.***, 2011). We hypothesized that cows with a 30-d DP and dried off without antibiotics might have a compromised udder health in the subsequent lactation compared with cows with a 0-d DP. In our study, postpartum SCC was lower in cows with a 30-d DP than in cows with a 0-d DP. The greater postpartum SCC in cows with a 0-d DP, compared with cows with a 30-d DP, is in line with previous research (**Kuhn *et al.***, 2006; **Pezeshki *et al.***, 2007; **Van Hoeij *et al.***, 2016), with the exception that in the current study omitting of the DP was compared with shortening the DP without the use of dry cow antibiotics. In an earlier study, omitting dry cow antibiotics increased SCC at calving in untreated quarters compared with quarters that were treated with dry cow antibiotics (**Scherpenzeel *et al.***, 2014). Earlier, we hypothesized that the greater SCC postpartum after a short or no DP, compared with a conventional DP was related with 1) lower milk production postpartum, 2) differences in proliferation rate of udder cells, 3) increase in IMI across the DP or precalving period, and 4) increase in IMI during the course of lactation (**Van Hoeij *et al.***, 2016).

Firstly, the greater SCC of cows with a 0-d DP could be related to the reduction in milk yield for cows with a 0-d DP, compared with cows with a 30-d DP. Postpartum FPCM yield was lower in cows with a 0-d DP than in cows with a 30-d DP, which is in line with previous studies (**Rastani *et al.***, 2005; **Klusmeyer *et al.***, 2009; **Van Knegsel *et al.***, 2014). Lower milk yield in cows with a 0-d DP could be related with the lower milk producing capacity of secretory udder cells in these cows (**Van Hoeij *et al.***, 2017a) and thus a lower dilution of SCC and a greater SCC in milk, compared with cows with a 30-d DP. In earlier studies, milk yield was negatively related with SCC, irrespective of the presence of an IMI (**Schepers *et al.***, 1997; **Green *et al.***, 2006a; **Boland *et al.***, 2013). Moreover, difference in SCC among dry period lengths disappeared when SCC was corrected for milk yield (**Steeneveld *et al.***, 2013). Also in the current study, SCC corrected for milk yield was not different between dry period lengths, which implies that the difference in milk yield between cows with a 0-d or 30-d DP contributed to the difference in SCC between DP lengths in the current study, and the greater SCC is therefore possibly related with the lower milk yield in cows with a 0-d DP in the current study.

Secondly, the greater SCC of cows with a 0-d DP could be due to differences in regeneration rate of udder secretory cells between cows with a 0-d DP or a 30-d DP. Proliferation rate and SCC in the periparturient period and in the subsequent early lactation did not differ between udder halves with a 60-d DP and udder halves with a 0-d DP, whereas the proportion of apoptotic and proliferating cells at 7 days postpartum was lower in quarters with a 0-d DP, compared with those with a 60-d DP (**Fitzgerald *et al.***, 2007). A lower proportion of apoptotic cells in the udder in cows without a DP may result in a lower SCC, and is, therefore, unlikely to be related with the greater SCC in cows with a 0-d DP, compared with cows with a 30-d DP in this study.

Thirdly, the greater SCC of cows with a 0-d DP, compared with cows with a 30-d DP, is unlikely to be related to bacterial infections across the DP. Withholding dry cow antibiotics increased SCC at calving and increased the incidence of clinical mastitis till 100 DIM (by 1.7 times) due to both more infections with major and minor mastitis pathogens in untreated quarters compared with quarters that were treated with dry cow antibiotics (**Scherpenzeel *et al.***, 2014). In the current study, the incidence of IMI, either based on elevated SCC in composite milk samples or on bacterial culture in quarter milk samples, was not different between DP lengths. Additionally, the proportions of quarters affected with major or a minor mastitis pathogens in wk 1 and wk 5 postpartum were not different among transition treatments. The lack of difference in proportion of quarters infected with a major or minor mastitis pathogen across the DP in the current study, however, may also be due to the relatively small number of cows in this study.

Lastly, the greater SCC of cows with a 0-d DP, compared with cows with a 30-d DP, could be hypothesized to be related with a difference in number of elevations of SCC or cases of clinical mastitis later in lactation after different DP lengths. Cows with a 0-d DP have a lower renewal rate of secretory udder cells compared with a 30-d DP (**Capuco *et al.***, 1997; **Annen *et al.***, 2007). Due to the greater proportion of senescent udder cells postpartum for cows with a 0-d DP, the integrity and defense mechanisms of udder tissue in the subsequent lactation may be decreased, compared with cows with a 30-d DP (**Collier *et al.***, 2012). In the current study, the proportion of cows with at least one elevation of SCC during wk 1 – 44 of lactation and the number of elevations or cases of clinical mastitis per affected cow were not different between DP lengths. However, the incidence of clinical mastitis during wk 1 – 44 was numerically greater in cows with a 0-d DP (33%), than in cows with a 30-d DP (18%). Additionally, from survival and Cox proportional hazards analysis, a tendency for a greater hazard for clinical mastitis was present for cows with a 0-d DP(STD), than for cows with a 30-d DP(STD), but not for cows with a 0-d DP(LOW). This implies that, although effects are very small, a 30-d DP without use of dry cow antibiotics seems more beneficial for udder health in the subsequent lactation, compared with a 0-d DP. Previous studies showed no effect of DP length on the incidence of clinical mastitis between cows with a 0-d or a 30-d DP with dry cow antibiotics (**Rastani *et al.***, 2005; **Schlamberger *et al.***, 2010; **Van Hoeij *et al.***, 2016). In previous studies as well as the current study, the number of cows per treatment group may have been insufficient to achieve sufficient statistical power to detect true differences in mastitis incidence due to DP length.

Summarizing, in the current study, postpartum SCC was greater for cows with a 0-d DP, than cows with a 30-d DP, but SCC corrected for milk yield was not different among dry period length groups. In this study, differences in postpartum SCC are not only related to difference in milk yield, but could also be a result from differences in incidence of clinical mastitis later in lactation. The difference in postpartum SCC seemed not to be related with a difference in IMI across the DP.

Dietary energy level

Feeding a STD dietary energy level to cows with a 0-d DP numerically increased cow composite SCC. Feeding a greater dietary energy level increased plasma IGF-1 which stimulates cell proliferation in the udder (**Flint and Knight**, 1997; **Reist *et al.***, 2003). It could be hypothesized that, in the current study, feeding a greater dietary energy level to cows with a 0-d DP increased udder cell proliferation. Furthermore, cows with a 0-d DP(STD) had a 0.3 kg/d numerically greater milk production (27.4 vs. 27.1 ± 0.9 kg/d), which allowed more dilution of the numerically greater cow composite SCC and resulted in a not different SCC corrected for milk yield.

Cows with a 0-d DP(STD), but not cows with a 0-d DP(LOW), had a greater hazard for clinical mastitis in the subsequent lactation compared with cows with a 30-d DP(STD). Although not significant, these results seem to indicate that increasing, instead of reducing, dietary energy level for cows with a 0-d DP increases the hazard for clinical mastitis, and are contrary to our hypotheses that better metabolic status improves udder health. In previous epidemiological study, feeding concentrate with the basal ration decreased the incidence rate of clinical mastitis, which was hypothesized to be related with better metabolic status (**Barnouin *et al.***, 2005). In contrast, in a case-control study, feeding concentrate increased the incidence rate of clinical mastitis, but not when more management factors were included in a multivariable model (**Waage *et al.***, 1998). Effects of dietary energy level on udder health need to be further elucidated.

Udder health and metabolic status

Previous studies suggested that a NEB is negatively related with udder health (**Leslie *et al.***, 2000; **Ingvartsen and Moyes**, 2013). In the current study, postpartum SCC was negatively associated with FPCM yield and energy intake, and positively associated with EB and the plasma insulin concentration in dairy cows during the complete lactation period. In previous studies, cows with a greater EB had a greater plasma natural antibody concentration (**Van Knegsel *et al.***, 2007a), a lower plasma FFA concentration and a greater neutrophil activity (**Suriyasathaporn *et al.***, 2000) that lowers the risk for an IMI and elevation of SCC (**Ingvartsen and Moyes**, 2013). In the current study, the relation between FFA and SCC was not present between wk 1 – 7 of lactation. The relation was, however, present in wk 1 – 4 of lactation, but not from wk 5 postpartum onwards (data not presented), which could be related with the fact that the EB in cows with a 0-d DP was not negative anymore from wk 5 onwards (**Van Hoeij *et al.***, 2017b). The relation between FFA and SCC are likely stronger in cows with a more negative EB. An elevation of SCC in wk 4 – 44 was related with a greater EB and energy intake, tended to be related with a greater plasma FFA concentration in wk 2 before an elevation, and was related with a numerical decrease of FPCM in the weeks before an elevation. It could be hypothesized that, in this study, calculated EB was greater due to greater energy intake in wk 2 before an elevation of SCC and a numerical decrease in FPCM yield, but that true EB was lower due to increased immune response related with subclinical disease

6

before an elevation of SCC, which is reflected in an increased plasma FFA concentration. Plasma IGF-1 concentration tended to be greater in the week of an elevation of SCC than in the last week before an elevation of SCC. A greater plasma IGF-1 concentration implies that during, but not before, an elevation of SCC more energy was partitioned to the body rather than the udder. In cows with a case of clinical mastitis in wk 4 – 44, EB was greater in wk 1 than in wk 2, and greater in wk 2 than in wk 3 before a case of clinical mastitis. A greater EB could result from lower FPCM yield, or greater energy intake. In wk -3 – -1, FPCM yield numerically decreased and energy intake numerically increased, but were not significantly different among weeks in this study, which implies large variation in FPCM yield and energy intake among cows in preparation for an upcoming case of clinical mastitis. In a previous study, clinical mastitis was related with lower DMI in the same week, although large variability in response of DMI to clinical mastitis was observed among cows (**González *et al.***, 2008). FPCM yield and energy intake were lower and the plasma GH concentration tended to be greater in the week of clinical mastitis compared with the last week before clinical mastitis, which is in line with results of **González *et al.*** (2008). A greater plasma GH concentration was previously observed in mastitis-induced cows, and was related with an increase in leucocytes that may be a result of the effect of GH on bone marrow to release stored immune cells (**Burvenich *et al.***, 1999). From this study it appears that the relation of clinical mastitis with metabolic status is more pronounced in the week of clinical mastitis, than in the weeks prior to clinical mastitis.

6.6 Conclusion

A 30-d DP without dry cow antibiotics, compared with a 0-d DP, resulted in a lower SCC in dairy cows during the subsequent lactation, but not when SCC was corrected for milk yield. Dry period length did not affect incidence of elevated SCC or IMI across the DP, but during the complete subsequent lactation cows with a 0-d DP fed a standard energy level had a greater hazard for a case of clinical mastitis than cows with a 30-d DP. Dry period length or feeding either a lipogenic or glucogenic diet did not affect incidence of elevations of SCC in the subsequent lactation. This implies that, although effects are very small, shortening the DP to 30-d without use of dry cow antibiotics seems more beneficial for udder health in the subsequent lactation, compared with complete omission of the DP.

6.7 Acknowledgements

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CHAPTER 7

General discussion

7.1 Introduction

Diseases in dairy cows occur mostly in early lactation and include ketosis, retained placenta, metritis, mastitis, milk fever, displaced abomasum, lameness, and impaired fertility (**Butler *et al.***, 1981; **Drackley**, 1999; **Ingvartsen *et al.***, 2003). Disease in early lactation may affect milk production performance not only in early lactation but also in later lactation including total lactation yield (**Fourichon *et al.***, 1999). Diseases in early lactation are often related with a negative energy balance (**EB**) and compromised metabolic status (**Collard *et al.***, 2000; **Butler**, 2003; **Ingvartsen *et al.***, 2003). Increasing disease incidence instigated a search for management strategies to improve EB for health and welfare of dairy cows. Previous studies reported that milking once daily in early lactation increased EB and plasma glucose concentration compared with milking twice or three times daily (**Rémond *et al.***, 2002; **Phyn *et al.***, 2014). Different feeding strategies, such as feeding glucogenic, compared with more lipogenic nutrients (**Drackley**, 1999; **Van Knegsel *et al.***, 2007) or addition of conjugated linoleic acid to dairy cow rations (**Harvatine and Allen**, 2006) were found to increase EB in early lactation. Shortening or omitting the dry period (**DP**) length was also opted as a management strategy to increase EB in early lactation (**Grummer and Rastani**, 2004). Shortening or omitting the DP increased EB in early lactation, through a reduction in postpartum milk yield (**Rastani *et al.***, 2005; **Van Knegsel *et al.***, 2014). In some cows without a DP (**0-d DP**), the increased EB remained throughout lactation and was associated with fattening and spontaneous drying off in late lactation (**Chen *et al.***, 2016b). In this thesis we hypothesized that adjustment of the dietary energy level to the expected milk yield of cows, i.e. aimed at zero EB would prevent fattening. Furthermore, we hypothesized that feeding a lipogenic ration, rather than a more glucogenic ration in later lactation, would prevent fattening and increase lactation persistency by increasing the plasma GH concentration in cows with a 0-d DP.

Behavioral indicators for metabolic status would allow earlier observation of a detrimental metabolic status. In previous studies, cows with a lower plasma free-fatty acid (**FFA**) concentration had greater postpartum walking activity (**Adewuyi *et al.***, 2006) and rumination (**Stangaferro *et al.***, 2016) compared with cows with a greater plasma FFA concentration. However, to our knowledge, the relation between feeding and lying behavior, and steps in dairy cows in early lactation with other plasma metabolites and hormones was not described previously. We hypothesized that a difference in metabolic state would be reflected in feeding behavior and activity of cows after a 0-d or 30-d DP partly through differences in EB.

Preventive use of dry cow antibiotics is not allowed anymore in several European countries including the Netherlands. This new legislation demands management interventions to maintain udder health during the DP and subsequent lactation in cows without intramammary infections (**IMI**) or cure IMI in infected cows. To our knowledge, all experiments that studied the effect of DP length on udder health were carried out using cows that were treated with dry cow antibiotics when dried off. Therefore DP length and use of antibiotics are confounded in these studies. We hypothesized that a 30-d DP without use of dry cow antibiotics, compared with cows with a 0-d DP, would negatively affect udder health in the subsequent lactation as existing IMI are neither flushed out or treated. Furthermore, we hypothesized that a 30-d DP without use, as compared to with use, of dry cow antibiotics might negatively affect udder health in the subsequent lactation due to more new IMI during a DP without dry cow antibiotics.

In a previous study, large variation in the effects of DP length on milk yield in the subsequent lactation was observed between individual cows, which could partly be explained by individual cow characteristics, such as parity or milk yield in the previous lactation (**Steeneveld *et al.***, 2014). We hypothesized that also effects of DP length on udder health in the subsequent lactation can be explained by prepartum cow characteristics such as parity, milk yield, and somatic cell count (**SCC**). The question remains what cows are suitable for a 0-d or a 30-d DP with regard to udder health in the subsequent lactation, and how these cows should be managed to optimize their performance in milk yield, udder health, metabolic status, and general health after a certain DP length.

The main aims of this thesis were 1) to evaluate effects of dietary energy level and source during lactation on milk production, metabolic status, fattening, and lactation persistency in cows with different dry period lengths, 2) to evaluate relationships between metabolic status and cow behavior of cows in early lactation, 3) to study dry period lengths with and without use of dry cow antibiotics on udder health of cows with different dry period lengths, and 4) to identify prepartum cow characteristics that determine with udder health after different dry period lengths.

To study these aims, two large experiments were conducted. Chapter 2, 3, 4, and 6 describe experiment 1. In this experiment, 123 cows were assigned randomly to a 0-d dry period (2/3 of cows) or a 30-d dry period (1/3 of cows). Cows with a 0-d dry period were fed either a (**LOW**) energy level, which was based on the requirement for their expected milk yield (35.4 kg FPCM/d) [**0-d DP(LOW)**] or a standard (**STD**) energy level [**0-d DP(STD)**] which was based on the energy level for the expected milk yield of cows with a 30-d DP [**30-d DP(STD)**] (40.4 kg/d). Chapter 2 describes the effects of different (LOW or STD) energy levels after a 0 or 30 days dry period on milk production, feed intake, and plasma metabolites in wk 1 – 7 of lactation. In Chapter 3 we aimed to prevent fattening and improve lactation persistency by lowering plasma insulin and increasing plasma GH concentration through 1) adjusting dietary energy level to expected milk yield of cows with a 0-d dry period, and 2) feeding a lipogenic ration, compared with a more glucogenic ration from wk 8 in lactation onwards. Chapter 3 describes the effects of different dietary energy levels and energy sources after a 0 and 30 days dry period on lactogenic hormones, EB during a complete lactation, and lactation curve characteristics. Chapter 4 focusses on the relations of feeding behavior, lying, and steps with plasma metabolites and hormones in wk 4 of lactation.

Chapter 5 and 6 focus on udder health. Chapter 5 describes experiment 2, in which 167 cows were assigned randomly to a 0-d, 30-d, or 60-d dry period. At drying off, cows with a 30-d or 60-d dry period were treated with dry cow antibiotics. Chapter 5 describes the effects of dry period length, with use of dry cow antibiotics, on udder health and the individual cow characteristics that are associated with udder health after a 0, 30 or 60 days dry period. Recently, preventive use of dry cow antibiotics is not allowed anymore in some European countries, among which the Netherlands (**EC**, 2/2015 299/04; **Dutch M. o E. a.**, 2017). Therefore, cows with a 30-d dry period in experiment 1 (Chapter 6), were not treated with dry cow antibiotics. Chapter 6 focusses on udder health across the dry period and in the subsequent lactation after a 0 or 30 days dry period without use of dry cow antibiotics. In chapter 7 results of this thesis are discussed. Specifically what cows are suitable for a 0-d or a 30-d dry period with regard to udder health in the subsequent lactation, and how these cows should be managed to optimize their performance in milk yield, udder health, metabolic status, and general health after a certain dry period length.

The first part of the current Chapter discusses the metabolic status of cows with different DP lengths and different dietary energy level and source during the subsequent lactation. Secondly, lactation curve characteristics including lactation persistency of cows with different DP lengths and different dietary energy level and source are discussed. Thirdly, the relation between metabolic status and behavior of cows with different DP lengths is discussed. Fourthly, udder health of cows with different DP lengths and different use of dry cow antibiotics is discussed. Finally, the practical implications of tailored dry periods will be discussed.

7.2 Metabolic status

Cows with an omitted DP have lower milk yield (**Grummer and Rastani**, 2004; **Rastani *et al.***, 2005; **Van Knegsel *et al.***, 2014), lower energy demands throughout lactation, and therefore potentially have an improved EB in early lactation and require less energy during later lactation than cows with a short or conventional DP. In all previous studies, cows with an omitted, short (~30-d) or conventional (~60-d) DP were fed a ration with the same energy level which contained energy for the expected milk yield of cows after a conventional DP (**Andersen *et al.***, 2005; **Rastani *et al.***, 2005; **De Feu *et al.***, 2009; **Van Knegsel *et al.***, 2014). In one of these studies, BCS of cows with a 0-d DP increased, and some cows dried off spontaneously during the subsequent lactation (**Chen *et al.***, 2016b). In Chapter 2, we aimed to evaluate whether feeding cows with a 0-d DP according to their expected milk yield would reduce either milk yield or EB, or both, and how different concentrate levels in wk 4 – 7 affect plasma metabolites FFA, BHB, glucose, insulin, IGF-1, and GH in wk 1– 7 of lactation. In Chapter 2 and 4 of this thesis, half of the cows with a 0-d DP were fed an energy level for their expected milk yield [LOW], and the other half were fed an energy level for the expected milk yield of cows with a 30-d DP [STD] from wk 4 onwards (**Table 7.1**). From wk 8 postpartum onwards, half of each group of cows received a glucogenic ration and the other half a lipogenic ration at the assigned postpartum energy level. In Chapter 3, we looked at long term effects during wk 1 – 44 of lactation and hypothesized that feeding cows after a 0-d DP an energy level for their expected milk yield would not affect milk yield, but would reduce the plasma insulin and IGF-1 concentration, limit EB to zero, and thereby reduce fattening, compared with feeding a greater energy level such as for cows with a 30-d DP. Greater prepartum fattening BCS was previously associated with postpartum a more negative EB (**Butler *et al.***, 1981; **Chen *et al.***, 2016b). In Chapter 3, we also hypothesized that feeding a lipogenic ration would limit the plasma insulin and IGF-1 concentration, increase GH concentration leading to a more persistent milk production, a less positive EB, and reduced fattening in the course of lactation, compared with feeding a more glucogenic ration.

This part of the general discussion will elaborate how DP length, dietary energy level, and dietary energy source (glucogenic or lipogenic) affect metabolic status, and fattening of cows with different DP lengths.

Table 7.1: Overview of dietary energy level and source fed to cows during and in different periods after a 0-d or 30-d dry period

Dry period length	Energy level[1]		Wk 4 – 1 prepartum	Wk 1 – 4	Wk 4 – 7	Wk 8 – 31	Wk 31 – 44
0-d DP	LOW	Max. concentrate[2]	1 kg/d	6.7 kg/d	6.7 kg/d	6.7 kg/d	0 kg/d
		Basal ration	Lactation ration (**LR**)	LR	LR	LR – LOW[3] G or L[4]	LR - LOW G or L
0-d DP	STD	Max. concentrate	1 kg/d	6.7 kg/d	8.5 kg/d	8.5 kg/d	0 kg/d
		Basal ration	LR	LR	LR	LR - STD G or L	LR - STD G or L
30-d DP	STD	Max. concentrate	From 10-d prepartum 1 kg/d	6.7 kg/d	8.5 kg/d	8.5 kg/d	0 kg/d
		Basal ration	Dry cow ration	LR	LR	LR - STD G or L	LR - STD G or L

[1]The low (LOW) dietary energy level was based on the requirement for their expected milk yield of cows with a 0-d DP, and the standard (STD) dietary energy level was based on the requirement for the expected milk yield of cows with a 30-d DP. [2]Experimental concentrate supply increased stepwise by 0.3 kg/d from 4 DIM up to 8.5 kg/d at 28 DIM for cows with a STD energy level or by 0.3 kg/d from 4 DIM up to 6.7 kg/d at 22 DIM for cows with the LOW energy level. Experimental concentrate decreased stepwise by 0.5 kg/wk starting in wk 15 to 0 kg/d in wk 31 for cows with a STD energy level and by 0.4 kg/wk in wk 15 down to 0 kg/d in wk 31 for cows with a LOW energy level. After wk 31 postpartum, difference in dietary energy level remained in the basal ration. [3]The lactation ration with a LOW dietary energy level contained more wheat straw than the ration with a STD energy level. [4]The glucogenic lactation ration consisted mainly of corn silage and grass silage. The lipogenic lactation ration consisted mainly of grass silage and sugar beet pulp.

7.2.1 Management measures to improve metabolic status

Dry period length

A 0-d DP reduced postpartum milk yield compared with a 30-d DP in the current study and previous studies, but this reduction is partly compensated with milk yield in the precalving period (**Rastani *et al.***, 2005; **Van Knegsel *et al.***, 2014). To make a proportionate comparison of total lactation yield, **Kok et al.** (2016) defined effective lactation yield as daily milk yield between 60-d before the expected calving day and 60-d before the expected calving day in the subsequent lactation. Based on these field data, postpartum 305-d yield was 4.6 kg FPCM/d lower for cows with a 0-d DP (23.8 ± 0.62 vs 28.4 ± 0.57 kg FPCM/d), but effective lactation yield was 2.5 kg FPCM/d lower for cows with a 0-d DP (22.4 ± 0.57 vs 24.9 ± 0.52 kg FPCM/d) compared with cows with a 30-d DP (**Kok *et al.***, 2016). In the current thesis, Wood's curves were used to estimate effective lactation yield, which was defined as daily milk yield between 30-d before calving and 30-d before expected calving in the subsequent lactation, because not all cows remained in the experiment till 30-d before next calving. Effective lactation yield tended to be 2.7 kg FPCM/d lower for cows with a 0-d DP compared with cows with a 30-d DP (0-d DP(LOW) 25.3 ± 0.9; 0-d DP(STD) 24.9 ± 1.0; 30-d DP(STD) 27.8 ± 1.0 kg FPCM/d; P = 0.08), which is quite similar to findings of Kok et al. (2016) based on field data. In conclusion, the loss of milk yield after a 0-d DP, compared with a 30-d DP, is substantially lower when the more accurate effective lactation yield is calculated compared with the measure 305-d milk yield.

Cows with an omitted DP have a greater EB than cows with a short or conventional DP (**Rastani *et al.***, 2005; **De Feu *et al.***, 2009; **Van Knegsel *et al.***, 2014), which potentially reduces the risk of metabolic diseases in early lactation (**Butler *et al.***, 1981; **Collard *et al.***, 2000; **Ingvartsen *et al.***, 2003). In Chapter 2, incidence of treated cases of milk fever, clinical mastitis, retained placenta, left displaced abomasum, endometritis, or pyometra in the first 7 weeks of lactation was not different between cows with a 0-d DP or a 30-d DP (**Table 7.2**). However, the incidence of at least 1 disease in wk 1 – 7 was numerically lower for cows with a 0-d DP (44% vs 57%; P = 0.18) than for cows with a 30-d DP, although the number of affected cows was low.

Table 7.2: Proportion of cows with a disease in early lactation after a 0-d or 30-d DP as compared to previous studies. Significance could not be determined in either experiment due to the low number of diseased animals

	Chapter 2			Rastani et al. (2005)		Schlamberger et al. (2010)		Vanholder et al. (2015)
	Wk 1-7[1]			Wk 1 - 10		Wk 1 – 4		
	0-d DP (LOW)	0-d DP(STD)	30-d DP	0-d DP	28-d DP	0-d DP	56-d DP	Conventional DP
Cows (n)	40	41	42					
Milk fever	8%	0%	5%					
Clinical mastitis	15%	17%	14%	14%	26%	25%	42%	
Retained placenta	18%	12%	10%	10%	4%	8%	17%	
Endometritis	8%	17%	19%	0%	0%			
Pyometra	5%	2%	5%					
Left displaced abomasum	3%	0%	5%	10%	4%			
Ketosis	3%	2%	0%	0%	4%	0%	25%	11.6%

In previous studies, omitting the DP resulted in a numerically lower incidence of cows with mastitis (**Rastani *et al.***, 2005; **Schlamberger *et al.***, 2010) or ketosis (**Rastani *et al.***, 2005; **Schlamberger *et al.***, 2010; **Vanholder *et al.***, 2015), a numerically greater (**Rastani *et al.***, 2005) or lower (**Schlamberger *et al.***, 2010) number of cows with retained placenta, and a numerically greater number of cows with displaced abomasums (**Rastani *et al.***, 2005), compared with a short or conventional DP. Additionally, a meta-analysis reported that shortening and omitting the DP tended to reduce the risk of ketosis in the subsequent lactation, but that shortening the DP did not alter the risk for mastitis, metritis, or reproductive failure in the subsequent lactation compared with a conventional DP (**Van Knegsel *et al.***, 2013). In conclusion, in this thesis, omitting the DP, compared with a short DP, did not reduce metabolic disorders and diseases in early lactation. Based on the greater EB in cows with an omitted dry period, positive effects on (metabolic) disease incidence can be expected, compared with cows with a short or conventional. Indeed, when subclinical ketosis was based on plasma BHB concentration ≥ 1.2 mmol/L (**Duffield *et al.***, 1998), incidence of subclinical ketosis was greater in cows with a 30-d DP (31%) than in cows with a 0-d DP (11%) in wk 1 – 7 of lactation (P < 0.01). However, in general, the combination of low numbers of animals and low incidences of diseases makes it difficult to estimate potential health effects in experimental datasets.

Dietary energy level

In Chapter 2, we aimed to limit the dietary energy level for half of cows with a 0-d DP to reduce the chance of fattening in late lactation. In Chapter 2, reducing postpartum concentrate level in early lactation for cows with a 0-d DP did not affect FPCM yield or plasma FFA, BHB, glucose, insulin, or IGF-1 concentration. Energy balance was -13 kJ/kg$^{0.75}$·d for cows with a 0-d DP(LOW) and more positive (51 kJ/kg$^{0.75}$·d) for cows with a 0-d DP(STD) in week 6 and 7 postpartum. These results shows that, though EB was lower in wk 6 and 7 for cows with a 0-d DP(LOW), postpartum metabolic status is similar compared with cows with a 0-d (STD), which indicates that cows are not more at risk for metabolic diseases. After wk 7 in lactation the contrast in concentrate levels remained, but concentrate level was build off between wk 15 - 31. From week 8 onwards, the contrast of STD and LOW energy level was established in the basal ration by adding more straw to the LOW basal rations.

In Chapter 3, we analyzed the effects of a LOW or STD energy level for cows with a 0-d DP cows between wk 1 - 44. Cows with a 0-d DP(LOW) had a lower energy intake (990 kJ/kg$^{0.75}$·d) than cows with a 0-d DP(STD) (1039 kJ/kg$^{0.75}$·d). The energy intake in cows with a 0-d DP(LOW) was only 5% smaller than that in cows with a 0-d DP(STD), which is a smaller difference than the 12% lower NE_L intake which was aimed for, and is likely due to the tendency to greater intake of basal ration of cows with a 0-d DP(LOW) (17.2 kg DM/d) than cows with a 0-d DP(STD) (16.7 kg DM/d). In cows with a 0-d DP, a LOW or STD dietary energy level did not result in different average body weight throughout lactation (699 and 699 kg) or BCS (3.04 and 3.02). However, weekly body weight gain was greater in cows with a STD energy level (2.6 ± 0.2 kg/wk) than in cows with a LOW energy level (2.0 ± 0.2 kg/wk) (P < 0.01). A STD energy level for cows with a 0-d DP supports the large energy demand for milk yield in the first weeks of lactation which resulted in a more positive EB in wk 6 and 7 postpartum (51 vs. -13 kJ/kg$^{0.75}$·d), compared with cows with a 0-d DP(LOW). In mid- and late-lactation, feeding a ration with a STD energy level to cows with a 0-d DP provided more energy than required for milk yield, resulting also in a more positive EB (114 vs. 74 kJ/kg$^{0.75}$·d) in wk 7 – 44 of lactation. Effects on BCS were not present compared with a LOW energy level, which could be related with the relatively small difference in EB between cows with a 0-d DP(LOW) or 0-d DP(STD). Reducing the dietary energy level for cows with a 0-d DP did not affect fattening by means of BCS or BW, which is likely related with greater basal ration and energy intake, compared with cows with a 0-d DP fed a STD energy level. Similarly, body condition score in our study was not different for cows with a 0-d DP (2.8) or 30-d DP (2.8 in wk 1 – 7 of lactation), compared with a previous study in which all cows were fed the energy level for milk yield of cows after a 60-d DP (3.0 and 2.7 in wk 1 – 9 of lactation for cows with a 0-d DP or 30-d DP, respectively) (**Chen *et al.***, 2016b).

In Chapter 3, cows with a 0-d DP had lower milk yield (27.2 ± 0.8 kg vs 32.3 ± 0.8 kg FPCM/d) compared with cows with a 30-d DP. Various studies seem to show that a short or no DP predisposes for a certain postpartum milk yield (**Table 7.3**).

Table 7.3: Fat-and-protein corrected milk production (kg/d) of cows with an omitted or short dry period

	DIM	0-d DP	30-d DP	Difference
Chapter 4	305	27.2	32.3	5.1
Annen et al., 2004 (primiparous)	119	33.7	36.7	3.0
Annen et al., 2004 (multiparous)	119	40.1	41.5	1.4
Rastani et al., 2005	70	33.3	37.5	4.2
Klusmeyer et al., 2009	294	32.2	36.6	4.4
Van Knegsel et al., 2016	98	35.4	40.4	5.0

Body weight gain and milk production. Cows with a 0-d DP had greater body weight gain (2.3 ± 0.1 kg/wk), than cows with a 30-d DP (1.0 ± 0.2 kg/wk; P < 0.01). This greater body weight gain could be the result of a lower milk production due to lower secretory capacity of udder cells and a larger energy intake than needed for milk production. The surplus of available energy that is not used for milk production is partitioned towards body reserves. In the current study, cows with a 0-d DP(LOW) (86 ± 8 kg) or with a 0-d DP(STD) (101 ± 9 kg) gained more BW than cows with a 30-d DP (63 ± 8 kg) between wk 8 – 44 of lactation (P < 0.01). Furthermore, 1 kg body weight gain after peak milk yield was associated with a greater reduction in milk yield for cows with a 0-d DP(LOW) (-0.55 ± 0.3 kg FPCM / kg BW) or 0-d DP(STD) (-0.54 ± 0.3 kg FPCM / kg BW) than for cows with 30-d DP (-0.39 ± 0.3 kg FPCM / kg BW) (P < 0.01). These results may imply that cows with a 0-d DP are more sensible for milk production loss due to weight gain than cows with a 30-d DP. Body weight gain in late lactation may possibly inhibit milk production and the feasibility of a short DP or continuous milking in the upcoming prepartum period. On the other hand, the correlation between body weight gain and milk production may simply be a result of cows having similar energy intakes and if not depositing energy in milk, it will go to body reserves. However, a previous study on extended lactations reported that feeding a high level of energy throughout lactation was related with a lower proportion of cows that could be milked till drying off (**Grainger *et al.***, 2009). This implies that excess energy, which cannot be used for milk production due to limited secretory capacity of the udder, negatively affects milk production.

In Chapter 3, EB in mid- and late- lactation tended to be greater in cows with a 0-d DP than in cows with a 30-d DP. In mid- and late- lactation, dietary energy level for cows with a 0-d DP may be further reduced to reduce inhibitory effects of body weight gain on milk production.

Dietary energy source

In Chapter 3 we hypothesized that feeding an iso-caloric ration with lipogenic nutrients, compared with more glucogenic nutrients, after peak milk yield would result in a similar DMI, greater milk yield, a less positive EB, lower plasma insulin, IGF-1 and greater plasma GH concentration. Previous studies reported that a lipogenic ration in mid- and late- lactation reduced the plasma glucose and insulin concentration compared with a more glucogenic ration (**Voelker**

and Allen, 2003; **Mahjoubi *et al.***, 2009) and a lower plasma insulin concentration likely stimulates nutrient partitioning towards the udder rather than towards the body (**Hart**, 1983). In early lactation, previous studies showed that feeding a lipogenic ration did not affect milk, lactose or protein yield, increased milk fat content, and decreased EB, compared with a glucogenic ration (**Van Knegsel *et al.***, 2007; **Garnsworthy *et al.***, 2009; **Mahjoubi *et al.***, 2009; **Van Knegsel *et al.***, 2014). In our study, contrary to our expectation, a lipogenic ration decreased DMI, energy intake, and milk yield in wk 8 – 44 of lactation, without decreasing EB, compared with feeding the more glucogenic ration. Lower DMI of cows fed a lipogenic ration could be related with the lower DM content, fill, or palatability of the ration (**Zom *et al.***, 2012). In addition, dietary energy source did not affect body weight or BCS. So, it appears that, the greater energy intake with a glucogenic ration was fully partitioned towards milk yield. Thus, cows with a glucogenic or lipogenic ration had similar nutrient partitioning to body reserves. A glucogenic ration decreased the milk fat content for cows with a 0-d DP(STD) or 30-d DP(STD) in wk 8 – 44 of lactation, which may be the result of a lower rate of mobilization of body fat reserves in early lactation and lower availability of lipogenic precursors in late lactation, compared with feeding a lipogenic ration. Dietary energy source did not affect milk fat content of cows with a 0-d D(LOW) which may be related with the greater amount of straw in the LOW ration that increased the amount of lipogenic precursors and decreased the amount of glucogenic precursors for these cows. In accordance with our hypothesis, a lipogenic ration decreased the plasma IGF-1 concentration and the plasma insulin concentration within cows with a 0-d DP(STD), and tended to increase the plasma GH concentration compared with a more glucogenic ration.

7.2.2 Cow factors that determine milk production and metabolic status after different dry period lengths

This thesis and previous studies have shown that the success of certain DP lengths with regard to udder health and milk yield in the subsequent lactation can be determined by certain prepartum cow characteristics. Cow characteristics parity and body condition score are discussed.

Parity

Parity affected milk yield and lactation curve characteristics in the subsequent lactation in this thesis. In Chapter 2 and 3, milk yield, but not FPCM yield, tended to be lower for cows with postpartum parity 2 compared with cows with parity ≥3 after a 0-d DP, while this effect was not present when cows were assigned to a 30-d DP, which is in line with previous studies (**Annen *et al.***, 2004; **Steeneveld *et al.***, 2014). Moreover, the difference in effective lactation yield, was larger for cows with a 0-d DP between parity 2 (23.0 kg/d) or parity ≥3 (25.4 kg/d) than for cows with a 30-d DP between parity 2 (25.1 kg/d) or parity ≥3 (26.5 kg/d) (**Kok *et al.***, 2017). In this thesis, estimated effective lactation yield was not different between cows with different parity (Parity 2 25.8 ± 0.8 vs. parity ≥3 26.2 ± 0.7; $P = 0.68$) and there was no effect for the interaction between parity and DP length ($P = 0.45$), likely due to low numbers of cows. Young cows may need a short or conventional DP for their ongoing body development and mammary growth (**Annen *et al.***, 2004).

Body condition

In cows with different DP lengths, a greater BCS across the DP was associated with greater milk yield (**Domecq *et al.***, 1997; **Chen *et al.***, 2016b) and a greater negative EB postpartum (**Butler *et al.***, 1981; **Chen *et al.***, 2016b). In a previous study, compared with a 60-d DP, a 0-d or 30-d DP reduced milk yield and increased EB in cows with prepartum a low or normal BCS cows, but this effect was not present for cows with prepartum a high BCS (**Van Knegsel and Kemp**, 2016). In Chapter 2, a 0-d DP reduced FPCM yield and increased EB in cows with prepartum a normal or high BCS before calving, compared with a 30-d DP, but this effect was not present for cows with prepartum a low BCS **(Figure 7.1.a-b)**. **Van Knegsel and Kemp** (2016) reported no effect of DP length or precalving BCS on DMI, while in Chapter 2, DMI was lower in cows with a 30-d DP that had a low or high BCS precalving ($P < 0.05$) **(Figure 7.1.c)**. Furthermore, in Chapter 2 energy level for cows with a 0-d DP was based on requirements for cows with a 0-d or 30-d DP, while in the study of **Van Knegsel and Kemp** (2016), all cows received the energy level for cows with a 60-d DP. Assigning cows with a high prepartum BCS to a 0-d DP, rather than a 30-d DP, can be suggested to improve DMI an EB in early lactation.

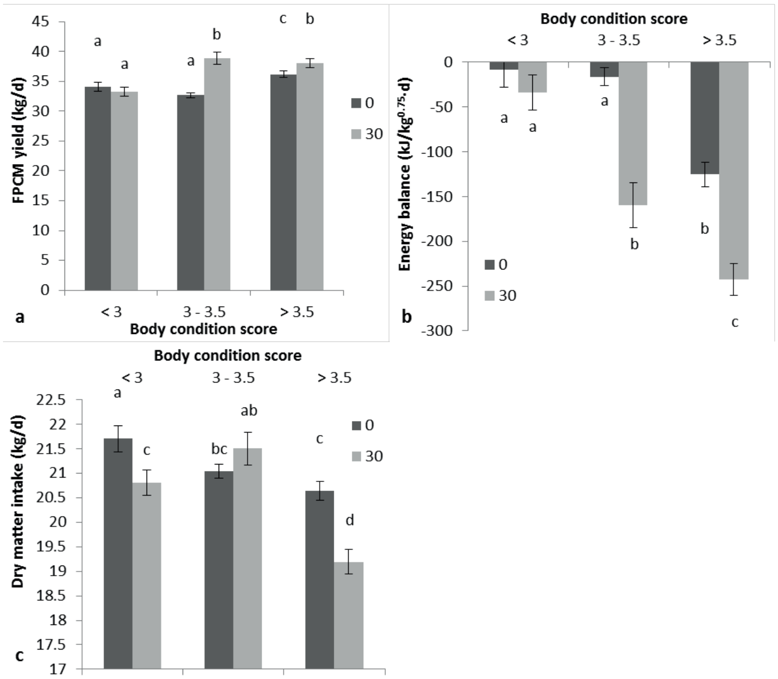

Figure 7.1 a-b: Fat-and-protein corrected milk (a), energy balance (kJ/kg$^{0.75}$·d) (b), and dry matter intake (c) in wk 1 – 7 for cows with a low (< 3), normal (3.0 – 3.5), or high (> 3.5) body condition score between wk 6 - 4 prepartum and a 0-d or 30-d dry period in Chapter 2.

7.2.3 Additional benefits of a short or omitted dry period

Voluntary waiting period and inter-calving period

Cows with a short or omitted DP had an earlier onset of first ovulation and a greater proportion of cows with normal cyclicity in early lactation than cows with a conventional DP (**Watters *et al.***, 2009; **Chen *et al.***, 2015). In the current study, cows with a 0-d DP(LOW) had a lower number of days until conception, which is likely related with a greater EB and better fertility than in cows with a 30-d DP(STD), which is in line with previous studies (**Butler and Smith**, 1989; **Gümen *et al.***, 2005; **Chen *et al.***, 2015). Better fertility, as reflected by a lower number of days till conception, in cows with a 0-d DP fed a LOW dietary energy level, may lead to the decision to shorten the voluntary waiting period, compared with cows with a 30-d DP. The voluntary waiting period is a set period at the beginning of lactation that allows time for uterine involution, resumption of normal cyclicity, and restoration of zero EB (**Löf *et al.***, 2012). A shorter voluntary waiting period could be economically optimal for cows with a 0-d DP due to their lower expected 305-d yield (Chapter 4), better metabolic status (Chapter 2), earlier first ovulation (**Chen *et al.***, 2015), and possibly lower disease incidence (§7.2.1 - dry period length) in early lactation, compared with a 30-d DP (**Inchaisri *et al.***, 2011).

Effects of milk yield on offspring

Effect of level of milk yield may negatively affect metabolic status of the dam and carry-over to her daughters. High milk yield during gestation in cows, but not heifers, resulted in calves with a greater basal plasma insulin concentration, lower insulin sensitivity (**Kamal *et al.***, 2015), and lower birth weight (**Kamal *et al.***, 2014), compared with cows that had lower milk yield during gestation. Furthermore, calves from cows with high milk yield grew faster, possibly due to predisposition to store body fat, and had a lower pregnancy rate after first service, compared with calves from cows with lower milk yield (**Opsomer, Ghent University, Ghent, Belgium, Pers. comm.**). High milk yield in the dam during gestation increased SCC, decreased fertility and milk yield, and reduced survival to second parity in daughters due to culling for aforementioned reasons (**Banos *et al.***, 2007; **Berry *et al.***, 2008; **Gonzalez-Recio *et al.***, 2012). It could be hypothesized that applying a short or no DP may improve metabolic status of dams and reduce carry-over effects to their offspring.

7.2.4 Conclusions

A 0-d DP reduced milk yield, increased EB, and improved metabolic status throughout lactation, compared with a 30-d DP. Reducing the dietary energy level for cows with a 0-d DP did not affect milk yield or metabolic status, but resulted in a more negative EB in early lactation and a less positive EB in mid- and late- lactation. In contrast to expectations, reducing dietary energy level did not prevent fattening, which was likely related with compensation of lower concentrate level with greater basal ration intake, which may have decreased effects of contrast in dietary energy level. In early lactation, feeding a high dietary energy level supports cows during the period of negative EB, but in mid- and late- lactation dietary energy level can likely be further reduced to reduce fattening of cows with a 0-d DP. In contrast to our hypothesis, feeding a lipogenic ration did not affect fattening, compared with a more glucogenic ration. Cows fed a lipogenic ration had lower DMI, milk yield, and metabolic status in mid- and late- lactation compared with cows fed a more glucogenic ration. A lipogenic ration with greater DM content and palatability can be hypothesized to have a similar effect on DMI, milk yield, and metabolic status, compared with a more glucogenic ration.

7.3 Lactation persistency

Previously, it was suggested that greater persistency of milk yield in the subsequent lactation could partially compensate for milk losses in early lactation after a short or omitted DP (**Grummer and Rastani**, 2004). Previous studies, however, reported ambiguous effects of DP length on lactation persistency (**Mantovani *et al.***, 2010; **Atashi *et al.***, 2013; **Chen *et al.***, 2016a). In Chapter 3 we hypothesized that a lower plasma insulin concentration after peak milk yield stimulates the plasma GH concentration and, thereby, potentially increases lactation persistency in cows with a 0-d. We aimed to lower the plasma insulin concentration by 1) adjusting dietary energy level to expected milk yield of cows with a 0-d DP, and 2) feeding a lipogenic ration, compared with a more glucogenic ration. Cows with a 0-d DP were fed an energy level aimed at zero EB [LOW], compared with an energy level based on the expected milk yield of cows with a 30-d DP [STD].

This part of the general discussion will discuss how adjustment of dietary energy level, and dietary energy source (glucogenic or lipogenic) affects lactation curve characteristics for cows with a 0-d or 30-d DP.

7.3.1 Management measures to improve lactation persistency

Dry period length

In chapter 3 (experiment 1), cows with a 30-d DP had a smaller increasing slope of the lactation curve [*b*, (**Wood**, 1967)] and smaller decline of milk production after peak yield [*c*, (**Wood**, 1967)] and lower relative rate of decline [Rd, (**Dijkstra *et al.***, 2010)] than cows with a 0-d DP. This is in line with a previous study that showed a lower decline of milk production after peak yield in cows after a conventional DP, compared with cows with an omitted DP (**Mantovani *et al.***, 2010). Also in Chapter 5 (experiment 2) (**Table 7.4**), cows with a 30-d or 60-d DP had greater lactation persistency by means of a smaller decreasing slope (*c*) of the lactation curve and lower relative rate of decline, compared with cows with a 0-d DP ($P < 0.01$). In Chapter 3 we hypothesized that the greater lactation persistency of cows with a 30-d DP was related with a less positive EB in these cows compared with cows with a 0-d DP, regardless of dietary energy level or source. In Chapter 5, compared with chapter 3, cows with a 0-d (39 vs. 10 kJ/kg$^{0.75}$·d) or 30-d DP (-59 vs. -101 kJ/kg$^{0.75}$·d) had a more positive or (less negative EB) in wk 1 – 14 of early lactation, and may be assumed to have had a more positive EB in late lactation. From comparison of the relationship of EB with lactation persistency from Chapter 3 and 5, it seems that the relation between EB and the declining slope (*c*) or relative decline of the lactation curve (rd) has the same direction within cows with a 0-d or 30-d DP (**Figure 7.2**). These results would support our hypothesis from Chapter 3 that the decreasing slope of the lactation curve is related with differences in postpartum EB after different DP lengths, as well as with effects of DP length alone.

Differences in lactation curve characteristics among cows with different dry period lengths may be related with differences in disease incidence. A previous study showed that cows that develop metabolic disorders or diseases such as milk fever, retained placenta, metritis, ketosis, displaced abomasum, or mastitis in early lactation have a more persistent lactation after peak milk yield than cows without disease in early lactation (**Hostens *et al.***, 2012). In this study, greater lactation persistency, based milk yield reduction after peak milk yield from the MilkBot model used by Hostens, was hypothesized to be due to a smaller increasing slope of the lactation curve and postponed pregnancy which reduces negative effects of pregnancy on lactation persistency (**Hostens *et al.***, 2012). For Chapter 3, a similar analysis was performed using Wood's lactation curve characteristics for cows with a 0-d or 30-d DP with or without disease in wk 1 – 7 postpartum. In Chapter 3, disease in early lactation did not affect total FPCM yield in wk 1 – 44. The declining slope of the lactation curve (*c*) or relative decline from the midway point between peak lactation and end lactation (rd) were not different among different transition treatments that had at least 1 metabolic disorder or associated diseases in early lactation, compared with cows without disease in early lactation (**Table 7.5**). An interaction of disease with DP length was present for persistency (*S*) ($P < 0.05$). Persistency (*S*) (**Wood**, 1967), was greater in cows with a 30-d DP and at least 1 metabolic disorder or disease in early lactation (**Figure 7.3**), which was related with a greater increasing slope (0.13 ± 0.03 vs. 0.21 ± 0.03) in these cows, compared with cows with a 30-d DP without disease in early lactation. These results suggest that persistency (*S*) may indeed differ for cows with a 30-d when affected by disease. Furthermore, cows with a 0-d DP(STD) without disease in early lactation had greater persistency than cows with a 0-d DP(LOW) without disease in early lactation, which supports a previous conclusion from §7.2.1 – dietary energy level, that cows with a 0-d DP should be fed a STD energy level in early lactation to support the demand for high milk production and to limit negative EB.

Table 7.4: Lactation curve characteristics during week 1 to 44 of lactation in cows after a 0-d, 30-d, or 60-d dry period (Chapter 5)

	Dry period length				*P*-values[1]					
	0-d	30-d	60-d	SEM	DP	R	P	DP × R	DP × P	R × P
Cows (n)	53	51	54							
a^2	16.9[a]	22.5[b]	27.9[c]	1.0	<0.01	0.05	0.30	0.25	0.19	0.52
b^2	0.31[a]	0.25[a]	0.20[b]	0.01	<0.01	0.09	0.76	0.38	0.32	0.67
c^2	0.0064[a]	0.0052[b]	0.0046[b]	0.0002	<0.01	0.48	<0.01	0.55	0.67	0.95
S^2	6.67[a]	6.60[ab]	6.49[b]	0.004	0.02	0.07	<0.01	0.68	0.29	0.41
Relative rate of decline (/d)[3]	-0.0046[a]	-0.0039[b]	-0.0035[b]	0.0002	<0.01	0.80	<0.01	0.65	0.70	0.83

[a-c] Values within transition treatment with different superscript letters differ ($P < 0.05$).[1]DP = Dry period length, R = ration, P = parity. [2]*a, b, c, S* represent initial fat and protein corrected milk (FPCM) yield (kg/d), the increasing slope, the decreasing slope, and persistency according to Wood's lactation curve, respectively. [3]Relative rate of decline midway between peak lactation and 44 weeks in lactation calculated according to Dijkstra et al. (2010).

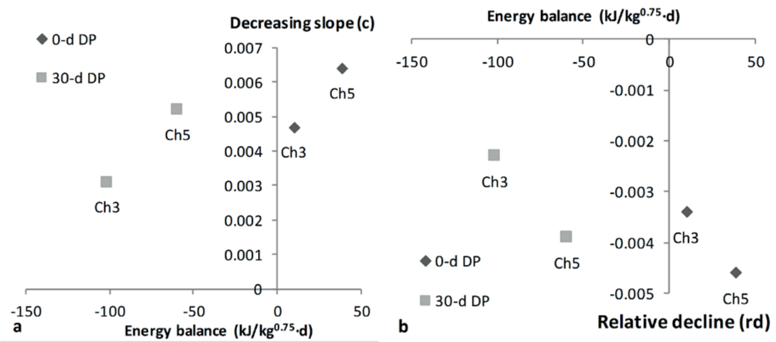

Figure 7.2: Relationship between energy balance and the decreasing slope of the lactation curve [*c*; (**Wood**, 1967)] (Figure a) and the relative rate of decline midway between peak lactation and 44 weeks in lactation (Figure b) (**Dijkstra *et al.***, 2010). Ch3 = Chapter 3 (experiment 1), Ch5 = Chapter 5 (experiment 2).

Table 7.5: Lactation curve characteristics for cows with or without a metabolic disorder or disease in wk 1 – 7 of lactation after a 0-d or 30-d DP. Cows with a 0-d DP were provided either a low [0-d DP(LOW)] dietary energy level, or the standard [0-d DP(STD)] dietary energy level that cows with a 30-d DP received [30-d DP(STD)]

	Disease wk 1 -7		Transition treatment[1]			P – value[2]		
	No	Yes	0-d DP (LOW)	0-d DP (STD)	30-d DP (STD)	D	DP	D × DP
Cows (n =)	53	53	35	34	37			
Total FPCM yield (kg) wk 1 - 44	8947	8520	8131	8252	9818	0.20	<0.01	0.10
b^3	0.20	0.21	0.23	0.23	0.17	0.57	0.04	0.02
c^3	0.0043	0.0041	0.0048[a]	0.0045[a]	0.0033[b]	0.63	<0.01	0.37
S^3	6.64	6.75	6.61	6.74	6.74	0.18	0.27	0.04
rd^4	-0.0032	-0.0030	-0.0036[a]	-0.0033[a]	-0.0024[b]	0.44	<0.01	0.64

[a-b] Values within transition treatment with different superscript letters differ ($P < 0.05$). [1]Transition treatment: Cows with a 0-d DP were fed a glucogenic or lipogenic ration with either a low [0-d DP(LOW)] energy level, based on their requirement for their expected milk yield, or a standard [0-d DP(STD)] energy level based on the expected milk yield of cows with a 30-d DP [30-d DP(STD)]. [2]D = disease in early lactation (milk fever, ketosis, clinical mastitis, retained placenta, metritis, cystic ovaria, left displaced abomasum, lameness), DP = Dry period length. [3]*b*, *c*, *S* represent the increasing slope, decreasing slope and persistency according to Wood's lactation curve. [4]rd = Relative rate of decline midway between peak lactation and 44 weeks in lactation calculated according to Dijkstra et al. (2010).

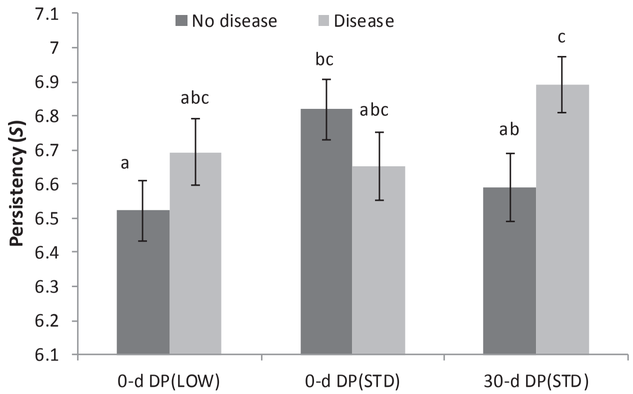

Figure 7.3: Persistency (*S*) for cows with or without disease in wk 1 – 7 of lactation and with different transition treatments (cows with a 0-d DP were fed a glucogenic or lipogenic ration with a low [0-d DP(LOW)] energy level, based on their requirement for their expected milk yield, or a standard [0-d DP(STD)] energy level that cows with a 30-d DP [30-d DP(STD)] received based on the requirement for their expected milk yield).

Dietary energy level

In a previous study on extending the lactation in a pasture-based system, cows with greater energy intake more often dried off spontaneously before the intended drying off day, compared with cows fed a control ration (**Grainger *et al.***, 2009). In contrast, in another study, feeding a dietary energy level below the requirement for milk production also negatively affected persistency (**Chilliard**, 1992). From Chapter 3, it appears that a STD dietary energy levels for cows with a 0-d DP (990 and 1039 kJ/kg$^{0.75}$.d for LOW and STD level, respectively) numerically increased lactation persistency, compared with cows with a 0-d DP fed a LOW energy level. Furthermore, cows with a 0-d DP(STD) without disease in early lactation had greater persistency than cows with a 0-d DP(LOW) without disease in early lactation (**Figure 7.3**). The contrast in dietary energy level between the LOW and STD ration (i.e., 49 kJ/kg$^{0.75}$ .d) for cows with a 0-d DP was possibly too small, due to compensation of low dietary energy level with greater basal ration intake, and number of cows may have been too low to significantly affect lactation curve characteristics in cows with a 0-d DP.

Dietary energy source

Feeding a lipogenic ration was hypothesized to improve lactation persistency, compared with a more glucogenic ration. Previous studies showed that a lipogenic ration in mid- and late- lactation reduced the plasma glucose and insulin concentration compared with a more glucogenic ration (**Voelker and Allen**, 2003; **Mahjoubi *et al.***, 2009). We hypothesized that a lower plasma insulin concentration after peak milk yield stimulates the plasma GH concentration. Plasma GH concentration regulates nutrient partitioning towards milk (**Hart**, 1983; **Lucy**, 2004). Morover, a higher level of endogenous GH (**Sorensen and Knight**, 2002) or administration of exogenous GH (**Van Amburgh *et al.***, 1997; **Bauman**, 1999) was positively related with lactation persistency. In Chapter 3, feeding a glucogenic ration increased plasma IGF-1 concentration for all transition treatments, increased plasma insulin concentration in cows with a 0-d DP(STD), and decreased plasma GH concentration in cows with a 0-d DP(STD) or 30-d DP(STD). The greater plasma IGF-1 concentration in cows fed a glucogenic ration was related with a greater DMI and energy intake, compared with cows fed a lipogenic ration. A greater plasma insulin and IGF-1, and lower GH concentration can be expected to result in fewer nutrients partitioned towards the mammary gland, resulting in a reduced milk yield (**Hart**, 1983) and reduced lactation persistency. However, in

Chapter 3, cows fed a glucogenic ration had greater DMI and milk yield than cows fed a lipogenic ration, while lactation curve characteristics were not affected by ration. Thus, cows with a glucogenic or lipogenic ration had similar nutrient partitioning to body reserves. The lack of effect of dietary energy source on lactation persistency could be related with introduction of ration contrast in wk 8, and not from calving onwards. However, a previous study showed that feeding a G or L concentrate from calving to 100 DIM did not affect lactation persistency (**Chen *et al.***, 2016a). Another explanation may be that the lipogenic ration, compared with a more glucogenic ration, resulted in a lower DMI, energy intake, as well as less glucogenic precursors, which decreased synthesis of lactose (**Chapter 3**), the major osmotic component for milk production (**Davis and Collier**, 1985). In conclusion, feeding a lipogenic ration during wk 8 – 44 of lactation did not affect lactation curve characteristics, compared with feeding a more glucogenic ration, which is possibly due to similar nutrient partitioning to body reserves with either ration.

7.3.2 Conclusions

A 30-d DP improved lactation persistency, compared with a 0-d DP. Disease in early lactation further improved persistency in cows with a 30-d DP. In cows with a 0-d DP without disease, feeding a greater energy level improved persistency, compared with feeding an energy level according to expected milk yield. Although a lipogenic ration reduced the plasma insulin concentration and increased the plasma GH concentration, a lipogenic ration did not affect lactation persistency, compared with a more glucogenic ration. A lipogenic ration with greater DM content and palatability may have resulted in similar DMI, and may have resulted in greater lactation persistency, compared with a more glucogenic ration.

7.4 Behavior and metabolic status

Diseases in early lactation in dairy cows are often related with a negative EB (**Collard *et al.***, 2000; **Butler**, 2003; **Ingvartsen *et al.***, 2003). Plasma metabolites and hormones can be an indicator for metabolic status, but require an invasive method for collection than behavioral observations. Previous studies reported that cows with a lower plasma FFA concentration had greater walking activity postpartum, compared with a greater plasma FFA concentration (**Adewuyi *et al.***, 2006) and that cows with ketosis had lower feed intake, feeding time, meal time, and less meals and visits than cows without ketosis (**González *et al.***, 2008). To our knowledge, the relation between behavior and other plasma metabolites and hormones was not described previously. In Chapter 4 we aimed to analyze the relation of metabolic status, based on plasma metabolites, with feeding behavior and daily motion and steps of dairy cows at week 4 postpartum after a 0-d or 30-d DP. This part of the general discussion will elaborate on behavioral indicators for metabolic status that may be relevant for metabolic status.

7.4.1 Indicators for metabolic status

Behavioral and plasma indicators for metabolic status

In Chapter 4, cows were clustered for metabolic status based on plasma FFA, BHB, glucose, insulin, IGF-1, and GH concentration which resulted in groups of cows with a good, average, poor, or very good metabolic status. Cows with good or very good metabolic status had lower milk yield and a greater EB. Good metabolic status was associated with greater dry matter intake (**DMI**), basal ration intake, more visits and meals, and more time spent lying, compared with a poor metabolic status. In Chapter 4, lying time, but not feeding behavior, had a positive, monotonous relation with metabolic status. Lying time may, therefore, be a reliable indicator for metabolic status.

Milk yield and EB do not necessarily hold a linear relationship, as it was previously reported that cows with similar milk yield had different EB (**Ingvartsen *et al.***, 2003; **McGuire *et al.***, 2004). It could be hypothesized that differences in EB, but not milk production, would result in differences in plasma metabolites that could be reflected in different feeding or lying behavior, or steps. To evaluate this hypothesis, data from Chapter 4 was used in which cows were paired for milk yield and rated (binary) for high or low EB in week 4 of lactation. Cows with a low EB had a greater plasma FFA concentration than cows with a greater EB (**Table 7.6**). This is in concordance with previous studies, that report on the strong relation between EB and plasma FFA concentration, as greater plasma FFA concentration is a reflection of body reserve mobilization due to energy shortage, i.e. negative EB (**Bell**, 1995; **Adewuyi *et al.***, 2005). In contrast to my hypothesis, plasma BHB, glucose, insulin, IGF-1, and GH concentration were not different for EB groups when milk yield was similar. Differences in behavior were present for cows with different EB but similar milk yield. Cows with a low EB had a lower DMI, basal ration intake, and lying time than cows with a greater EB.

Differences in DMI and lying time related with EB from this analysis are not due to difference in milk yield, are therefore unlikely related with differences in udder pressure (**Bertulat *et al.***, 2017), but could be related with social dominance (**Grant and Albright**, 1995) because in experiment 1, every 2 cows shared a feed trough. In a previous study, multiparous cows, but not primiparous cows, fed in a competitive setting had a greater feeding rate and shorter meal time due to more displacements, and spent more time standing-not-feeding postpartum, compared with cows fed in a non-competitive setting (**Proudfoot *et al.***, 2009). In my study, feeding rate or meal time were not affected, which makes competition at the feed bunk a less likely explanation for difference in DMI and lying time between cows with a low or high EB. It was previously reported that DMI varies among cows (**Drackley**, 1999), that low DMI and shorter lying time are likely related with discomfort and the challenge of physiological and social changes, including dominance, around transition (**Huzzey *et al.***, 2005), and individual differences in resilience to adapt to these challenges (**Aleri *et al.***, 2016). Although underlying mechanisms are unclear, results from this analysis support our conclusion from Chapter 4 that lying time and DMI are related with metabolic status and EB regardless of milk production level.

Table 7.6: Feeding and lying behavior, and steps for cows with a high or low energy balance but similar FPCM yield in wk 4 postpartum

	Energy balance group			P-value
	Low	High	SEM	EB group
Cows	40	40		
Dry matter intake (kg DM/d)	21.1	22.9	0.3	<0.01
Basal ration intake (kg DM/d)	14.1	16.0	0.3	<0.01
Feeding rate (kg/min)	0.21	0.23	0.01	0.15
Meals (n/d)	7.63	7.67	0.19	0.63
Visits (n/d)	28.1	30.0	1.6	0.41
Meal time (min/d)	212	228	8	0.16
Lying time (h/d)	10.8	11.8	0.3	0.02
Lying bouts (n/d)	11.7	13.0	0.6	0.11
Steps (n/d)	1213	1258	52	0.54
Motion	4849	5172	212	0.28
FFA (mmol/L)[1]	0.19 (0.15 – 0.25)	0.11 (0.09 – 0.14)		<0.01
BHB (mmol/L)[1]	0.76 (0.65 – 0.89)	0.67 (0.57 – 0.78)		0.24
Glucose (mmol/L)	3.68	3.85	0.07	0.13
Insulin (μU/mL)	13.6	15.0	1.5	0.48
IGF-1 (ng/mL)[1]	114	122	7	0.42
GH (μg/L)[1]	4.35 (3.76 – 5.03)	4.00 (3.44 – 4.65)		0.43
FPCM (kg/d)[1]	37.2	37.2	1.0	0.96
EB (kJ/ $kg^{0.75}$·d)[1]	-128	-31	27	0.01

[1]FFA = Free fatty acids, BHB = β-hydroxybutyrate, IGF-1 = insulin-like growth factor 1, GH = growth hormone. FFA, BHB, and GH were log transformed for analyses, but are shown as actual values with confidence interval, FPCM = fat-and-protein corrected milk. EB = energy balance.

7.4.2 Conclusions

Cows with a good metabolic status had lower milk yield and greater EB, and had greater DMI, basal ration intake, more visits and meals, and more time spent lying, compared with a poor metabolic status. Cows with lower EB, but similar milk yield, had greater plasma FFA concentration, although other plasma metabolites and hormones were not affected. Cows with lower EB, but similar milk yield, had lower DMI and lying time than cows with a greater EB. Differences in DMI and lying time may indicate differences in metabolic status or EB.

7.5 Udder health

Until now, effects of DP length on udder health are not fully clear. Studies showed contrasting results of DP length on SCC, new intramammary infections (**IMI**) and clinical mastitis postpartum of cows (**Gulay *et al.***, 2003; **Rastani *et al.***, 2005; **Kuhn *et al.***, 2006; **Pezeshki *et al.***, 2007; **Church *et al.***, 2008; **Watters *et al.***, 2008; **Bernier-Dodier *et al.***, 2010; **Pinedo *et al.***, 2011; **Steeneveld *et al.***, 2013; **Shoshani *et al.***, 2014). Chapter 5 of this thesis focused on udder health, more specifically SCC, elevations of SCC and cases of clinical mastitis, of cows subjected to a 0-d, 30-d or 60-d DP, including dry cow antibiotics for cows with a 30-d or 60-d DP. In line with our study in Chapter 5, in all previous studies on the effect of DP length on udder health, cows with a DP were treated with dry cow antibiotics (**Gulay *et al.***, 2003; **Rastani *et al.***, 2005; **Kuhn *et al.***, 2006; **Pezeshki *et al.***, 2007; **Church *et al.***, 2008; **Watters *et al.***, 2008; **Bernier-Dodier *et al.***, 2010; **Pinedo *et al.***, 2011; **Santschi *et al.***, 2011; **Steeneveld *et al.***, 2013; **Shoshani *et al.***, 2014). Therefore DP length and use of antibiotics are confounded in these studies. Moreover, recently, preventive use of antimicrobials in the EU has been restricted (**EC**, 2/2015 299/04). Therefore, in Chapter 6, udder health was studied in cows subjected to a 0-d or 30-d DP without use of dry cow antibiotics. In Chapter 6, we aimed to compare the effect of a 0-d or a 30-d DP without use of dry cow antibiotics on udder health during the peripartum period, and in the subsequent lactation and to evaluate relationships between udder health and metabolic status of dairy cows.

Chapter 5 also aimed to determine cow characteristics in the previous lactation that determine udder health of cows after a 0-d, 30-d or 60-d DP. In previous studies, postpartum udder health was related with cow characteristics including parity, calving month, occurrence of clinical mastitis in the previous lactation, SCC before drying off, and milk yield during the days before drying off (**Green *et al.***, 2007; **Pantoja *et al.***, 2009).

This part of the general discussion will elaborate on 1) what management measures affect udder health of cows with a 0-d or 30-d DP treated with or without dry cow antibiotics, 2) cow characteristics relevant for effects of DP length on udder health across the DP and in the subsequent lactation, 3) what other management factors can be relevant for udder health of cows with different DP lengths.

7.5.1 Optimizing management

Dry period length

In Chapter 5 and 6, cows with a 0-d DP had a greater SCC in wk 1 – 44 postpartum than cows with a 30-d DP, and in Chapter 5 cows with a 30-d DP had a greater postpartum SCC than cows with a 60-d DP. In Chapter 5 and 6 we discussed that differences in postpartum SCC among DP lengths could be related with 1) use of dry cow antibiotics, 2) lower milk yield postpartum (**Steeneveld *et al.***, 2013), 3) increase in IMI across the DP or precalving period, and 4) increase in IMI in the subsequent lactation.

In Chapter 6, but not in Chapter 5, the difference in postpartum SCC between cows with a 0-d and 30-d DP was not present anymore when postpartum milk yield (kg/d) was taken into account. In Chapter 5, cows with a 30-d or 60-d DP were treated with dry cow antibiotics, which was related with less variation in postpartum SCC (CV% LnSCC 9.49 in Chapter 5 vs. 29.04 in Chapter 6). The low variation in SCC in Chapter 5, possibly related with use of dry cow antibiotics, left no variation for the effect of differences in milk production on postpartum SCC, which may have been present if no dry cow antibiotics had been used. In a previous study on effects of different DP lengths including use of dry cow antibiotics, SCC did not differ anymore among DP lengths when postpartum milk yield was taken into account (**Steeneveld *et al.***, 2013) This study was, however, based on a larger field study and included more cows than Chapter 5.

In the peripartum period, the proportion of cows with a chronic IMI was greater, and the cure of existing IMI was lower for cows with a 0-d DP, than for cows with a 30-d or 60-d DP in Chapter 5, but was not different between cows with a 0-d or 30-d DP in Chapter 6. In both Chapter 5 and 6, the proportion of cows with a new or no IMI across the DP was not different among DP length groups. These results suggest that use of dry cow antibiotics is relevant for cows with an existing IMI at the beginning of the DP, but has no preventive effect for cows without an IMI at the beginning of the DP.

Previous studies reported no difference in mastitis incidence in cows with a shortened (**Pezeshki *et al.***, 2007; **Watters *et al.***, 2008; **Santschi *et al.***, 2011), or omitted DP (**Rastani *et al.***, 2005), compared with cows with a conventional DP length. In concordance with these studies, in Chapter 5 and 6, the postpartum occurrence of at least 1 elevation of SCC, the number of elevations per affected cow, and the number of cases of clinical mastitis per affected cow were not different among DP lengths. However, the postpartum proportion of cows with at least 1 case of clinical mastitis tended to be lower in cows with a 30-d DP (18%), compared with a 0-d DP (33%) in Chapter 6, while there was no difference in the proportion of cows with at least 1 case of clinical mastitis (27, 25, and 25% for cows with a 0-d, 30-d, or 60-d, respectively) in Chapter 5. Furthermore, cows with a 0-d DP fed the energy level for the expected milk yield of cows with a 30-d DP tended to have a greater hazard for a case of clinical mastitis in the subsequent lactation than cows with a 30-d DP. The numerical, but not statistical, difference in occurrence of clinical mastitis between cows with a 0-d or 30-d DP in Chapter 5 and 6 could be hypothesized to be related with the low number of affected cows in either study, the different period in time in which experiments for Chapter 5 and 6 were conducted which also implies different cows the herd and change from blanket to selective use of dry cow antibiotics in the herd. Over time, and especially due to change from blanket to selective use of dry cow antibiotics, presence of different udder pathogens in the herd may have changed (**Zadoks and Fitzpatrick**, 2009). Additionally, no DP is associated with a lower renewal of epithelial and parenchymal udder cells, which may decrease integrity and defense of udder tissue and may, to some extent, interact with abovementioned environmental and management factors. In conclusion, SCC was greater in cows with a 0-d DP compared with a 30-d DP, although no difference in IMI across the DP were observed. Furthermore, in Chapter 6, but not in Chapter 5, a 0-d DP tended to increase the hazard for clinical mastitis in the subsequent lactation. Larger field studies are needed to evaluate effects of DP length on clinical mastitis in the subsequent lactation.

7.5.2 Cow characteristics that determine udder health in the subsequent lactation after different dry period lengths

Cow characteristics in the previous lactation that may affect udder health in the subsequent lactation were identified in Chapter 5 and are here in the general discussion also analyzed for the experiment in Chapter 6. First, cow characteristics associated with SCC in the subsequent lactation are discussed, followed by cow characteristics associated with elevations of SCC and cases of clinical mastitis in the subsequent lactation. Cow characteristics parity and milk yield in previous late lactation are discussed in more detail.

Cow characteristics that determine SCC after different dry period lengths

In Chapter 5, postpartum SCC was related with cow characteristics parity, elevations of SCC in the previous lactation, last FPCM before a 60-d DP, average SCC between 150 and 37 days prepartum, and average SCC for lactation, and included interactions of these factors with DP length (**Table 7.7**). For this general discussion, similar analysis was performed for Chapter 6. In Chapter 6, postpartum SCC was associated with cow characteristics DP length, parity, average SCC for lactation, and SCC and milk yield between 150 and 37 days before the expected calving date (**Table 7.7**). In contrast to cow characteristics from Chapter 5, in Chapter 6 no interactions were present between these prepartum cow characteristics and DP length. Comparing these two Chapters, it could be hypothesized that the presence of interactions with DP length in Chapter 5 is related with use of dry cow antibiotics during the DP, rather than with DP length itself. Dry period length, including use of dry cow antibiotics in Chapter 5 was associated with a better cure rate of existing IMI across the DP in cows with a 30-d DP (92%) than cows with a 0-d DP (50%), while no difference in cure of IMI between cows with a 0-d DP (62%) or 30-d DP (64%) was observed without use of dry cow antibiotics in Chapter 6. Overall, in both experiments, parity and average SCC in the previous lactation were cow characteristics that determined the effect of a 0-d or 30-d DP on udder health in the subsequent lactation.

In Chapter 6, cows were not treated with dry cow antibiotics, while postpartum udder health may have been better after application of dry cow antibiotics to cure existing IMI in cows with a high SCC, as previously suggested in §7.5.1. Among farms, postpartum SCC after selective use of DCT was greater (**Rajala-Schultz *et al.***, 2011; **Scherpenzeel *et al.***, 2014), or not different (**Rajala-Schultz *et al.***, 2011; **Cameron *et al.***, 2015) compared with blanket use of dry cow antibiotics. Dutch legislation only allows selective use of dry cow antibiotics in cows with postpartum parity 2 with prepartum SCC ≥150.000 cells/mL and in cows with postpartum parity ≥3 with prepartum SCC ≥50.000 cells/mL (**KNMvD**, 2013; **Dutch M. o E. a.**, 2017). In Chapter 6, 57% of cows with a 30-d DP and 52% of cows with a 0-d DP had a prepartum SCC above these thresholds, but were not treated with dry cow antibiotics. In cows with a prepartum SCC above the threshold, a 30-d DP without use of dry cow antibiotics reduced SCC across the DP, compared with a 0-d DP (**Figure 7.4a-b).**

Table 7.7: The multivariable model with cow characteristics that determine postpartum SCC between week 1 and 44 of lactation of cows different dry period lengths in Chapter 5 and 6. Values represent LSM ± SEM or regression coefficient (β) with standard error

Prepartum variable	Category	Postpartum SCC[1,2] Chapter 5 LSMEANS (SEM)	*P*-value	Postpartum SCC[1,2] Chapter 6 LSMEANS (SEM)	*P*-value
Dry period length (DP)[3]	0	5.03 (0.08)	0.52	5.11 (0.23)	<0.01
	30	4.56 (0.09)		4.72 (0.24)	
	60	4.49 (0.09)		N.p.	
Postpartum parity[4]	Parity 2	4.61 (0.09) [a]	<0.01	4.79 (0.24)	0.01
	Parity 3	4.58 (0.08) [a]		5.04 (0.23)	
	Parity >3	4.88 (0.09) [b]		N.p.	
DP × Parity	0 × 2	5.02 (0.10) [a]	<0.01	N.p.	
	0 × 3	4.77 (0.10) [b]		N.p.	
	0 × >4	5.30 (0.11) [c]		N.p.	
	30 × 2	4.42 (0.10) [d]		N.p.	
	30 × 3	4.56 (0.11) [d,e]		N.p.	
	30 × >4	4.69 (0.11) [e]		N.p.	
	60 × 2	4.40 (0.13) [d]		N.p.	
	60 × 3	4.42 (0.10) [d]		N.p.	
	60 × >3	4.65 (0.11) [e]		N.p.	
Elevation of SCC[5]	No	4.62 (0.08) [a]	0.02	N.p.	
	Yes	4.77 (0.09) [b]		N.p.	
DP × Elevation of SCC	0 × No	4.76 (0.09) [a]	<0.01	N.p.	
DP × Elevation of SCC	0 × Yes	5.30 (0.10) [b]		N.p.	
DP × Elevation of SCC	30 × No	4.64 (0.10) [a]		N.p.	
DP × Elevation of SCC	30 × Yes	4.47 (0.11) [c]		N.p.	
DP × Elevation of SCC	60 × No	4.46 (0.09) [c,d]		N.p.	
DP × Elevation of SCC	60 × Yes	4.52 (0.11) [a,d]		N.p.	

[a-e]Values with different superscript letters differ ($P < 0.05$). [1]SCC was analyzed as the natural logarithm of SCC. [2]SCC was monitored between2 and 44 weeks in lactation. [3]In Chapter 5 cows had a 0-d, 30-d, or 60-d DP. Cows with a 30-d or 60-d DP were treated with dry cow antibiotics. In Chapter 6, cows had a 0-d or 30-d DP and cows were not treated with dry cow antibiotics. [4]For Chapter 6, parity classes were postpartum parity 2 and postpartum parity ≥3. [5]An elevation of SCC in milk was defined as SCC in milk ≥200,000 cells/mL after two previous weeks with SCC<200,000 cells/mL. [6]Average SCC for lactation is shown as SCC. N.s. = not significant. N.p. = not present.

7

Continuation of table 7.7

Prepartum variable	Category	Postpartum SCC[1,2] Chapter 5		Postpartum SCC[1,2] Chapter 6	
		β (SE)	*P*-value	β (SE)	*P*-value
Last FPCM before 67-d DP (kg/d)	Average 25	-0.19 (0.04)	<0.01	N.p.	
Last FPCM × SCC[1]		0.03 (0.01)	<0.01	N.p.	
Average FPCM yield between 150 - 37d (kg/d)		N.p.		-0.08 (0.05)	0.14
FPCM reduction 150 – 67-d (kg)	Average -4.77	-0.06 (0.02)	<0.01		
Average SCC[1] between 150 – 37-d		N.p.		0.21 (0.09)	0.02
DP × FPCM reduction (kg)	0	0.03 (0.01) [a,b]	<0.04	N.p.	
DP × FPCM reduction (kg)	30	0.05 (0.02) [a]		N.p.	
DP × FPCM reduction (kg)	60	Ref [b]		N.p.	
Average SCC for lactation[1]	Average[6] 113	-0.70 (0.26)	0.05	-0.31 (0.35)	0.35
Average FPCM between 150 – 37-d × Average SCC[1] for lactation		N.p.		0.023 (0.012)	0.03
Parity × average SCC for lactation[1]	>3	Ref [a]		N.p.	
DP × Parity × average SCC for lactation[1]	0 × 2	0.18 (0.27)	<0.01	N.p.	
DP × Parity × average SCC for lactation[1]	0 × 3	0.02 (0.27)		N.p.	
DP × Parity × average SCC for lactation[1]	30 × 2	-0.08 (0.28)		N.p.	
DP × Parity × average SCC for lactation[1]	30 × 3	-0.89 (0.29)		N.p.	
DP × Parity × average SCC for lactation[1]	0 × >3, 30 × >3, 60 × 2, 60 × 3, 60 × >3	Ref		N.p.	

[a-e]Values with different superscript letters differ ($P < 0.05$). [1]SCC was analyzed as the natural logarithm of SCC. [2]SCC was monitored between2 and 44 weeks in lactation. [3]In Chapter 5 cows had a 0-d, 30-d, or 60-d DP. Cows with a 30-d or 60-d DP were treated with dry cow antibiotics. In Chapter 6, cows had a 0-d or 30-d DP and cows were not treated with dry cow antibiotics. [4]For Chapter 6, parity classes were postpartum parity 2 and postpartum parity ≥3. [5]An elevation of SCC in milk was defined as SCC in milk ≥200,000 cells/mL after two previous weeks with SCC<200,000 cells/mL. [6]Average SCC for lactation is shown as SCC. N.p. = not present.

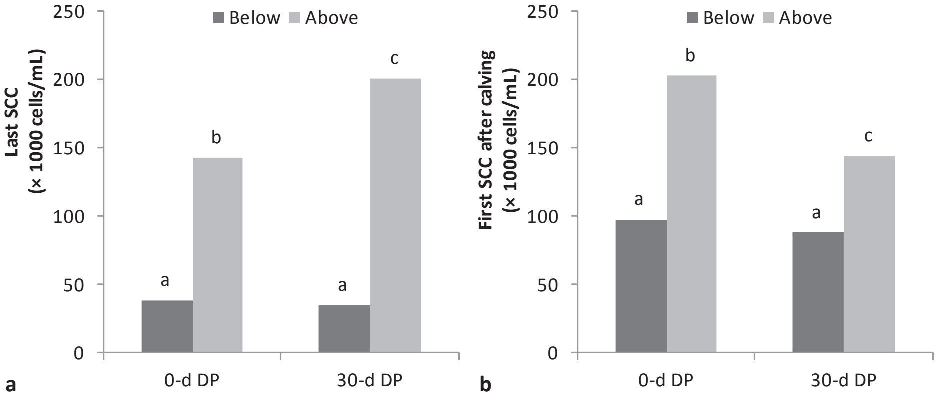

Figure 7.4: Last SCC prior to 37 days before calving (a) and first SCC after calving (after 10 DIM) (b) for cows assigned to a 0-d or 30-d DP with a prepartum SCC below the threshold for dry cow antibiotics (parity 2: <150.000 cells/mL, parity ≥3: <50.000 cells/mL; dark grey) or above the threshold (light grey). [a – c]Values with different superscripts differ ($P < 0.05$).

Cow characteristics that determine elevations of SCC and clinical mastitis after different dry period lengths

In Chapter 5, the cow characteristic that determined at least 1 elevation of SCC in the subsequent lactation was parity. A similar analysis was performed for Chapter 6, and cow characteristics that determined postpartum at least 1 elevation of SCC included parity and average SCC between 150 and 37d prepartum (**Table 7.8**). Effects of parity on udder health will be discussed below. In Chapter 6, cows with a greater SCC between 150 and 37 days prepartum had a greater odds for at least 1 elevation of SCC postpartum than cows with a lower SCC, which is in line with previous studies that identified a high SCC before drying off (≥200,000 cells/mL) as a risk factor for high SCC in the subsequent lactation (**Green *et al.***, 2008; **Rajala-Schultz *et al.***, 2011).

In Chapter 5, the cow characteristic that determined at least 1 case of clinical mastitis was the last SCC at 67d before the expected calving date, which is in line with previous studies (**Green *et al.***, 2007; **Pantoja *et al.***, 2009). A similar analysis was performed for Chapter 6, and cow characteristics that determined at least 1 case of clinical mastitis included DP length and prepartum incidence of clinical mastitis (**Table 7.8**). Clinical mastitis before drying off was previously reported as a risk factor for postpartum clinical mastitis (**Green *et al.***, 2002).

Table 7.8: Multivariable models with prepartum cow characteristics that determine occurrence of at least 1 elevation of SCC or 1 case of clinical mastitis between week 1 and 44 postpartum for cows after a 0-d or 30-d dry period in Chapter 6. (Incidence or odds ratio (OR) with confidence interval)

		Postpartum variables							
		Chapter 5				Chapter 6			
		Elevation of SCC[1,2]		Clinical mastitis[2]		Elevation of SCC[1,2]		Clinical mastitis[2]	
Prepartum variable	Category	Incidence	*P*-value	Incidence	P-value	Incidence	*P*-value	Incidence	P-value
Dry period length	0	79%	0.99	27%	0.99	54%	0.72	33%	0.03
	30	78%		25%		51%		18%	
	60	79%		25%					
Postpartum parity[3]	Parity 2	67%	0.03	13%	0.56	41%	0.02	19%	0.10
	Parity 3	83%		30%		64%		35%	
	Parity ≥3	87%		35%					
		OR		OR		OR		OR	
Last SCC[1] before 67-d prepartum	Average[4] 102			2.04 (1.22 – 3.43)	<0.01				
Average SCC[1] between 150 – 37-d prepartum	Average[4] 66					1.93 (1.19 – 3.16)	<0.01		
At least 1 case of clinical mastitis in the previous lactation								3.00 (1.22 – 8.03)	0.03

[1]SCC was analyzed as the natural logarithm of SCC. [2]An elevation of SCC or clinical mastitis was analyzed as at least 1 elevation or at least 1 case of clinical mastitis in the full lactation (0 or 1). A postpartum elevation was defined as an elevation ≥200,000 cells/mL after two weeks SCC < 200.000 cells/mL. [3]For Chapter 6, parity classes were postpartum parity 2 and postpartum parity ≥3. [4]Average SCC for lactation is shown as SCC.

Green et al. (2002) also reported that 90% of quarters with clinical mastitis within 150 DIM, was related with a major mastitis pathogen earlier identified during the DP (**Green *et al.***, 2002). In Chapter 6, most cases of clinical mastitis in cows with a 0-d or 30-d DP occurred in early lactation (42% in wk 1 – 7, **Figure 7.5**) and likely originate from an infection already present during the dry period, although pathogens were not cultured in this study.

In Chapter 6, omission of dry cow antibiotics for cows with a 30-d DP may have increased the odds for a postpartum elevation of SCC or case of clinical mastitis, as an existing IMI, marked by high SCC, or a case of clinical mastitis in the previous lactation may not have cured over the DP (**Green *et al.***, 2008). The proportion of cows with chronic IMI (SCC ≥200,000 cells/mL) across the DP was lower in cows with a 30-d DP treated with dry cow antibiotics in Chapter 5 (8%) than in cows with a 30-d DP not treated with dry cow antibiotics in Chapter 6 (36%). Bacterial infections with mastitis pathogens across the DP may be resolved through use of dry cow antibiotics. Cows with aforementioned risk factors for an elevation of SCC or a case of clinical mastitis postpartum should be properly diagnosed and treated with the designated antibiotic treatment, including a DP matching the withdrawal period.

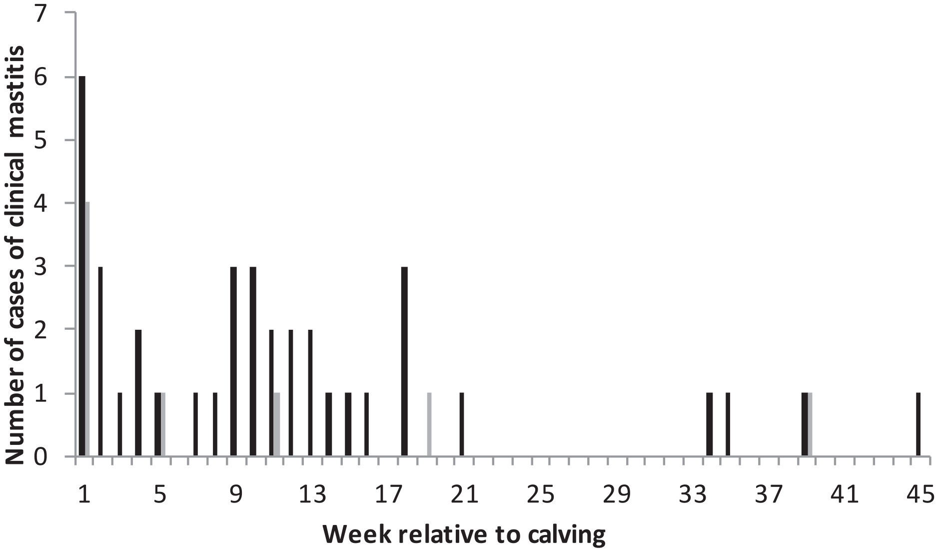

Figure 7.5: Occurrence of a case of clinical mastitis per week relative to calving for cows after a 0-d (black; n = 81 cows) or 30-d (gray; n = 42 cows) dry period.

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Parity

Chapter 5 and Chapter 6 discussed that SCC and the incidence of at least 1 elevation of SCC or at least 1 case of clinical mastitis was lower in cows with postpartum parity 2 than in cows with greater parity (**Figure 7.6**). Previous studies also showed that low parity cows have better udder health than greater parity cows (**Schepers *et al.***, 1997; **Green *et al.***, 2007), which may be related with the greater EB (Chapter 2), with a greater plasma IGF-1 (Chapter 2 and 4) and lower plasma FFA concentration (Chapter 2), and a lower immunosuppressive effect of plasma FFA's on neutrophils (**Ingvartsen and Moyes**, 2013) in low parity cows than in greater parity cows. Lower daily milk yield in young cows also reduces the risk of damage to the teat sphincter and pathogens entering the teat canal, and leaves more of the bacteriostatic keratin lining of the teat canal in place which has antibacterial activity and traps invading pathogens (**Suriyasathaporn *et al.***, 2000; **Ingvartsen *et al.***, 2003). Furthermore, presence of high SCC (≥ 200,000 cells/mL) in the previous late lactation increases the risk for clinical mastitis in the following lactation (**Green *et al.***, 2007; **Pantoja *et al.***, 2009). Cows with greater parity have experienced a period of late lactation more often, have a greater daily milk yield throughout lactation that may damage the teat sphincter or keratin lining and, therefore, may have a greater risk for IMI in late lactation and thus a greater risk for IMI in the subsequent lactation than cows with lower parity. Due to the greater risk for IMI in cows with greater parity, these cows may more often need treatment with dry cow antibiotics with a matching DP length than cows with lower parity.

Milk yield in previous late lactation associated with postpartum udder health

In Chapter 5 and 6, a large FPCM reduction in late lactation and lower FPCM yield at the last test-day prior to 67-d before calving (last FPCM) (Chapter 5) or prior to 37-d before calving (Chapter 6) was associated with increased incidence of IMI prepartum (SCC ≥ 200,000 cells/mL at the last test-day before the conventional drying off day). **Table 7.9** shows that in both Chapter 5 and 6, the prepartum FPCM reduction was greater and the last FPCM was lower for cows with a prepartum IMI than for cows without a prepartum IMI ($P < 0.05$, for all analyses). An existing IMI prepartum negatively affects secretory capacity of udder cells and milk yield (**Benites *et al.***, 2002). A greater reduction of milk yield or low FPCM yield in late lactation may, therefore, indicate an IMI. Moreover, in Chapter 6, a lower average FPCM yield between 150 and 37 days prepartum was related with a greater SCC postpartum regardless of DP length (previously reported in **Table 7.7**), which was likely related with an uncured IMI across the DP.

Cows with high daily milk yield in late lactation, drying off may be difficult without distress and impaired animal welfare, and should therefore be discouraged (**Leitner *et al.***, 2007; **Zobel *et al.***, 2013). High milk yield hampers formation of a bacteriostatic keratin plug in the first week of the DP (**Capuco *et al.***, 1990) and was related with more open teat ends (**Dingwell *et al.***, 2004). Open teat ends leave a port of entry for mastitis pathogens (**Dingwell *et al.***, 2001) and exhibit a risk for obtaining a new IMI during the DP (**Dingwell *et al.***, 2004; **Rajala-Schultz *et al.***, 2005; **Green *et al.***, 2008). High milk yield in late lactation can be reduced using a prolactin inhibitor, feeding a ration low in energy, or less frequent milking. Prolactin inhibitors, such as Cabergoline or Quinagolide, reduced milk yield, milk leaking, and udder pressure after abrupt drying off, compared with untreated cows (**Lacasse *et al.***, 2011; **Ollier *et al.***, 2014; **Boutinaud *et al.***, 2016; **Bertulat *et al.***, 2017). At this point in time, the use of Cabergoline for drying off cows is, however, not allowed in the EU due to adverse effects in cows, including recumbency and death (**EC**, 2016; **EMA**, 2016).

In Chapter 5 and 6, in order to reduce milk yield in the week before drying off, cows were fed a dry cow ration from 7 days before the drying off day and were gradually ceased through milking once daily from 4 days before the drying off day till drying off. In previous studies, gradual cessation of milk before drying off decreased or did not affect IMI across the DP or SCC in the subsequent lactation (**Gott *et al.***; **Newman *et al.***, 2010). Compared with a lactation ration, feeding a dry hay ration before drying off was associated with a lower prolactin concentration, which facilitate drying off, but also with a lower plasma glucose, and greater FFA and BHBA concentration (**Ollier *et al.***, 2014). A greater plasma FFA concentration was previously associated with decreased neutrophil activity (**Suriyasathaporn *et al.***, 2000) which increases the risk for an IMI and elevation of SCC (**Ingvartsen and Moyes**, 2013). In Chapter 5 and 6, feeding a dry cow ration and gradual cessation decreased milk yield reduced to < 10 kg/d at the end of the drying off week for cows with a 30-d or 60-d DP (**Figure 7.7**). Milk yield at the end of the drying off week did not affect postpartum SCC, or postpartum occurrence of at least 1 elevation of SCC or at least 1 case of clinical mastitis. Gradual cessation supports milk yield reduction and closure of teat ends (**Dingwell *et al.***, 2004) and, thereby, reduces the risk of high SCC and clinical mastitis in early lactation postpartum (**Green *et al.***, 2007, 2008).

Table 7.9: Reduction in fat-and-protein corrected milk (FPCM) yield and last FPCM prior to 67-d before calving (Chapter 5) or prior to 37-d before calving (Chapter 6) for cows with or without an IMI (SCC ≥ 200,000 cells/mL at the last test-day before the conventional drying off day)

	Chapter 5			Chapter 6		
	No IMI	IMI	*P* - value	No IMI	IMI	*P* - value
FPCM reduction between 150 and 67-d (Ch5) or 37-d (Ch 6) prepartum (kg/d)	4.4 ± 0.4	6.2 ± 0.7	0.03	5.2 ± 0.5	11.5 ± 1.0	<0.01
Last FPCM (kg/d)	25.3 ± 0.5	22.5 ± 0.9	0.03	24.3 ± 0.5	18.1 ± 1.0	<0.01

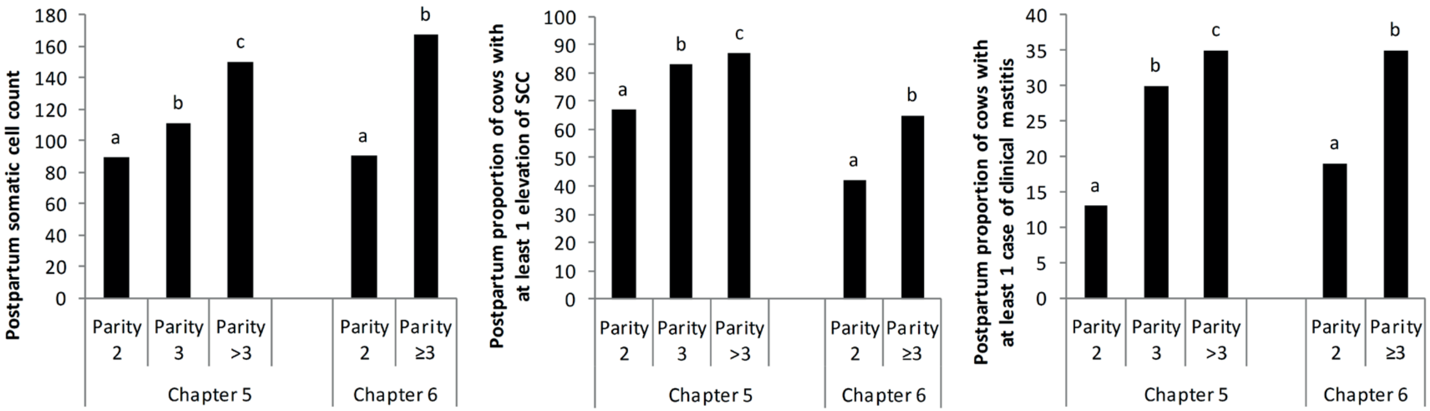

Figure 7.6: Somatic cell count (SCC), proportion of cows with at least 1 elevation of SCC, and proportion of cows with at least 1 case of clinical mastitis for cows with different postpartum parity in Chapter 5 and Chapter 6. [a-c]Values within Chapter with different superscript letters differ ($P < 0.05$).

7.5.3 Other management factors relevant for udder health of cows for a 0-d or 30-d DP and use of dry cow antibiotics

In addition to the management factors studied in this thesis, it can be hypothesized that use of teat sealant or teat dip can be relevant for udder health of cows with different DP lengths. Preventive use of teat sealant in cows with a low SCC reduced the number of new IMI across the DP and cases of clinical mastitis postpartum, compared with cows not treated with teat sealant (**Rabiee and Lean**, 2013). Daily teat-end dip during the DP reduced the presence of environmental mastitis pathogens on the udder and the incidence of new IMI with these mastitis pathogens (**Whist *et al.***, 2006; **Rabiee and Lean**, 2013).

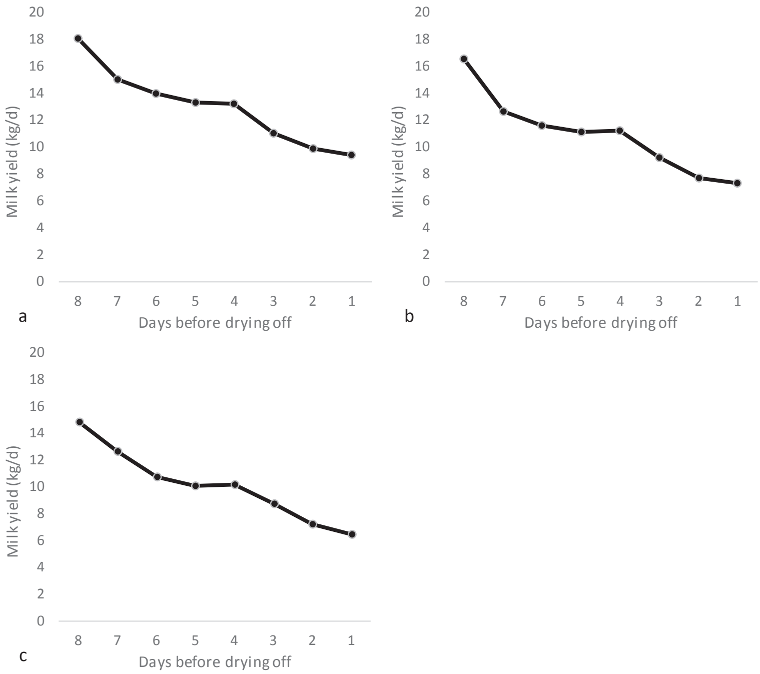

Figure 7.7: Milk yield reduction in the drying off week for cows with a 60-d DP in Chapter 5 (a), a 30-d DP in Chapter 5 (b), or a 30-d DP in Chapter 6 (c).

7.5.4 Conclusions

For cows with different DP lengths, udder health across the DP was not different when no dry cow antibiotics for cows with a DP were used. When dry cow antibiotics were used for cows with a 30-d or 60-d DP, incidence of new IMI decreased across the DP, compared with cows with a 0-d DP. Postpartum SCC was greater in cows with a 0-d DP, which was related with lower milk yield and no use of dry cow antibiotics, but elevations of SCC and cases of clinical mastitis were not different compared with cows with a 30-d DP or 60-d DP. However, cows with a 0-d DP tended to have a greater hazard for clinical mastitis in the subsequent lactation than cows with a 30-d DP. Effects of DP length on clinical mastitis in the subsequent lactation should be further evaluated in larger field studies. Additional to DP length, postpartum SCC, with or without use of dry cow antibiotics during the DP, was also determined by parity and average SCC for the previous lactation. Postpartum clinical mastitis was determined by clinical mastitis in the previous lactation or high SCC before drying off. Cows with average high SCC for previous lactation, high SCC before drying off, or a case of clinical mastitis in the previous lactation should be treated with dry cow antibiotics during a DP length matching the withdrawal period. Cows with a low SCC and no clinical mastitis in the previous lactation, no application of dry cow antibiotics does not have detrimental effects for udder health in the subsequent lactation. Furthermore, a 30-d DP without use of dry cow antibiotics decreased the SCC across the DP, compared with a 0-d DP.

7.6 Applying a Tailored Dry Period

This thesis evaluated if adjustments in dietary energy level or dietary energy source in the subsequent lactation can support cows with a short or no DP to prevent fattening and to increase lactation persistency, what effects different DP lengths, with or without use of dry cow antibiotics, have on udder health, and what cow characteristics determine udder health after different DP lengths. Based on findings from the current and previous studies (**Steeneveld *et al.***, 2014; **Van Knegsel *et al.***, 2014; **Kok *et al.***, 2016), a decision tree was composed that can support farmers in choosing the proper DP management for individual cows, including decisions on DP length (0, 30, or 60 days) and use of dry cow antibiotics. Additional to cow characteristics in the previous lactation that determine udder health in the subsequent lactation, milk yield at the decision moment is of importance for udder health in the subsequent lactation (**Dingwell *et al.***, 2004; **Green *et al.***, 2008). Studies in this thesis did not evaluate effects of different levels of milk yield at the decision moment or the moment of drying off on udder health postpartum. For the decision tree, a threshold of 12 kg/d was chosen as an arbitrary measure for the decision to drying off cows. It can be hypothesized that the decision for a 30-d or 0-d DP, with regard to milk yield and udder health in the subsequent lactation, is only appropriated when milk yield and lactation persistency in the period between the decision moment and the chosen DP length is sufficient. Variables in the decision tree included parity, and last SCC and last daily milk yield before the DP decision. This decision tree was the basis for a mobile application to support farmers in choosing the proper DP management for individual cows.

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The decision tree for DP management was by default programmed to start at 67 days before the expected calving date to account for a drying off week, although farmers could adjust the decision moment according to their management **(Figure 7.8)**. The advice for DP length at the decision moment at 67 days before the expected calving date can imply either application of a 60-d DP or to continue milking till 37 days before the expected calving date. Based on this thesis, application of dry cow antibiotics during a 60-d DP would be appropriate in cows with 1) at least 1 elevation of SCC (SCC ≥ 200.000 cells/mL) or 2) a case of clinical mastitis in the previous lactation or 3) in cows with postpartum parity 2 that have a SCC ≥ 150.000 cells/mL or 4) cows with parity ≥ 3 that have a SCC ≥ 50.000 cells/mL (**KNMvD**, 2013) and likely have an IMI that can be cured with treatment using dry cow antibiotics (**Scherpenzeel *et al.***, 2016), and 5) cows with low milk yield (§ 7.5.2 – milk yield in previous late lactation). Application of a 60-d DP would also be suitable for cows that do not have a history of ketosis (§ 7.2.1 – dry period length), impaired fertility (Chapter 4), or high BCS (§7.2.2). According to a review of the optimal DP length, a 40 to 60-d DP resulted in little or no loss in postpartum milk yield (**Bachman and Schairer**, 2003). Thus, cows assigned to a 60-d DP can be milked up till 40-d before calving without detrimental effects on milk yield in the subsequent lactation or impairing use of dry cow antibiotics during the DP. A second decision moment can be installed at 37 days before the expected calving date for cows that were milked after the decision moment at 67 days before the expected calving date. The advice for DP length at the decision moment at 37 days before the expected calving date can imply either application of a 30 DP or to continue milking till calving. Based on this thesis, application of a 30-d DP would be appropriate for 1) cows that have an elevated SCC at the second but not the first decision moment, 2) cows that did not have a case of clinical mastitis in the past lactation, 3) cows with postpartum parity 2, because cows with parity 2 have greater expected milk loss when given a 0-d DP compared with a 30-d DP, and 4) cows that have low milk yield at the decision moment. Furthermore, application of a 30-d DP or 0-d DP would be suitable for cows that have a history of ketosis or impaired fertility, or have a high BCS at the decision moment. Application of a 0-d DP would be appropriate for cows that do not have a high SCC at the second decision moment, did not have clinical mastitis in the previous lactation, postpartum have parity ≥3, and have high milk yield.

When this decision tree had been applied to the current experiments, in Chapter 5, a 60-d DP would have been appropriate for 36% of cows due to a high SCC and for 9% of cows due to low milk yield (**Table 7.10**). A 30-d DP cannot include use of dry cow antibiotics due to the withdrawal period for these antibiotics, which is longer than the DP. However, in Chapter 5, 12% of cows assigned to a 30-d DP had a new high SCC, which should have been treated with dry cow antibiotics during a DP matching the withdrawal period. Furthermore, 11% of cows should have been dried off due to low milk yield. In Chapter 6, 47% of cows assigned to a 30-d DP had a high SCC which should have been treated with dry cow antibiotics. However, even without dry cow antibiotics , a 30-d DP lowers SSC compared with a 0-d DP. Furthermore, 15% of cows should have been dried off due to low milk yield. The proportion of cows with high SSC at 37-d prepartum in Chapter 6 is likely overestimated because cows were only assigned to a 30-d or 0-d DP, data around 67-d before calving was not available, and cows suitable for a 60-d DP with dry cow antibiotics could, therefore, not be eliminated from the dataset. Based on Chapter 5, 30% of all cows (45 / 152), and based on Chapter 6, 50% of all cows (62 / 124) were suitable for a 0-d DP.

Table 7.10: Number and percentage of cows with a high SCC (parity 2: ≥150.000 cells/mL, parity ≥3: ≥50.000 cells/mL) before the drying off week for cows with a 60-d dry period in Chapter 5, for cows with a 30-d dry period in Chapter 5, and for cows with a 30-d dry period in Chapter 6

	Chapter 5 67-d prepartum		Chapter 5 37-d prepartum		Chapter 6 37-d prepartum	
	Milk yield > 12 kg	Milk yield < 12 kg	Milk yield > 12 kg	Milk yield < 12 kg	Milk yield > 12 kg	Milk yield < 12 kg
No high SCC	91 (60%)	6 (4%)	45 (79%)	5 (9%)	62 (50%)	4 (3%)
High SCC	47 (31%)	8 (5%)	6 (10%)	1 (2%)	44 (35%)	14 (12%)
Total		152		57		124

[1]For 152 of 167 cows in Chapter 5, SCC was available for the week before the drying off week for cows with a 60-d DP. [2]Cows assigned to a 60-d DP in Chapter 5, cows suitable for a 60-d dry period based on high SCC, or cows without available SCC from the week before the drying off week for cows with a 60-d dry period were eliminated for this analysis.

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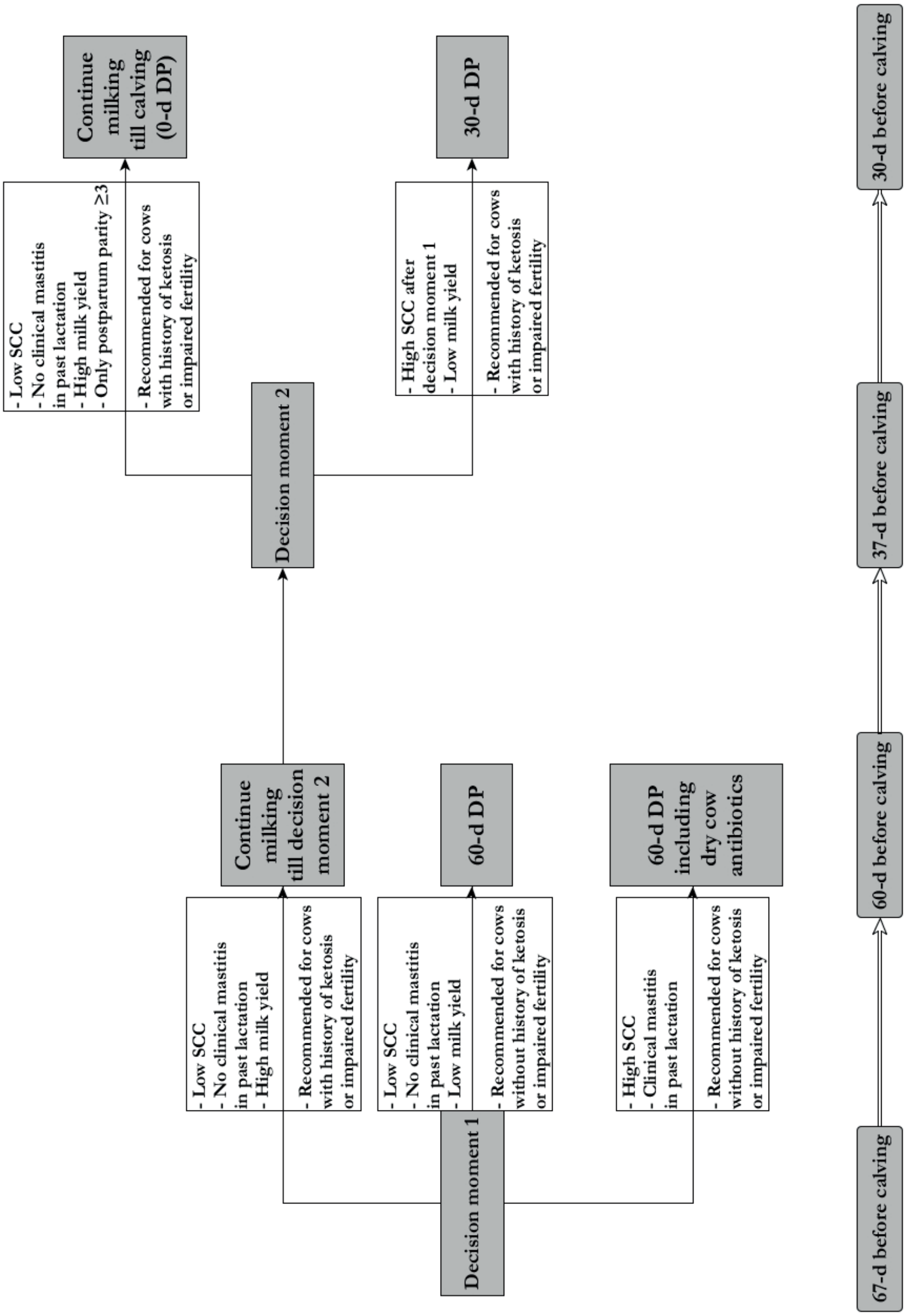

Figure 7.8: Decision tree for assigning individual cows to different dry period (DP) lengths based on udder health variables evaluated in Chapter 5 and 6.

7.7 Conclusions

A 0-d DP reduced milk yield and lactation persistency, resulting in 2.7 kg/d lower FPCM yield, improved metabolic status, and resulted in increased body weight gain and greater milk yield reduction in wk 1 – 44, compared with a 30-d DP. Reducing the postpartum dietary energy level for cows with a 0-d DP increased basal ration intake and a lower than expected contrast in dietary energy level. The lower than expected contrast may have resulted in no difference in FPCM yield or lactation persistency, body weight, BCS, or body weight gain, or metabolic status as reflected by the plasma FFA, BHB, glucose, insulin or IGF-1 concentrations throughout lactation. However, cows with a high energy level had a more positive EB in wk 6 and 7 of lactation than cows with a reduced energy level. In early lactation, feeding a high dietary energy level supports cows during the negative EB, but in mid- and late- lactation dietary energy level can likely be further reduced to limit body weight gain and to prevent greater milk yield reduction.

Feeding a glucogenic ration after peak milk yield increased DMI and FPCM yield, and improved metabolic status as reflected by a greater plasma insulin and IGF-1 concentration, although EB was not different, compared with a more lipogenic ration. Furthermore, a glucogenic ration did not affect body weight or fattening by means of greater BCS or body weight gain, compared with a lipogenic ration. Feeding a glucogenic ration after peak milk yield also did not affect lactation curve characteristics, compared with a more lipogenic ration. So, nutrient partitioning to body reserves in cows with a glucogenic or lipogenic ration was similar and greater DMI of cows with a glucogenic ration was fully partitioned to milk yield. A lipogenic ration with greater DM content and palatability can be hypothesized to increase DMI, and possibly metabolic status, milk production, and lactation persistency, compared with a more glucogenic ration.

In this thesis, a 0-d DP increased postpartum SCC compared with a 30-d or 60-d DP with dry cow antibiotics and compared with a 30-d DP without dry cow antibiotics. The difference in postpartum SCC between cows with a 0-d DP and a 30-d DP was partly explained by lower milk yield of cows with a 0-d DP compared with cows with a 30-d DP. Dry period length, without use of dry cow antibiotics, did not affect udder health across the DP. In cows with a low SCC before drying off, no application of dry cow antibiotics during a 30-d DP was not detrimental for udder health, and udder health across the DP was comparable with cows with a 0-d DP. Effects of DP length on udder health in the subsequent lactation are unclear; cows with a 0-d DP fed a high energy level tended to have a greater hazard for a case of clinical mastitis during the subsequent lactation than cows with a 30-d DP not treated with dry cow antibiotics. Cow characteristics that determined udder health in the subsequent lactation after different DP lengths included variables average SCC in the previous lactation, average SCC between 150 and 37 days prepartum, last SCC at 67d before the expected calving date, clinical mastitis in the previous lactation, reduction of milk yield in late lactation, and parity.

In conclusion, a 0-d DP will lower milk production, but improves metabolic status, compared with a DP. A 0-d DP may reduce the number of cows with metabolic disease in farms with high prevalence of metabolic disease in early lactation, which may compensate lower income due to foregone milk production. However, not all cows are suitable for a 0-d DP. Cows with postpartum parity 2 are unsuitable for a 0-d DP due to greater expected postpartum milk loss in the subsequent lactation than in greater parity cows, and cows with high SCC (at least ≥200,000 cells/mL) or a great reduction in milk yield before drying off, or a case of clinical mastitis in the previous lactation should be treated with dry cow antibiotics during a DP length matching the withdrawal period.

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7


1066
1139
1292

CHAPTER 8

Summary

Diseases in dairy cows occur mostly in early lactation and include ketosis, retained placenta, metritis, mastitis, milk fever, displaced abomasum, lameness, and impaired fertility (**Butler *et al.***, 1981; **Drackley**, 1999; **Ingvartsen *et al.***, 2003). Diseases in early lactation are often related with a negative energy balance (**EB**) (**Collard *et al.***, 2000; **Butler**, 2003; **Ingvartsen *et al.***, 2003). Shortening or omitting the dry period (**DP**) length was opted as a management strategy to improve EB in early lactation (**Grummer and Rastani**, 2004). The improved EB, after a 0-d dry period was, however, associated with fattening and spontaneous drying off in late lactation (**Chen *et al.***, 2016b). However, these cows were fed a dietary energy level for the estimated milk production after a conventional (60-d) DP. In this thesis we hypothesized that adjustment of the dietary energy level to the expected milk yield of cows with an omitted dry period, and feeding a lipogenic ration, rather than a more glucogenic ration, would prevent fattening and increase lactation persistency of cows with a 0-d DP. Additionally, we hypothesized that improved metabolic status in early lactation would be related with changes in feeding and lying behavior and steps, compared with cows with poorer metabolic status. Effects of dry period length on post-partum udder health are still ambiguous, possibly partly because in many studies DP length and use of dry cow antibiotics were confounded. Recently, preventive use of antimicrobials in the EU has been restricted. To our knowledge no study had yet evaluated effects of DP length without use of dry cow antibiotics on udder health. In a previous study, large variation in the effects of DP length on milk production in the subsequent lactation was observed between individual cows, which could partly be explained by individual cow characteristics, such as parity or milk yield in the previous lactation (**Steeneveld *et al.***, 2014). We hypothesized that prepartum cow characteristics could also determine postpartum udder health after different dry period lengths.

The aims of this thesis were 1) to evaluate effects of dietary energy level and source during lactation on milk production, metabolic status, fattening, and lactation persistency in cows with different dry period lengths, 2) to evaluate relationships between metabolic status and cow behavior of cows with different dry period lengths, 3) to study dry period lengths with and without use of dry cow antibiotics on udder health of cows with different dry period lengths, and 4) to identify cow characteristics related with udder health after different dry period lengths.

To study these aims, two large experiments were conducted. Chapter 2, 3, 4, and 6 describe experiment 1. In experiment 1, 123 cows were assigned randomly to a 0-d DP (2/3) or a 30-d DP (1/3). Cows with a 0-d DP were fed either a (**LOW**) energy level, which was based on the energy requirements for their expected milk yield [**0-d DP(LOW)**] or a standard (**STD**) energy level [**0-d DP(STD)**] based on the requirement for the expected milk yield of cows with a 30-d DP. From wk 8 onwards, cows were fed either a glucogenic or lipogenic ration with a LOW or STD energy content. In experiment 1, cows with a 30-d DP were not treated with dry cow antibiotics at drying off. In experiment 2, 167 cows were assigned randomly to a 0-d, 30-d, or 60-d DP. At dry off, cows with a 30-d or 60-d DP were treated with dry cow antibiotics.

Chapter 2 describes effects of dietary energy level on milk production, EB, and metabolic status in wk 1 – 7 of lactation. Cows with a 0-d DP had lower fat-and-protein-corrected milk (**FPCM**) and a better metabolic status as reflected by lower plasma free-fatty acid (**FFA**) and β-hydroxybutyrate (**BHB**) concentrations, and greater dry matter intake (**DMI**), EB, plasma glucose, insulin and IGF-1 concentrations than cows with a 30-d DP. Cows with a 0-d DP received either a LOW or STD concentrate level. Reducing concentrate level for cows with a 0-d DP resulted in a less positive EB in week 6 and 7 postpartum, but did not affect milk yield or plasma metabolites. We concluded that dietary energy level can be reduced to the requirements for expected milk yield of cows with a 0-d DP, without detrimental effects on milk yield or metabolic status.

In **Chapter 3,** effects of dietary energy level and dietary energy source on fattening and lactation curve characteristics, such as persistency, after wk 7 in lactation are described. Compared with a 0-d DP, a 30-d DP resulted in a lower body weight gain, and greater milk yield and lactation persistency. Greater lactation persistency in cows with a 30-d DP was likely related with their lower metabolic status as reflected by a lower EB, lower plasma insulin and IGF-1, and greater plasma GH concentration. Reducing dietary energy level for cows with a 0-d DP decreased energy intake, EB, and weekly body weight gain, but did not affect milk production and lactation curve characteristics. Feeding a glucogenic ration increased DMI and energy intake, milk yield, and metabolic status as reflected by greater plasma IGF-1 concentration and lower milk fat percentage than in cows fed a more lipogenic ration. Fattening or lactation curve characteristics were not affected by dietary energy source. In conclusion, dry period length, dietary energy

8

level, but not dietary energy source, affected the EB and fattening of dairy cows during wk 1 – 44 of lactation. Dry period length, but not dietary energy level or dietary energy source affected lactation persistency of dairy cows.

In **Chapter 4,** relations between metabolic status and behavior were studied. Cows were clustered for metabolic status based on plasma metabolite and hormone concentrations, which resulted in groups of cows with good, average, poor, or very good metabolic status (only cows with a 0-d DP). Better metabolic status was associated with greater DMI, basal ration intake, more visits and meals, and more time spent lying, compared with a poor metabolic status. Lying time had a positive, monotonous relation with metabolic status among clusters and may be a reliable indicator for metabolic status. Lying time can be measured using sensor technology and can be a practical tool to monitor metabolic status in dairy cows in early lactation.

In **Chapter 5**, udder health of cows with a 0, 30, or 60-d DP was assessed. Cows with a 30-d or 60-d DP were treated with dry cow antibiotics at dry off. Cows with a 0-d DP had a greater occurrence of chronic intra-mammary infections (**IMI**) and a lower occurrence of cured IMI across the DP, than cows with a 30-d or 60-d DP. Postpartum average somatic cell count (**SCC**) for lactation was greater in cows with a 0-d DP than in cows with a 30-d or 60-d DP, although incidence of elevations of SCC (SCC ≥ 200,000 cells/mL) or clinical mastitis were not different among cows with different DP lengths. Postpartum SCC was determined by parity, elevations of SCC in the previous lactation, last FPCM before a 60-d DP, average SCC between 150-37 days prepartum, and average SCC for the previous lactation. Occurrence of postpartum elevations of SCC was determined by parity. Occurrence of clinical mastitis in the subsequent lactation was determined by a greater last SCC before the conventional drying-off day. The identified cow characteristics that predict postpartum udder health could be used in a decision support model to optimize DP length for individual cows.

In **Chapter 6**, udder health of cows with a 0-d or 30-d DP without use of dry cow antibiotics was assessed. Prevalence of udder quarters with elevated SCC or with mastitis pathogens was not different in wk 5 prepartum, or wk 1 or 5 postpartum between cows with a 0-d DP or a 30-d DP. Furthermore, occurrence of chronic or new IMI across the DP was not different between cows with a 0-d or 30-d DP. During wk 1 – 44 of lactation, cows with a 0-d DP had a greater SCC than cows with a 30-d DP, but not when SCC was corrected for milk yield. During wk 1 – 44 of lactation, the occurrence of at least 1 elevation of SCC (≥200,000 cells/mL) and clinical mastitis were not different between DP lengths, although cows with a 0-d DP had a numerically greater occurrence of clinical mastitis and tended to have a greater hazard for clinical mastitis than cows with a 30-d DP. These results should be confirmed in a larger field study.

Comparing **Chapter 5 and 6**, a 0-d DP seems to decrease udder health, compared with a 30-d or 60-d DP with dry cow antibiotics. However, when none of the cows were treated with dry cow antibiotics, cows with a 0-d DP did not differ from cows with a 30-d DP for all assessed udder health variables across the DP and for most of the assessed udder health variables in the subsequent lactation. Cows with a 0-d DP tended to have greater hazard for clinical mastitis and a numerically greater incidence of clinical mastitis, compared with cows with a 30-d DP not treated with dry cow antibiotics (Chapter 6). Incidence of clinical mastitis did not differ between cows with a 0-d DP and cows with a 30-d DP treated with dry cow antibiotics (Chapter 5), while in the latter situation a greater incidence of clinical mastitis was expected for cows with a 0-d DP.

Chapter 7 is the general discussion, which elaborated on what cows are suitable for different DP lengths and how these cows should be managed. In conclusion, a 0-d DP will lower milk production, but improves metabolic status in the subsequent lactation, compared with a DP. A 0-d DP may reduce the number of cows with metabolic disease in farms with high prevalence of metabolic disease in early lactation, which may compensate lower income due to foregone milk production. However, not all cows seem to be suitable for a 0-d DP. Cows with postpartum parity 2 are less suitable for a 0-d DP due to lower postpartum milk yield than in cows of greater parity. Cows with a great reduction in milk yield before drying off are unsuitable for a 0-d DP because they do not produce milk till calving. Cows with high SCC or a case of clinical mastitis in the previous lactation are also unsuitable for a 0-d DP because they should be treated with dry cow antibiotics during a DP length matching the withdrawal period to prevent udder health problems in the subsequent lactation. With regard to metabolic status, cows with a high prepartum BCS benefit from a 0-d DP because postpartum a greater DMI and EB can be expected, compared with giving cows with a high BCS a 30-d DP. Postpartum, feeding a high energy level to cows with a 0-d DP improved EB and seemed to improve lactation persistency, compared with a reduced energy level. In mid- and late- lactation, however, dietary energy level may need to be further reduced for cows with a 0-d DP to prevent fattening compared with a 30-d DP. Postpartum feeding a lipogenic ration decreased DMI, milk yield, and metabolic status, and did not improve lactation persistency, although plasma GH concentration was greater and plasma insulin concentration was lower, compared with a more glucogenic ration. Improvement of palatability and increasing dry matter content of the lipogenic ration may have resulted in similar DMI and improved lactation persistency, compared with a glucogenic ration.

Ziekten bij melkkoeien komen met name voor tijdens de vroege lactatie en omvatten onder andere ketose (slepende melkziekte), aan-de-nageboorte-staan, metritis (baarmoederontsteking), melkziekte, lebmaagdislocatie, kreupelheid, en verminderde vruchtbaarheid (**Butler *et al.***, 1981; **Drackley**, 1999; **Ingvartsen *et al.***, 2003). Ziekten tijdens de vroege lactatie zijn vaak gerelateerd aan een negatieve energiebalans (**EB**) (**Collard *et al.***, 2000; **Butler**, 2003; **Ingvartsen *et al.***, 2003). Verkorten of weglaten van de droogstandsperiode (**DP**) is eerder gesuggereerd als een managementstrategie om de EB tijdens de vroege lactatie te verbeteren (**Grummer and Rastani**, 2004). De verbeterde EB na een 0-d DP bleek echter geassocieerd met vervetting en spontane prepartum droogstand (**Chen *et al.***, 2016b). De betreffende koeien hadden echter een rantsoen gekregen met een energie inhoud overeenkomstig de verwachte melkproductie van koeien met een conventionele (60-d) DP. In deze thesis hebben we de hypothese gehanteerd dat aanpassen van het energieniveau in het rantsoen aan de verwachte melkproductie van koeien zonder droogstand, of het voeren van een lipogeen- in plaats van een glucogeen- rantsoen, vervetting zou voorkomen en de lactatiepersistentie zou verbeteren. Daarnaast hanteerden we de hypothese dat een verbeterde metabole status van koeien geassocieerd zou zijn met veranderingen in eet-, lig- en loopgedrag vergeleken met het gedrag van koeien met een mindere metabole status. Het is bovendien nog steeds niet duidelijk welke invloed droogstandslengte heeft op postpartum uiergezondheid, waarschijnlijk mede doordat in veel studies naar droogstandslengte droogzetantibiotica gebruikt werden. Recent is het preventieve gebruik van droogzetantibiotica aan banden gelegd. Voor zover wij weten is de invloed van droogstandslengte zonder gebruik van droogzet antibiotica op uiergezondheid echter nog niet eerder onderzocht. In een eerdere studie werd bij verschillende droogstandslengtes een grote variatie gevonden in de melkproductie van individuele koeien in de volgende lactatie. Deze variatie kon deels worden toegeschreven aan individuele koe-eigenschappen zoals pariteit of melkproductie in de vorige lactatie (**Steeneveld *et al.***, 2014). Onze hypothese was dat dergelijke prepartum koe-eigenschappen de postpartum uiergezondheid na verschillende droogstandslengten kunnen bepalen.

De doelen in deze thesis waren 1) evaluatie van invloeden van energieniveau en energiebron in het rantsoen tijdens lactatie op melkproductie, metabole status, vervetting, en lactatie persistentie van koeien na verschillende droogstandslengten, 2) evaluatie van de relatie tussen metabole status en gedrag van koeien met verschillende droogstandslengten, 3) onderzoeken van het de invloed van verschillende droogstandslengten met en zonder droogzetantibiotica op pre- en postpartum uiergezondheid, 4) identificeren van prepartum koe-eigenschappen die gerelateerd zijn aan uiergezondheid na verschillende droogstandslengten.

Om deze doelen te onderzoeken werden twee grote experimenten uitgevoerd. Hoofdstuk 2, 3, 4, en 6 beschrijven experiment 1. In experiment 1 werden 123 koeien random toegewezen aan een 0-d DP (2/3) of een 30-d DP (1/3). Koeien met een 0-d DP kregen een rantsoen met een laag (**LOW**) energieniveau dat gebaseerd was op de behoefte voor hun verwachte melkproductie [**0-d DP(LOW)**] of een standaard (**STD**) energieniveau [**0-d DP(STD)**] gebaseerd op de behoefte voor de verwachte melkproductie van koeien met een 30-d DP [**30-d DP(STD)**]. Vanaf 8 weken in lactatie kregen de koeien een glucogeen- of lipogeen- rantsoen met een LOW of STD energieniveau toegewezen. In experiment 1 werden koeien met een 30-d DP niet behandeld met droogzetantibiotica. In experiment 2 werden 167 koeien random toegewezen aan een 0-d, 30-d, of 60-d DP. Bij het droogzetten werden koeien met een 30-d of 60-d DP behandeld met droogzetantibiotica.

Hoofdstuk 2 beschrijft de invloed van het energieniveau in het rantsoen op melkproductie, EB, en metabole status in week 1 – 7 van de lactatie. Koeien met een 0-d DP hadden een lagere vet- en eiwit-gecorrigeerde melkproductie (**FPCM**) en een betere metabole status, zoals op te maken was uit de lagere plasma concentratie vrije-vetzuren (**FFA**) en β-hydroxyboterzuur (**BHB**), en grotere drogestof opname (**DMI**), EB, en plasma glucose-, insuline-, en IGF-1-concentratie, dan bij koeien met een 30-d DP. Koeien met een 0-d DP kregen een rantsoen met een LOW of STD energieniveau. Het verlagen van het energieniveau voor koeien met een 0-d DP resulteerde in een minder positieve EB in week 6 en 7 postpartum, maar had geen invloed op melkproductie en plasma metaboliet concentraties. Onze conclusie was dat het energieniveau van het rantsoen verlaagd kan worden tot de behoefte voor de verwachte melkproductie van koeien met een 0-d DP, zonder negatieve invloeden op melkproductie of metabole status.

In **Hoofdstuk 3** wordt de invloed van de energieniveau en energiebron in het rantsoen op vervetting en lactatiecurve-karakteristieken zoals persistentie, na week 7 van lactatie beschreven. In vergelijking met koeien met een 0-d DP resulteerde een 30-d DP in een lagere toename in lichaamsgewicht en grotere melkgift en lactatiepersistentie. De grotere lactatiepersistentie van koeien met een

30-d DP was waarschijnlijk gerelateerd aan de lagere metabole status, zoals bleek uit de lagere EB, lagere plasma insuline- en IGF-1-concentratie en hogere plasma GH concentratie. Het verlagen van het energieniveau voor koeien met een 0-d DP verlaagde de energie-inname, EB, en wekelijkse toename in lichaamsgewicht, maar had geen invloed op melkproductie en lactatiecurve- karakteristieken. Het voeren van een glucogeen rantsoen verhoogde de DMI en energie-inname, melkgift, en metabole status, zoals bleek uit de hogere plasma IGF-1 concentratie en het lagere melkvet percentage, vergeleken met koeien die een lipogeen rantsoen kregen. In conclusie bleek dat droogstandslengte en energieniveau, maar niet de energiebron, EB en vervetting van koeien tussen week 1 en 44 van lactatie beïnvloeden. Droogstandslengte, maar niet energieniveau of energiebron in het rantsoen, beïnvloeden lactatiepersistentie van melkkoeien.

In **Hoofdstuk 4** werd de relatie tussen metabole status en gedrag bestudeerd. Koeien werden geclusterd voor metabole status op basis van plasma metaboliet- en hormoonconcentraties, wat resulteerde in vier groepen koeien met een goede, gemiddelde, slechte, of hele goede metabole status (alleen koeien met een 0-d DP). Een betere metabole status was geassocieerd met een hogere DMI, hogere opname van het basisrantsoen, meer bezoeken aan de voerbak, meer maaltijden, en meer lig- en minder looptijd, in vergelijking met koeien met een slechte metabole status. Ligtijd had een positieve relatie met metabole status tussen clusters en zou een betrouwbare indicator kunnen zijn voor metabole status. Ligtijd kan worden gemeten met sensortechnologie en kan een praktisch hulpmiddel zijn voor het monitoren van metabole status van melkkoeien in vroege lactatie.

In **Hoofdstuk 5** werd de uiergezondheid van koeien met een 0, 30, of 60 dagen DP onderzocht. Koeien met een 30-d of 60-d DP werden bij het droogzetten behandeld met droogzetantibiotica. Koeien met een 0-d DP hadden meer chronische intramammaire infecties (**IMI**) en minder genezen IMI tijdens de prepartum periode dan koeien met een 30-d or 60-d DP. Postpartum was het gemiddelde somatisch celgetal (**SCC**) hoger voor koeien met een 0-d DP dan voor koeien met een 30-d of 60-d DP, maar de incidentie van verhogingen van celgetal (SCC ≥ 200,000 cellen/mL) en klinische mastitis was niet verschillend voor koeien met een verschillende droogstandslengte. Postpartum SCC werd bepaald door pariteit, verhogingen van SCC in de vorige lactatie, laatste FPCM voor een 60-d DP, gemiddelde SCC tussen 150 – 37 dagen prepartum, en gemiddeld SCC in de

8

vorige lactatie. De incidentie van postpartum verhogingen van SCC werd uitsluitend bepaald door pariteit. De incidentie van postpartum klinische mastitis werd bepaald door een hoger laatste SCC vóór de conventionele droogzetdag. De hiervoor geïdentificeerde koe-eigenschappen die postpartum uiergezondheid bepalen kunnen gebruikt worden in een beslismodel voor optimalisatie van droogstandslengte voor individuele koeien.

In **Hoofdstuk 6** werd de uiergezondheid van koeien met een 0-d or 30-d DP zonder gebruik van droogzetantibiotica onderzocht. Prevalentie van uierkwartieren met een verhoogd celgetal of mastitispathogenen was niet verschillend in week 5 prepartum, of week 1 of 5 postpartum tussen koeien met een 0-d of 30-d DP. Bovendien was het percentage koeien met een chronische of nieuwe IMI tijdens de DP niet verschillend voor een 0-d of 30-d DP zonder droogzetantibiotica. Tijdens week 1 – 44 van de lactatie hadden koeien met een 0-d DP een hoger celgetal dan koeien met een 30-d DP, maar niet wanneer het celgetal werd gecorrigeerd voor melkgift. Tijdens week 1 – 44 van de lactatie was het vóórkomen van tenminste 1 verhoging van celgetal (SCC ≥200,000 cellen/mL) en klinische mastitis statistisch niet verschillend tussen droogstandslengten, hoewel koeien met een 0-d DP numeriek meer klinische mastitis hadden en een groter risico leken te hebben om klinische mastitis te krijgen dan koeien met een 30-d DP. Deze resultaten moeten nog worden bevestigd in een grotere veldstudie.

Bij vergelijking van **Hoofdstuk 5 en 6** lijkt een 0-d DP de postpartum uiergezondheid te verminderen in vergelijking met een 30-d of 60-d DP met gebruik van droogzetantibiotica. Hoewel er een grotere incidentie van klinische mastitis verwacht werd bij koeien met een 0-d DP bleek de incidentie van klinische mastitis niet te verschillen tussen koeien met een 0-d- en koeien met een 30-d DP die behandeld waren met droogzetantibiotica (Hoofdstuk 5). Wanneer geen droogzetantibiotica gebruikt werden, waren alle uiergezondheidsvariabelen tijdens de droogstand en de meeste postpartum uiergezondheidsvariabelen niet verschillend tussen koeien met een 0-d or 30-d DP. Koeien met een 0-d DP hadden wel een tendens tot een groter risico op klinische mastitis vergeleken met koeien met een 30-d DP die niet behandeld waren met droogzetantibiotica (Hoofdstuk 6).

Hoofdstuk 7 is de algemene discussie waarin wordt aangegeven welke koeien geschikt zijn om een bepaalde droogstandslengte te ondergaan en hoe deze koeien behandeld moeten worden. In conclusie leidt een 0-d DP tot een lagere melkproductie, maar ook tot een verbeterde metabole status in de volgende lactatie, in vergelijking met koeien die een droogstandsperiode hebben gehad. Een 0-d DP kan het aantal koeien met metabole ziekten verlagen op bedrijven met een hoge prevalentie van metabole ziekten tijdens de vroege lactatie, hetgeen mogelijk de lagere inkomsten door gederfde melkproductie als gevolg van postpartum metabole ziekten compenseert. Echter, niet alle koeien zijn geschikt voor een 0-d DP. Koeien met postpartum pariteit 2 zijn minder geschikt voor een 0-d DP vanwege een lagere verwachte melkproductie in vergelijking met koeien met een hogere pariteit. Koeien met een grote melkproductiedaling vóór het droogzetten zijn ook ongeschikt voor een 0-d DP, omdat ze geen melk kunnen produceren tot 0-d voor afkalven. Koeien met een hoog celgetal of een geval van klinische mastitis in de vorige lactatie zijn eveneens ongeschikt voor een 0-d DP, omdat ze behandeld dienen te worden met droogzetantibiotica, tijdens een droogstandsperiode die minstens overeenkomt met de voorgeschreven wachttijd, om uiergezondheidsproblemen in de volgende lactatie te voorkomen. Met het oog op een goede metabole status hebben koeien met een hoge prepartum BCS juist baat bij een 0-d DP, omdat dan een grotere postpartum DMI en EB wordt verwacht dan wanneer deze koeien een 30-d DP krijgen. Het postpartum voeren van een rantsoen met een hoog energieniveau aan koeien met een 0-d DP resulteerde in een verbeterde EB en leek de lactatiepersistentie te verbeteren. Echter, in mid- en laat- lactatie moet het energieniveau voor koeien met een 0-d DP waarschijnlijk juist nog verder verlaagd worden voor koeien met een 0-d DP om vervetting te voorkomen. Het postpartum voeren van een lipogeen-, in vergelijking met een glucogeen- rantsoen aan koeien met een 0-d DP verlaagde de DMI, melkgift en metabole status, en verbeterde de lactatiepersistentie niet, hoewel de plasma GH concentratie wel groter en de plasma insuline concentratie lager was. Verbetering van de smakelijkheid en verhoging van het drogestofgehalte van het lipogene rantsoen zou wellicht kunnen zorgen voor een gelijke DMI en verbeterde lactatiepersistentie vergeleken met het glucogene rantsoen.

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CHAPTER 9

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‘Mijn’ experiment was groot en ik had het nooit alleen kunnen bolwerken. **Martin de Bree en Edwin Bleumer**, bedankt voor jullie hulp bij het overzien van dit project. **Martin**, dankjewel dat je net zoveel gaf om mijn koeien, mijn experiment, en alle metingen als ik. Het was een waar genoegen om met je samen te werken, al hoop ik dat het experiment en alles wat erbij kwam kijken, inclusief verhuizen van de koeien, je niet al te veel grijze haren heeft bezorgd. **Gerrit Remmelink**, hartelijk dank dat je me hebt geleerd de rantsoenen te formuleren, voor je hulp met het poolen van voermonsters, voor je lessen over diervoeding in de praktijk (organische producten ‘werken’ of je het nu wilt of niet…), voor je advies als ik experimentele twijfels had en voor je grote toewijding aan het experiment. **Karel van Houwelingen**, dank voor je geweldige werk met de gigantische dataset. Ik zal de uitgebreide mogelijkheden van ‘Access’ in geen duizend jaar doorgronden en daarom ben ik buitengewoon dankbaar voor de manier waarop jij alle data samen wist te brengen. Daarnaast wil ik alle **medewerkers van Dairy Campus Lelystad** bedanken, die er voor zorgden dat ‘mijn meiden’ elke dag hun eten op tijd kregen, hun melk kwijt konden en ook met grote zorg over hun gezondheid waakten. Jullie stellen werkelijk een geweldige standaard voor de melkveehouderij! Mijn speciale dank gaat uit naar **Jan Zandbergen**, die altijd paraat stond om mijn vragen te beantwoorden en niet te beroerd was om af en toe een bloedmonster te nemen als ik even pauze nodig had. Naar **Meint en Gerard**, voor hun kritische blik op mijn koeien en mijn experiment, en voor hun zorg voor ‘mijn lieve meiden’ als ze pijn in hun poten hadden of mastitis hadden en voor het altijd weten waar ze waren als ze in het afkalfhok zaten! En **Ebele**, dankjewel voor je betrokkenheid en hulp bij het project, het vangen van koeien, en de gezelligheid in de onderzoeksruimte; tige tank!

Ik wil de stuurgroep van Droogstand op Maat bedanken voor hun waardevolle aanvullingen en suggesties voor het project.

I want to thank the WHYDRY team; **Juncai, Novi, Akke, and Wei**, for being such a supportive dairy team. I have loved our tea-meetings, and it’s true what they say: sharing and discussing PhD troubles really helps!

Special thanks go to our esteemed colleagues from Bern, **Prof. Dr. Rupert Bruckmaier and Dr. Josef Gross**, for the collaboration in the project and more so, for all the samples they analysed for me. When I wanted to analyse Growth Hormone concentrations, they asked if we could maybe downsize the numbers,

because there were so many... But eventually the **Bern lab-ladies** analysed all samples and I'm still immensely grateful for that!

Ook vanuit onze groep Adaptatiefysiologie kwam hulp. **Ger**, dankjewel voor je hulp met het pipetteren van de plasma monsters in Lelystad, voor je geduld terwijl je mij leerde hoe ik een ELISA moest doen (wat een behoorlijke prestatie is!), dat je er altijd was om mijn praktische vragen te beantwoorden, en natuurlijk voor de 600 'blokken' die je vanuit alle hoeken bij elkaar sprokkelde. **Lora**, dankjewel voor het printen van al die kleine stickertjes, elke week gedurende 2,5 jaar. Ik heb onze wekelijkse printfeestjes gemist toe we klaar waren. Natuurlijk wil ik **Lora en Nanette** ook samen bedanken voor hun geweldige werk als secretaresses van ADP. Ongelofelijk hoe jullie, altijd met grote zorg, tijd en ruimte maken voor regelwerkzaamheden en het beantwoorden van alle vragen. En **Rudi**, dankjewel dat je de RIA's hebt gedaan op die eindeloze hoeveelheid monsters van mij. Het was een rijstebrij berg, maar je bent er doorheen gekomen! Ik had mijn artikelen zeker niet op tijd afgekregen zonder jouw geweldige werk en tomeloze inzet.

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I would like to thank my **fellow WAPS council members** for our cooperation. We have established things within the animal sciences graduate school that we can be really proud of! I would like to thank WIAS, both WIAS directors that I worked with, **Johan Verreth** and **Johan van Leeuwen,** and both WIAS secretaries **Denise Magendans** and **Janneke van Seters** for the pleasant cooperation. I also want to thank my **fellow members of the Wageningen PhD Council (WPC)** for the good times we have had and for the many things we achieved within Wageningen, but also

nationally. I am really grateful for the opportunity the WPC gave me to be representative of the WPC in the PhD Network of the Netherlands. It did truly widen my perspective on the work we were doing in the WPC, but also very simple, within my own group.

Mijn MSc studenten, Monica en Tim, hielpen mij op donderdag met het vangen van koeien, ze kalmeren en het dragen van de bloedmonsters op ijs. **Monica**, enorm bedankt voor je gezelschap, voor de gesprekken die we hadden op de vroege donderdagochtend, en voor het aaien en geruststellen van mijn koeien. Ze vonden het heerlijk en het maakte het veel makkelijker om met ze te werken. **Tim**, dankjewel voor al je hulp. Jij maakte het werk heel gemakkelijk; een ware cowboy! Als veehouder heb je mij vele waardevolle lessen geleerd over de melkveehouderij. My MSc student **Francesca** became my PhD colleague after she finished her above excellent thesis on persistency with me. For her thesis she helped Rudi with the RIA's with the most immaculate precision. Thank you so much for your commitment to the work and the great times we had together.

Hilda krijgt een speciaal plekje. Al sinds de kleuterschool trekken we samen op en nu deden we zelfs tegelijk een PhD in Wageningen. Ik heb genoten van onze wekelijkse wandelingetjes over de campus waarin we weer uren konden kletsen! Ik heb je gemist toen jij klaar was en ik nog een half jaar doorging!

Natuurlijk wil ik mijn schoonfamilie bedanken die vier jaar lang mijn verhalen heeft aangehoord. **Mieke en Max**, dankjewel voor alle gezellige uitjes en bijeenkomsten die jullie organiseren en dat we altijd bij jullie terecht kunnen voor een kopje thee en goede raad. **Mick en Gerjanne**, dank jullie wel voor de lekkere maaltijden en jullie leuke verhalen. Het is nooit saai als jullie er zijn! En tenslotte, **Ivo en Erik**, jullie zijn de liefste zwagers die ik me kan wensen! En als mede-dierenartsen-buiten-de-praktijk voel ik me helemaal bij jullie thuis.

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CHAPTER 10

Curriculum Vitae
Publications
Training and supervision plan
Colophon

Rienkje Johanna (Renny) van Hoeij was born on June 30th 1987 in Utrecht, The Netherlands. After completing secondary school in 2005 she studied Veterinary Medicine at the University of Ghent. In 2006 she transferred to Utrecht to continue her studies in Veterinary Medicine at Utrecht University. Already during her studies Renny grew more interested in animal nutrition and research. She participated in dairy nutrition research at the Veterinary Training and Research Centre of the University of California Davis in Tulare, USA in 2010, and did field research and data analysis at De Heus Voeders B.V., Ede in 2011 and 2012. Renny obtained her degree in Veterinary Medicine at Utrecht University in 2013 after which she started her PhD research at the Adaptation Physiology Group (ADP) of Wageningen University in the 'Customized dry period' project. The aim of her PhD project was to study the effect of a short or omitted dry period and different dietary energy levels and sources on udder health, metabolic health in early lactation, and lactation persistency in late lactation. Results of this research are presented in this thesis. Her first article, on cow characteristics associated with udder health after different dry period lengths, was honored as 'Editor's choice of the month' in September 2016. In January 2017 Renny received a scholarship to present her work at the National Mastitis Council annual meeting in St. Pete beach, Florida, USA.

PUBLICATIONS

Peer-reviewed scientific articles

Van Hoeij, R. J., J. Dijkstra, R. M. Bruckmaier, J. J. Gross, T. J. Lam, G. J. Remmelink, B. Kemp, and A. T. M. Knegsel (2017). Consequences of dietary energy source and energy level on energy balance, lactogenic hormones and lactation curve characteristics of cows after a short or omitted dry period. J. Dairy Sci.

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Kok, A., R. J. Van Hoeij, B. J. Tolkamp, M. J. Haskell, A. T. M. Van Knegsel, I. J. M. De Boer, and E. A. Bokkers. 2016. Behavioural adaptation to a short or no dry period in dairy cows. Appl. Anim. Behav. Sci. http://dx.doi.org/10.1016/j.applanim.2016.10.017

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Conference proceedings

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Kok, A., R.J. van Hoeij, B. J. Tolkamp, M. J. Haskell, A. T. M. van Knegsel, I. J. M. de Boer, E. A. M. Bokkers (2016). Effects of omission of the dry period on behaviour of dairy cows. Proceedings of the 4th DairyCare COST ACTION Conference 2016. - DairyCare COST Action: 14

Kok, A., R.J. van Hoeij, B. J. Tolkamp, M. J. Haskell, A. T. M. van Knegsel, I. J. M. de Boer, E. A. M. Bokkers (2016). Effects of short and no dry period on milk yield and behaviour of dairy cows. Proceedings of the 50th Congress of the International Society for Applied Ethology, Edinburg, 2016-07-12/2016-07-15

Van Knegsel, A. T. M., N. Mayasari, J. Chen, R. J. van Hoeij, A. Kok, B. Kemp (2016). Customising dry period length to improve adaptation to lactation in dairy cows, Proceedings of the 16th International Conference on Production Diseases in Farm Animals, Wageningen, the Netherlands

Xu, W., A. T .M. van Knegsel, D. B. de Koning, R. J. van Hoeij, B. Kemp, J. J. M. Vervoort (2016). Metabolite profiles in blood and milk for cows with different dry period lengths in early lactation. Proceedings of the 16th International Conference on Production Diseases in Farm Animals, Wageningen, the Netherlands

Rapporten

van Hoeij, R.J., T.J.G.M. Lam, D.B. de Koning, W. Steeneveld, B. Kemp, A.T.M. van Knegsel (2016). Uiergezondheid na verschillende droogstandslengtes: I. met gebruik van droogzetantibiotica. Rapport Droogstand op Maat.

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Van Hoeij, R.J., J. Dijkstra, G.J. Remmelink, B. Kemp en A.T.M. van Knegsel (2016). Het voorspellen van de energiebalans na verschillende droogstandslengtes. Rapport Droogstand op Maat.

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Van Hoeij, R.J., J. Dijkstra, R.M. Bruckmaier, J.J. Gross, T.J.G.M. Lam, G.J. Remmelink, B. Kemp, en A.T.M. van Knegsel (2017). Effect van energieniveau en energiesoort van het rantsoen op de energiebalans en lactatiepersistentie bij melkvee na een verkorte of geen droogstand. Rapport Droogstand op Maat.

Van Hoeij[1], R. J., A. Kok[1], B.J. Tolkamp, M.J. Haskell, B. Kemp, R.M. Bruckmaier, I.J.M. de Boer, E.A.M. Bokkers, A.T.M. van Knegsel (2017). Effecten van geen en korte droogstand op gedrag van koeien, en relaties met metabolieten. Rapport Droogstand op Maat. [1]Auteurs hebben evenveel bijgedragen.

TRAINING AND SUPERVISION PLAN OF THE WIAS GRADUATE SCHOOL

Compulsory courses (3 ECTS)

- WIAS Introduction Course (2015)
- Course on philosophy of science and ethics (2015)

Writing of project proposal (4 ECTS)

The effect of a short or omitted dry period and a different level of dietary energy and different energy sources on udder health, metabolic health in early lactation, and persistency in late lactation.

Post-graduate courses (4.3 ECTS)

- Rundveevoeding, Wageningen Academy (2014), 0.6 ECTS
- Advanced statistics: Design of Experiments, WGS (2014), 1.0 ECTS
- Energy metabolism and body composition, VLAG (2016), 1.0 ECTS
- Epigenesis and epigenetics, VLAG (2017), 0.8 ECTS
- Applied biotatalysis, VLAG (2017), 0.9 ECTS

Competence and skills courses (6.7 ECTS)

- Course on laboratory animal science, Utrecht University (2014), 3 ECTS
- Teaching and supervising thesis students, ESD (2014), 0.6 ECTS
- Project and time management, WGS (2014), 1.5 ECTS
- PhD competence assessment, WGS (2014), 0.3 ECTS
- Techniques for writing and presenting a scientific paper, WGS (2015), 1.2 ECTS
- Career orientation, WGS (2015), 1.5 ECTS
- Presenting with impact, Wageningen Graduate Schools (WGS) (2016), 1.0 ECTS
- Career assessment, WGS (2016), 0.3 ECTS

WIAS science day (0.9 ECTS)

- WIAS Science day (2014, 2015, 2016)

Discussion groups/local seminars/scientific meetings (3.1 ECTS)

- RFS 'de Veetelers' Lustrum symposium 'Limits to produce', Wageningen (2014)
- PhD Workshop Carousel, Wageningen (2014)
- Symposium droogstandslengte, Wageningen (2015)
- WPC PhD Symposium, Wageningen (2015)
- Mastitis meeting, Utrecht University (2015-2017)

International Conferences (17.4 ECTS)

- Animal Nutrition Research Forum (2014 – 2016)
- National Mastitis Council regional meeting, Gent, Belgium (2014)
- European College of Bovine Health Management, Maribor, Slovenia (2015)
- EU COST action 'DairyCare' conference, Zadar, Croatia (2015)
- 16th International Conference on Production Diseases in Farm animals, Wageningen, the Netherlands (2016)
- American Dairy Science Association Join Annual Meeting, Salt lake City, Utah, USA (2016)
- National Mastitis Council Annual meeting, St. Pete Beach, Florida, USA (2017) **Scholarship**
- 50th European Veterinary Conferences Voorjaarsdagen, Den Haag, the Netherlands (2017) **Invited speaker**

Supervision of practicals and tutoring (3.6 ECTS)

- Introduction to animal sciences (2013 – 2014)
- Applied animal biology practical (2015)
- Integrated course on ruminants (2014 – 2015)
- Adaptation physiology 2 (2015)

Supervision of thesis students (8.5 ECTS)

- Contributing to decision making by determining sensitivity rates of sensors to predict dairy cow diseases
- The effect of nutrition on health parameters after different dry period lengths
- The effect of nutritional energy source and dry period length on lactation persistency
- The effect of nutritional energy source and dry period length on plasma hormones related with persistency
- The effect of dry period length on cloths in milk of dairy cows in the precalving period
- Meta-analysis of effects of dry period length on udder health

Management tasks (6 ECTS)

- Convener of session 'Animals in the future', WPC PhD Symposium, Wageningen (2015)
- Secretary of WAPS Council (2015)
- Chair of WAPS Council (2016)
- Board member of Wageningen PhD council (WPC) and representative at PhD Netwerk Nederland (PNN) (2015 – 2017)

COLOFON

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Omslagontwerp: Marieke van Bergen – Vennix; ProefschriftMaken, Wageningen

Thesisontwerp en -opmaak: Renny van Hoeij; ProefschriftMaken, Wageningen

Drukwerk: ProefschriftMaken, Wageningen

Propositions

1. Lactation persistency of cows with an omitted dry period is not enhanced by adjusting the dietary energy levels to requirements for their expected milk yield, or by feeding a lipogenic ration in subsequent lactation.
   (this thesis)

2. The appropriate dry period management for an individual cow is not only dependent on the expected effect of dry period length on milk production and metabolic status in the subsequent lactation, but also on udder health prior to the dry period.
   (this thesis)

3. No sophisticated technique is available for treatment of esophageal achalasia.

4. Practicing performing arts stimulates brain development, social skills, and fine motor skills.

5. Striving to cite most recent references leads to bias of long forgotten but valuable insights, and re-invention of the wheel.

6. Science is not equal to truth, but merely a reflection of the interpretation of data by the scientist.

7. Women's emancipation in business has been a greater success than men's emancipation in domestic affairs.

8. Sailing is applied mindfulness training.

Propositions belonging to the thesis, entitled:

Metabolic status, lactation persistency, and udder health of dairy cows after different dry period lengths

Renny van Hoeij

Wageningen, 20 December 2017